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Exploring Traditional Medicine

Report of a Symposium



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Editors:
P.F. McGrath and P. Tout



Cover pictures:

The small burr parsley, *Caucalis platycarpos*. See chapter 18.

Image courtesy of the Croatian Academy of Sciences and Arts.

IAP symposium participants visiting Giang'anmen Hospital, Beijing, September 2015.

Image courtesy of the Chinese Academy of Engineering.

Exploring Traditional Medicine: Report of a Symposium

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Contents

Foreword		6
Acknowledgements		9
Introduction by Peter F. McGrath		11
Remembering Philippe Rasoanaivo		14
Section 1 Clinical Trials		16
01 A Chinese Patent Medicine for the Secondary Prevention of Myocardial Infarction		
Boli Zhang & Junhua Zhang	China	18
02 The Efficacy of <i>Prunus africana</i> (Rosaceae) in the Management of Symptomatic Benign Prostatic Hyperplasia		
Rogasian Mahunnah & Jichola J.J. Masanja	Tanzania	27
03 Traditional Chinese Medicine in the Treatment of Influenza		
Chen Wang	China	34
04 Non-clinical and Clinical Evaluation of <i>Alternanthera sessilis</i> for Haemoglobin Augmentation in Anaemia		
Erna C. Arollado, Ma. Isabel Pablo Savillo & Marina O. Osi	Philippines	44
05 Construction of the Vessel-collateral Theory and its Guidance for Prevention and Treatment of Vasculopathy		
Yiling Wu	China	52

06	A Research Strategy for the Development of Clinical Evidence for Traditional Herbal Medicine	Tsitsi G. Monera-Penduka, Charles Nhachi, Chiedza Maponga & Gene Morse	Zimbabwe	58
07	From Zheng Classification to New Drug Discovery in Chinese Medicine	Aiping Lu & Ge Zhang	China	67
Section 2 Physical Methods				74
08	The Clinical Efficacy, Evaluation and Central Mechanism Study on Acupuncture for Treating Functional Dyspepsia	Fanrong Liang	China	76
09	Wet-cupping Removes Oxidants and Decreases Oxidative Stress	Müzeyyen Arslan, Suleyman Murat Tagil, Huseyin Tugrul Celik <i>et al.</i>	Turkey	85
10	Electroacupuncture for Moderate and Severe Benign Prostatic Hyperplasia: A randomized controlled trial	Zhishun Liu, Yan Wang, Jinna Yu & Yulong Ding	China	91
11	The Efficacy of Acupuncture for Migraine Prophylaxis	Lin-Peng Wang	China	102

Section 3 | Public Health 108

- 12 **Incorporating Traditional Health Practitioners into Community-based Healthcare in KwaZulu-Natal, South Africa**
Nceba Gqaleni South Africa 110
-

- 13 **Integrating Principles of Modern Epidemiology, Health Promotion and Public Health Practices to Establish a Health Unit System Based on Ayurveda Health Promotion**
Senaka Pilapitiya Sri Lanka 124
-

Section 4 | Pharmacology 132

- 14 **Management of Convulsion and Migraine by Inhalation Therapy**
Philippe Rasoanaivo, Emmanuel Randrianarivo, Rianasoambolanoro Rakotosaona, Filippo Magi & Marcello Nicoletti Madagascar 134
-

- 15 **Ayurvedic *Amalaki Rasayana* and *Rasa-Sindoor* Suppress Neurodegeneration in Fly Models of Huntington's and Alzheimer's Diseases**
Subhash C. Lakhotia India 147
-

- 16 **A Naturally-occurring Antioxidant and Anti-inflammatory Bioflavonoid from the Seed of *Garcinia kola***
Ebenezer Olatunde Farombi & Isaac A. Adedara Nigeria 158
-

- 17 **Uncovering Potential of Indonesian Medicinal Plants for Dual Actions of Anti-diabetes and Anti-obesity**
Lucy Lahrita Japan/
Indonesia 167
-

18	From the Traditional Recipe to the Modern Remedy: The antitumor activity of the small burr parsley (<i>Caucalis platycarpus</i>)	Stella Fatović-Ferenčić, Nicola Kujundžić & Marko Pećina	Croatia	174
19	Antiplasmodial Activities of Fractions and Natural Compounds from <i>Icacina senegalensis</i> (Icacinaceae)	Serigne Omar Sarr, Sylvie Perrotey, Minjie Zhao <i>et al.</i>	Senegal	184
20	Network target: A novel approach to decipher traditional Chinese medicine	Shao Li	China	195
21	Zedupex, an Anti-herpes Herbal Medicine for Management of Human Herpes	Festus M. Tolo, Rukunga M. Geoffrey, Lucia K. Keter <i>et al.</i>	Kenya	206
22	Exploring Medicinal Plants for Better Health: Antiplasmodial properties of <i>Clerodendrum myricoides</i> and <i>Dodonaea angustifolia</i>	Yalemtsehay Mekonnen, Teferra Work, Gebremichael Gebretsadik & Yemane Tadesse	Ethiopia	218
23	Exploring Cameroonian Medicinal Plants for a New Generation of Anti-malarial Compounds	Vincent Pryde Kehdingha Titanji, Denis Zofou, Moses N. Ngemenya & Pierre Tane	Cameroon	227
24	Protective Effect from Ulcerative Colitis to Colitis-associated Cancer in Experimental Models with Huangqin Decoction	Peng Cao, Gang Chen, Yang Yang, Xiaolan Cheng, Yuehua Xu & Min Wang	China	238

Foreword

Since its foundation in 2000, IAP for Health (formerly the InterAcademy Medical Panel, IAMP) has developed a track record of interlinking the boundaries between medical practice, medical research, healthcare delivery and policy for the common good. Our regular IAMP Statements, for example, prepared by expert working groups nominated by member academies, provide recommendations to policy-makers based on unbiased, credible and up-to-date scientific information.

As with our Statements, the strength of IAP for Health and its programmes relies heavily on the strength of our membership – a network of 78 of the world’s national medical academies and academies of science and engineering with medical sections that is committed to improving health worldwide. When planning this project, ‘Exploring Traditional Medicine’, therefore, we did not hesitate to reach out to our member academies to help us identify not only expert reviewers, but also suitable case studies – the best of which are presented here in this book.

As healthcare costs in many countries are soaring, traditional medicine continues to be practiced, especially in developing countries, often with a long history of written prescriptions and other methodologies, as in traditional Chinese medicine and the Indian Ayurveda system, among others. There is a lot that can be learned, therefore, from these effective but potentially more economical health solutions.

Indeed, millions of people, especially in low and middle-income countries, do not have access to conventional medical care. Instead, they rely on a rich culture of traditional medical practices. In addition, it is estimated that about one-quarter of so-called ‘modern medicines’ are derived from plants used by traditional medical practitioners. Investigating traditional medicines, therefore, can help identify novel compounds that could lead to new pharmaceutical products.

In recent years, against a background of a changing spectrum of diseases and the demographic shift towards ageing societies, traditional medicine is receiving increasing attention worldwide. Building on this, as well as an initial World Health Organization (WHO) traditional medicine strategy (2002-2005), certain countries have moved towards integrating traditional and complementary medicine into their national healthcare services.

Much more needs to be done, however. In September 2015, world leaders signed up to a series of 17 Sustainable Development Goals, the third of which calls for “ensuring healthy lives and promoting well-being for all at all ages.”

Traditional medicine – as used by millions of people worldwide who do not have access to or who cannot afford ‘Western’ or ‘allopathic’ medicine – can play a large part in reaching this goal by the target date of 2030.

However, according to the WHO, a significant challenge to the wider integration of traditional medical practices into ‘mainstream’ medical care is the lack of scientific data and evidence to support its development.

A better understanding of the scientific basis of traditional medicine practices, including their safety and effectiveness, will therefore be useful in exploring their application to the prevention, treatment and rehabilitation of human disease and the sustainability of human health.

In order to promote the scientific study of traditional medicine – and answering calls made at the 67th World Health Assembly (2014) for the promotion of international cooperation and collaboration in the sharing of evidence-based information in the area of traditional medicine – the IAP for Health Executive Committee (EC) agreed to establish the ‘Exploring Traditional Medicine’ project. The project centred on an international symposium organized in collaboration with the Chinese Academy of Engineering (CAE) and with the support of the China Academy of Chinese Medical Sciences (CACMS), attended by some 200 researchers, practitioners, administrators of healthcare institutions and students from China.¹

Coincidentally, the symposium itself took place in Beijing during the same week that world leaders were at the United Nations headquarters in New York, half a world away, agreeing to the Sustainable Development Goals.

Taking advantage of the expertise found within its member academies, this IAP for Health project aims to review and showcase successful examples of research into and implementation of traditional medicine practices, with the aim of building a platform to communicate and share these experiences.

The project explores the contribution of traditional medicines and medical practices to human healthcare as well as the methodologies used to assess the science, safety, quality and efficacy of the products and processes.

To build on the presentations and discussions of the symposium, each case study has now been edited into a non-technical version, making it accessible to a wider audience, and published in this volume. The book will be distributed free of charge, especially through the member academies of IAP for Health. By providing such a collection of case studies,

¹ A summary of the symposium is available at: <http://www.iamp-online.org/content/exploring-traditional-medicine>

it is hoped that developing countries, with their limited human resources and limited access to allopathic medicines, as well as high-income countries, will be able to select the most appropriate examples and adapt them to their own particular national challenges.

As with all publications produced by the InterAcademy Partnership, the present volume is also being made available online

(see [www.http://www.interacademies.net/Publications/31264.aspx](http://www.interacademies.net/Publications/31264.aspx)

and

<http://www.iamp-online.org/content/exploring-traditional-medicine>)

with the hope that the case studies it contains will be able to reach an even wider audience and thus have a greater impact.

IAP for Health co-chairs

Lai Meng Looi

2010-2016



Detlev Ganten

2013-



Depei Liu

2016-



Acknowledgements

The present publication is the result of a series of deliberations by members of the Executive Committee of the InterAcademy Partnership for Health (formerly the InterAcademy Medical Panel, IAMP). A major goal of IAP for Health is to improve health world-wide.

Given that millions of people around the world do not have access to affordable 'allopathic' or 'Western' healthcare, but rely on time-honoured traditional practices, the aim of this project was to seek out successful examples of traditional medical practices, especially those that have been validated using modern scientific methods. In this way, we hope that this volume will not only add to our knowledge of traditional medicine and the benefits it can bring, but also help to shine a light on proven practices, introducing them to a wider audience – scientists and medics, as well as decision-takers and policy-makers.

We would therefore like to extend our gratitude to the many individuals and organizations that contributed to the planning and execution of the process that has resulted in the publication of this volume.

The project was largely funded by IAP for Health, with financial and logistical support from the Chinese Academy of Engineering (CAE) and the China Academy of Chinese Medical Sciences (CACMS), especially in regard to the organization of a workshop held at CACMS, Beijing, China, on 22-24 September 2015. We would therefore like to thank colleagues in Beijing, especially Depei Liu (CAE), Boli Zhang (president, CACMS), Baoyan Liu, (principal researcher, CACMS) and Ping Song (CACMS).

A total of 24 case studies were presented at the workshop, nine from China and 15 from 15 other countries. We thank all the delegates for sharing their work.

These case studies were selected following a call for proposals coordinated by the IAP for Health secretariat. Proposals received were reviewed by an Expert Committee, and the best selected for presentation at the Beijing symposium. The project would not have been possible without the efforts of the Expert Committee, so our sincere thanks go out to Depei Liu (Chair); Liaquat Ali, Vice-Chancellor, Bangladesh University of Health Sciences (BUHS) and Honorary professor, Department of Biochemistry & Cell Biology; Mustafa Ali Mohd, deputy director, University of Malaya Medical Centre, Malaysia; Armando Carceres, chemical biologist, Faculty of Chemical Sciences and Pharmacy, University of San Carlos, Guatemala; Jules A. Desmeules, professor of clinical pharmacology and toxicology, Multidisciplinary Pain Centre, Geneva University Hospitals, Geneva, Switzerland; Fola Esan, professor of haematology, Nigerian Academy of Sciences, Nigeria; Vijay Kumar, president, Science Council of Asia and president, National Academy of Sciences of Sri

Lanka; Job I. Jondiko Ogoche, Professor of Chemistry, Maseno University, Kenya; Shing-Tung Yau, Department of Physics, Harvard University, USA; Yung Chi (Tommy) Cheng, pharmacologist, Henry Bronson Professor of Pharmacology, Yale University School of Medicine, USA; Baoyan Liu, principal researcher, CACMS; and Boli Zhang, member of CAE, president, CACMS, and president, Tianjin University of Traditional Chinese Medicine.

The IAP for Health secretariat played a critical role in establishing the Expert Committee, disseminating the call for case studies, working with the selected authors to collect and finalise their case studies, and working with an editor and designer to prepare the book for printing. We are therefore grateful to the members of the secretariat, including Peter McGrath, coordinator, Muthoni Kareithi, project assistant, and Sabina Caris, temporary project assistant. Together they have provided the administrative assistance that has enabled the project to stay on track despite the complications caused by its global reach.

The 24 case studies presented in this volume were initially prepared by the scientists involved and, once again, we extend our thanks to them. Paul Tout, a freelance editor based in Trieste, Italy, and Peter McGrath then adapted the texts to the format presented in this book, which we hope will make them accessible to a wider, non-technical audience.

The talent and commitment of each member of this diverse group helped to move the project forward efficiently and effectively. We express our thanks to them all.

IAP for Health co-chairs

Lai Meng Looi

2010-2016



Detlev Ganten

2013-



Depei Liu

2016-



Introduction

In September 2015, at the headquarters of the United Nations in New York, world leaders agreed to a suite of 17 Sustainable Development Goals¹. These SDGs, and the 169 targets that were also agreed, lay out a roadmap for global development from 2015 to 2030. While targets to ensure good health and well-being are embedded across the 17 SDGs, SDG3, in particular, aims to “Ensure healthy lives and promote well-being for all at all ages.”

Among the targets of SDG3 are access to safe, effective, quality and affordable essential medicines for all. While some may read this as access to ‘Western’ or ‘allopathic’ medicines, it is clear that the number of people with limited or no access to such medicines means that the scale of this challenge is enormous unless other forms of medicine are considered. In Africa, Asia, Latin America and the Middle East, for example, between 70 and 95 per cent of the population still use traditional medicine for their primary healthcare². But while the details of Ayurveda in India, Traditional Chinese Medicine, and other systems elsewhere, have been written down and codified for centuries, other traditional practices emerge and evolve more organically and have fewer controls over their safety and practice, or guarantees of their effectiveness. In addition, while traditional medical practices are regarded as an essential component of healthcare in many low and middle-income countries, there is a parallel trend in their increasing popularity and uptake in high-income countries.

The World Health Organization (WHO) defines traditional medicine as “the sum total of the knowledge, skills and practices based on the theories, beliefs and experiences indigenous to different cultures, whether explicable or not, used in the maintenance of health as well as in the prevention, diagnosis, improvement or treatment of physical and mental illness.”³

Indeed, pre-empting the SDGs, the WHO had a traditional medicine strategy in place between 2002 and 2005 and has since updated it in a new 2014-2023 strategy.⁴ This current strategy aims to support WHO Member States in developing proactive policies and implementing action plans to strengthen the role traditional medicine plays in keeping people healthy. And while it builds on the work carried out under the previous strategy, it places more emphasis on prioritizing health services and systems, including traditional and complementary medicine products, practices and practitioners.

¹ <https://sustainabledevelopment.un.org/?menu=1300>

² Molly Meri Robinson and Xiaorui Zhang. The world medicines situation 2011 (WHO, 2011)

³ <http://www.who.int/medicines/areas/traditional/en/>

⁴ http://www.who.int/medicines/publications/traditional/trm_strategy14_23/en/

The strategy has two key goals:

- to support Member States in harnessing the potential contribution of traditional and complementary medicine to health, wellness and people-centred healthcare; and
- to promote the safe and effective use of such medicine through the regulation of products, practices and practitioners.

To achieve these goals, the WHO is focusing on three strategic objectives:

- building the knowledge base and formulating national policies;
- strengthening safety, quality and effectiveness through regulation; and
- promoting universal health coverage by integrating traditional and complementary medicine services and self-health care into national health systems.

However, achieving these objectives relies on countries identifying and evaluating strategies and criteria for integrating traditional medicine into their national healthcare systems and practices. While steady progress has been made in many countries, many others are still hesitant to embrace traditional medicine due to uncertainties about the safety, efficacy and quality of such medicines.

In short, there is a need for more rigorous scientific research and clinical trials into the traditional medicines and medical practices.

The WHO also recognizes the need “to promote international cooperation and collaboration in the area of traditional and complementary medicine in order to share evidence-based information.”⁵

In this instance, promoting international cooperation and collaboration also answers to SDG17, which focuses on building partnership to help implement the other 16 SDGs.

Another reason to explore traditional medicine is the fact that about one-quarter of ‘modern medicines’ are derived from plants used by traditional medical practitioners. Indeed, the 2015 Nobel Prize in Physiology or Medicine was shared by Youyou Tu, a member of CACMS, “for her discoveries concerning a novel therapy against malaria, artemisinin, derived from a plant used in traditional Chinese medicine.” Investigating traditional medicines, therefore, can help identify novel compounds that could lead to new pharmaceutical products⁶.

And there is an ongoing need for new pharmaceutical products, as highlighted by the 2013 IAP Statement, ‘Antimicrobial Resistance: A Call to Action’, which called for “Encouraging

⁵ <http://apps.who.int/medicinedocs/en/d/Js21462en/>

⁶ <http://www.scidev.net/global/medicine/feature/traditional-medicine-modern-times-facts-figures.html>

industry innovation, new business and collaborative R&D models, in partnership with the public sector, to develop novel anti-infective drugs”⁷

Indeed, on the subject of antimicrobial resistance, in September 2016 (almost exactly a year after the IAP symposium in Beijing), United Nations Member States met in New York and agreed to a strong political declaration that provides a basis for the international community to move forward together, and reaffirming their commitment to develop national action plans on antimicrobial resistance, based on the 2015 ‘*Global Action Plan on Antimicrobial Resistance*’, developed by the WHO, the Food and Agriculture Organization of the United Nations (FAO) and the World Organisation for Animal Health (OIE)⁸.

As per the 2014-2023 WHO strategy, traditional medicine can play a part in this global effort.

“Traditional medicine attracts both uncritical enthusiasm and uninformed criticism,” said Bernhard Schwartländer, representative of the World Health Organization (WHO) in China, during his opening address at the IAP for Health symposium in Beijing. “But it is arrogant to ignore thousands of years of wisdom. We need to explore it and exploit it. After all, 40% of the population of China and 80% of the population of Africa are using traditional medicines.”

In answer to this – and a justification for the IAP for Health project, of which this current volume is an integral part – IAP for Health co-chair Lai Meng Looi (2010 - 2016) confirmed: “We will continue to talk, to share and to collaborate with our partners and develop new ways to work together. It will be important to cultivate the new friendships formed in Beijing and to follow up with the different projects. I hope this symposium will mark a new beginning and offer new impetus to overcome the remaining barriers between different medical systems, allowing us to further tap and harness traditional medicine and provide it with the recognition that it deserves.”

In this way, IAP for Health can help contribute – along with its member academies and the institutions represented by the authors of the case studies presented herein – to the WHO 2014-2023 strategy for traditional and complementary medicine, and the SDGs, especially SDG3 that targets ‘health for all’.

Peter F. McGrath

IAP Coordinator

Trieste, Italy

February 2017

⁷ <http://www.iamp-online.org/antimicrobial-resistance-call-action>

⁸ <http://www.un.org/pga/71/2016/09/21/press-release-hl-meeting-on-antimicrobial-resistance/>

Remembering Philippe Rasoanaivo



Philippe Rasoanaivo (centre) with Dr Kadetz and staff at Mahitsy hospital, Madagascar.

Leading African scientist Philippe Rasoanaivo, a native of Madagascar and research director at the Malagasy Institute of Applied Research, died on 13 July 2016. He was also a Fellow of Madagascar's National Academy of Arts, Letters and Sciences (AcNALS), the African Academy of Sciences (AAS) and The World Academy of Sciences (TWAS).

In the course of his investigations, Rasoanaivo addressed a broad spectrum of topics, from natural product chemistry, to bioprospecting (the discovery and commercialization of new products based on biological resources), ethnobotany (the scientific study of the traditional knowledge of plants and their medical, religious, and other uses) and novel drugs, as well as studying substances targeting malaria and brain diseases. A traditional Malagasy plant called hazolava, which showed promising therapeutic properties in the treatment of psychiatric and sleep disorders, convulsions and male sexual dysfunctions, was a recent target of his investigations. He held several patents and manufactured eight phytomedicines.

In 2015, the AAS awarded Rasoanaivo the Olusegun Obasanjo Prize for his study of traditional medicine aimed at improving the efficacy of existing drugs for brain disorders and the treatment of male sexual problems.

His case study presented in this volume on 'Management of convulsion and migraine by inhalation therapy' (see chapter 14) provides a snapshot of how he successfully combined chemistry and ethnobotany to develop and eventually market phytomedicines.

Section 1: Clinical Trials

From preliminary tests of safety to large-scale trials to test for efficacy and potential side-effects, it is probably fair to say that clinical trials are the cornerstone of modern medical practices.

The following seven case studies – from China, the Philippines, Tanzania and Zimbabwe – describe clinical trials of various sizes aimed at confirming the beneficial effects of traditional medicines in patients.

01	A Chinese Patent Medicine for the Secondary Prevention of Myocardial Infarction	China	18
02	The Efficacy of <i>Prunus africana</i> (Rosaceae) in the Management of Symptomatic Benign Prostatic Hyperplasia	Tanzania	27
03	Traditional Chinese Medicine in the Treatment of Influenza	China	34
04	Non-clinical and Clinical Evaluation of <i>Alternanthera sessilis</i> for Haemoglobin Augmentation in Anaemia	Philippines	44
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06	A Research Strategy for the Development of Clinical Evidence for Traditional Herbal Medicine	Zimbabwe	58
07	From Zheng Classification to New Drug Discovery in Chinese Medicine	China	67

A Chinese Patent Medicine for the Secondary Prevention of Myocardial Infarction

China

Boli Zhang and Junhua Zhang.

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China 300193

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URL: iec.tjutcm.edu.cn/english2015/HOME.htm

Email: zjhtcm@foxmail.com

Duration: 2004-2009

Total cost: USD 1,000,000 (Ministry of Science and Technology, State Administration of Traditional Chinese Medicine of PR China)

Summary

Survivors from acute myocardial infarction (MI) remain at greatly increased risk of serious vascular events. Thus, secondary prevention aimed to decrease mortality and morbidity in survivors after acute MI is of great, and increasing, significance around the world. Several types of chemical drugs have been recommended for the secondary prevention of MI. However, these conventional strategies have several limitations, such as low adherence, high cost, and side effects during long-term use. Novel approaches to this problem are still needed. Our study aimed to evaluate the effectiveness and safety of Qi-Shen-Yi-Qi Dripping Pills (QSYQ), a multi-ingredient Chinese patent medicine, for the secondary prevention of MI.

A total of 3,505 eligible patients were randomly assigned to a QSYQ group (1,746 patients) and an aspirin group (1,759). Patients took their treatments for 12 months. The final follow-up visit took place 6 months later. The 12-month and 18-month estimated incidences of the primary outcome were 2.98% and 3.67%, respectively, in the QSYQ group. The figures were 2.96% and 3.81% in the aspirin group. These data did not show significant difference of primary and secondary outcomes between aspirin and QSYQ in patients who have had an MI. The result suggested that QSYQ has similar effects to aspirin in the secondary prevention of MI.

Background and Justification

Acute myocardial infarction is a leading cause of death worldwide. More than 7 million people a year have a myocardial infarction (MI). Over the past three decades, MI has emerged from an illness seen predominantly in developed countries to becoming more common also in developing countries. Progress in emergency management led to substantial reductions in the mortality rate of acute MI. However, survivors from acute MI remain at greatly increased risk of serious vascular events. Thus, secondary prevention aimed at decreasing mortality and morbidity in survivors of acute MI is of great, and increasing, significance around the world.

Platelets play a key role in the development of thrombotic and ischaemic diseases. Anti-platelet therapy is a major strategy for treating and preventing MI. Anti-platelet drugs have been shown to have definite and substantial net benefits for people who have occlusive vascular disease. Aspirin is a safe and cost-effective anti-platelet drug and current guidelines recommend low-dose aspirin (75-150 mg daily) for the secondary prevention of MI in many countries. However, there are several limitations related to this drug. Long-term therapy with aspirin is associated with an increase in the incidence of symptomatic

peptic ulcer, duodenal ulcers, and gastrointestinal and intracranial haemorrhage, even when used at low doses or in buffered or enteric-coated formulations. In a population-based cohort with 4.1 million citizens in Italy, for example, aspirin increased the risk of major gastrointestinal or cerebral bleeding episodes, and patients with diabetes had a high rate of bleeding. Furthermore, aspirin resistance has become a notable problem. Several studies have found that about 1 in 4 individuals may express biochemically defined aspirin resistance. Patients who are resistant to aspirin are at greater risk of recurrent serious vascular events than those who are sensitive to aspirin.

Current anti-platelet therapies are generally based on a specific signaling pathway in platelet activation, that is a single agent acting on a single target. Hence, the limitations associated with aspirin also exist for other anti-platelet agents, such as clopidogrel. Agents with multiple ingredients acting on multiple targets may be more effective and less harmful.

In Traditional Chinese Medicine (TCM), the key pathogenesis of MI is mainly “Qixu” (vital energy deficiency) and “Xueyu” (blood stagnation), that is, degradation of body function and thrombosis. Qi-Shen-Yi-Qi Dripping Pills (QSYQ), a Chinese patent medicine for adding “qi” and resolving stasis, was approved for clinical use for coronary heart disease and MI rehabilitation by the State Food and Drug Administration of China in 2003. QSYQ is made of extractions from Danshen (*Radix Salviae Miltiorrhizae*), Sanqi (*Radix Notoginseng*), Jiangxiang (*Lignum Dalbergiae Odoriferae*), and Huangqi (*Radix Astragali*). The quality control of QSYQ is good. Herbal materials were cultivated according to the good agriculture practices (GAP), and manufacturing processes strictly followed the standard of good manufacturing practices (GMP). Mass spectroscopy was used to analyse seven quality control markers of QSYQ and showed good consistency of the active markers among different batches. Pharmacological studies revealed that constituents of QSYQ could inhibit the platelet aggregation and the over release of β -TG (a platelet protein). Clinical studies have suggested that QSYQ had similar effect to aspirin in inhibiting platelet aggregation.

QSYQ is widely used for the secondary prevention of MI in China. However, there is insufficient evidence to know whether QSYQ can be used as an alternative to aspirin. This multi-centre randomized clinical trial aimed to test whether the regular administration of QSYQ would result in a significant reduction in total serious vascular events in patients who had experienced at least one documented MI.

Description

A randomised, double-blind, parallel controlled study was carried out. With such a large scale clinical trial, the quality control of process management was key for the validity and reliability of the results.

The study protocol was reviewed and approved by the Ethics Committee of Tianjin University of Traditional Chinese Medicine and the trial registered with the World Health Organization Clinical Trial Registering Platform. Patients with a definite diagnosis of ST-elevation or non-ST-elevation MI (ST is a diagnostic section of an ECG readout) were potentially eligible for this study if they met the following criteria: (1) the last documented MI was 4 weeks to 24 months earlier; (2) Traditional Chinese Medicine symptoms were “Qixu-Xueyu” (vital energy deficiency combining with blood stagnation); (3) aged from 18 to 75 years (the maximum age was adjusted from 65 years to 75 years in July 2006 because of inadequate recruitment).

Patients were required to be free of other life-threatening diseases or problems which might have limited the ability to obtain long-term follow up and to be free of any condition which would mean that regular use of the trial drugs was contraindicated. Patients with any of the following conditions were excluded: (1) a history of percutaneous coronary intervention (PCI) or coronary artery bypass graft surgery (CABG); (2) pregnant women or those who were breast feeding; (3) contraindication to aspirin (e.g., asthma, active phase peptic ulcer, and haemorrhagic disease); (4) heart function of grade IV (NYHA grade); (5) uncontrolled systemic hypertension (contractive pressure ≥ 180 mmHg or diastolic pressure ≥ 110 mmHg); (6) uncontrolled serious cardiac arrhythmias (e.g., atrial fibrillation and supraventricular tachycardia); (7) serious primary disease of liver, kidney, and hemopoietic system, or psychosis, or malignant tumor; (8) allergic history to study drugs; (9) participation in other clinical trials during the previous three months.

After a comprehensive medical evaluation, patients were given a full explanation of the study by investigators. Each patient was asked for their written informed consent before joining the study. Recruitment took place in 88 hospitals across China. Patients were randomly assigned to two groups (ratio 1:1): QSYQ group and aspirin group. In the QSYQ group, patients took 0.5 g (one package) QSYQ three times per day and 100 mg (in four tablets) simulated enteric-coated aspirin once a day. Patients in the aspirin group took 0.5 g (one package) simulated QSYQ three times per day and 100 mg (in four tablets) enteric-coated aspirin daily. Placebos for QSYQ and aspirin were developed which had the same appearance, colour, and taste as the relevant drug. Patients were prohibited from taking other anti-platelet drugs or “Yiqi-Huoxue” Chinese medicines during the treatment period. Concomitant medications, such as antihypertensive (e.g., beta-blockers and ACE

inhibitors), hypoglycaemic agents, and lipid-lowering drugs, could be prescribed at the discretion of the attending physicians and had to be recorded in detail (including drug name, period taken, dosage, and purpose). The treatment period for the trial drugs was 12 months. After this time (or if the trial drugs were stopped for some other reason), patients could be prescribed treatments by their physicians without any limitation.

The primary endpoint was a composite of cardiovascular death, non-fatal re-infarction (documented by ECG and enzyme changes), and non-fatal stroke (diagnosed by CT or MRI). After a first visit for collecting baseline data after randomization, enrolled patients, their dependents, or both, were asked to visit clinical centres monthly. If no primary endpoints occurred, there were 12 visits during the treatment period and a final visit (6 months after the termination of the trial drugs). When a patient had one of the primary endpoints, the case was considered completed and there was no further follow-up visit.

The number of patients for each group at each stage of the trail are shown in Fig. 1. At the end of the trial period, statistical analyses were carried out on the date from 1,456 patients in the QSYQ cohort and 1,500 in the aspirin group.

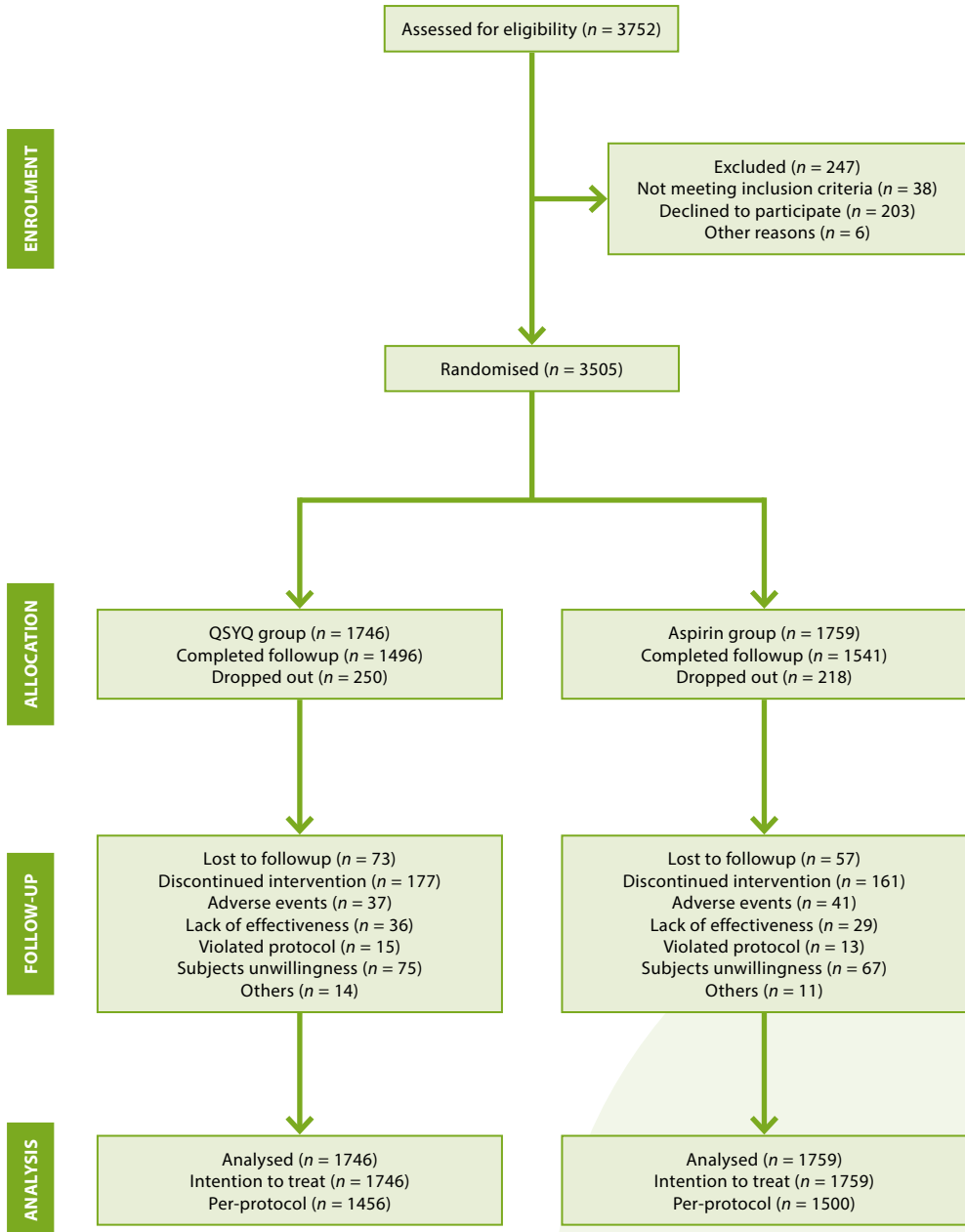


Figure 1: Flow diagram of participants through each stage of the trial.

Results

In the trial, QSYQ showed similar effects to aspirin for the prevention of recurrent vascular events in patient with a previous MI (Figure 2). The rate of composite endpoints (cardiovascular death, non-fatal MI, and non-fatal stroke) after 12 months of treatment was 2.98% in the QSYQ group and 2.96% in the aspirin group. The incidence of serious vascular events of this trial was lower than previous studies of secondary prevention for MI, which may be due to several factors.

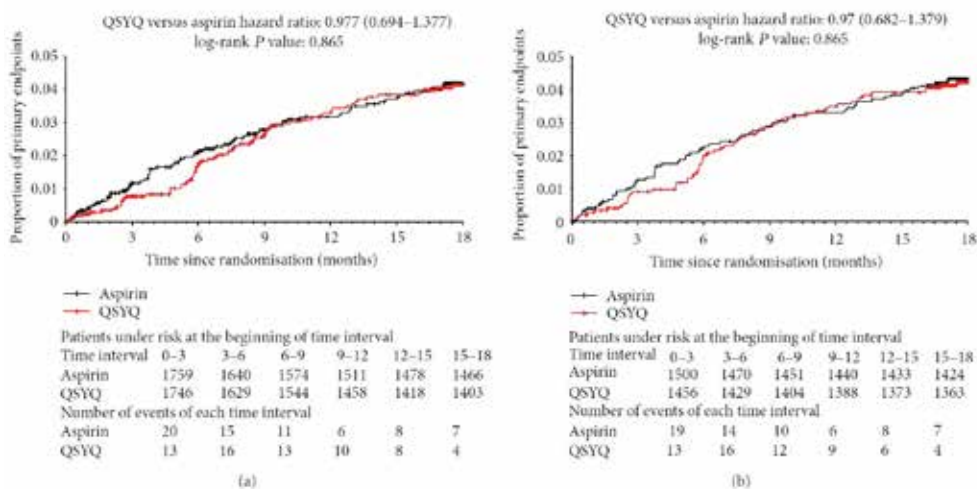


Figure 2: Cumulative incidence curves of the primary outcome composed of cardiovascular death, non-fatal myocardial infarction, and non-fatal stroke: (left) intention-to-treat analysis; (right) per-protocol analysis.

Partnerships

Among the main partners of this trial were Peking University First Hospital, the Chinese Academy of Traditional Chinese Medicine and Xiyuan Hospital, Beijing.

The protocol of the trial was revised more than ten times after consulting with multi-discipline experts from clinical epidemiology, evidence-based medicine, statistics and cardiology, etc. Before patients were recruited, the protocol of this trial was evaluated by Italian and US experts. The trial was designed, executed and analysed by a steering committee, a clinical monitoring centre, an endpoints committee, a drug management centre, a data management centre, and a biostatistics centre. Investigators in each clinical centre were trained before beginning the study.

Impact

- After completing this trial, we established a series of criteria and methods for completing large scale clinical trial. This trial won a National Award of Science and Technology.
- The methods and experience of this trial have been widely used in post-marketing evaluation of traditional Chinese medicines. After rigorous clinical evaluation, sales of Chinese patent medicines have developed greatly.
- The trial was successful mainly because it received support from the Ministry of Science and Technology and the State Administration of Traditional Chinese Medicine of China.

Replicability

The experience, especially the efforts taken to ensure a robust design and analysis of the trial, will be valuable for clinical trials of other traditional medicines.

Lessons Learned

How to manage the process of randomisation, drug allocation, timely data collection and audit were the main obstacles for the trial. We used a Clinical Research Interactive Voice Respond System (CRIVRS) for dynamic management of this trial. Investigators connected to the CRIVRS by telephone when a patient was ready to be randomised and the CRIVRS then provided a subject number, randomisation code, and drug number to the investigator by voice, email, and SMS.

Because of the care taken to develop the trial's randomized control design, the results have been widely accepted by clinical practitioners and researchers worldwide.

Future Plans

We intend to evaluate more Chinese patent medicines. For future clinical trials, we will design the protocols with international collaboration.

Publications

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The Efficacy of *Prunus africana* (Rosaceae) in the Management of Symptomatic Benign Prostatic Hyperplasia

Tanzania

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Duration: April 2006 - January 2007

Summary

A placebo-controlled, double blind randomized clinical trial was conducted among patients with symptomatic benign prostatic hyperplasia (BPH) at Muhimbili National Hospital, Dar es Salaam, Tanzania, from April 2006 to January 2007. The aim of the study was to evaluate the efficacy and safety of *Prunus africana* (Hook. f.) Kalm (family Rosaceae) in the treatment of symptomatic BPH.

Patients were divided into two groups in order to compare the drug to a placebo. Patients allocation to treatment and placebo were done by permuted blocks randomization.

In the treatment group, there were 26 (65.0%) patients who reported improvement in International Prostate symptom Score (IPSS) from the baseline and 21 (52.5%) patients reported improvement in quality of life.

In the placebo group there were 28 (73.7%) patients who reported improvement in IPSS from the baseline and 22 (57.9%) patients reported improvement in quality of life. Among all patients, the proportion of patients who reported improvement in IPSS in the treatment group was 26 (48.1%) as compared to 28 (51.9%) in the placebo group; the difference was not statistically significant. There was also no significant difference in the proportion of patients with improvement in quality of life between the treatment group 21(48.8%) as compared to 22 (51.2%) in the placebo group.

Generally the trend of the results in this study showed that the drug is not superior to the placebo.

Background and Justification

Medical therapy for clinical has a major role in the improvement of symptoms associated with bladder outlet obstruction. Medical therapy focuses on two aspects of the pathophysiology of BPH: (i) a dynamic (physiological, reversible) component related to the tension of prostatic smooth muscle in the prostate and bladder neck and (ii) a fixed (structural) component related to the bulk of the enlarged prostate compressing the urethra. Medical therapy for clinical BPH has developed over decades and the general mechanism of action of prescribed drugs is either to relax the smooth muscle tone and/or reduce the size of the prostate. Pharmaceutical extracts derived from plants are widely used throughout the world for the treatment of various medical conditions. The phytotherapeutic agents used in the treatment of lower urinary tract symptoms (LUTS) secondary to BPH are extracted from the roots, seeds, barks, or fruits of plants. Active components in plant extracts include phytosterols, fatty acids, lectins, flavonoids, plant oils and polysaccharides.

A clinical trial was conducted to evaluate the efficacy of *Prunus Africana* (Hook. F.) Kalm. (family Rosaceae) for clinical BPH

Description

A randomized double blind placebo controlled clinical trial was conducted from April 2006 to January 2007. The study population were divided into two treatment groups in which one group received a drug derived from *P. Africana* and the other group received a placebo. Both drug and placebo were made in capsules with similar colour (red) but coded by different names, "amest" and "alba". The disclosure of the codes was done by the manufacturer after the analysis.

After meeting patients, they were given an International Prostate Symptom Score (IPSS) and their quality of life (QoL) was assessed.

A total of 82 patients were enrolled and followed for a period of 10 months. Each patient was followed at 4-week intervals for a duration of 3 months. Four patients were lost to follow up and excluded from the study.

No any adverse drug reactions were reported in either group and there were no reported deaths.

Results

As shown in Table 1, of the 78 patients who completed the study, the largest number, 29 (37.2%) belonged to the group with a mean age 65.5 years. Among whole population, 40 (51.3%) patients were allocated to the treatment group, and 38 to the placebo group.

Mean age (years)	Drug	Placebo	Total
≤ 50	3	0	3 (3.8%)
55.5	5	10	15 (19.2%)
65.5	17	12	29 (37.2%)
75.5	11	14	25 (32.2%)
> 80	4	2	6 (7.7%)
Total	40 (51.3%)	38 (48.7%)	78 (100.0%)

Table 1: Distribution of study population by mean age and treatment group.

Baseline IPSS	Drug	Placebo	Total
0-7	6 (37.5%)	10 (62.5%)	16
8-19	33 (54.1%)	28 (45.9%)	61
20-35	1 (100.0%)	0 (0.0%)	1
Total	40	38	78

Table 2: Baseline International Prostate Symptom Score (IPSS) among the study population by group allocation.

IPSS:	Treatment allocation		Total
	Drug	Placebo	
Improved	26 (48.1%)	28 (51.9%)	54
Not improved	14 (58.3%)	10 (41.7%)	24
Total	40	38	78

Table 3: Comparison in improvement in International Prostate Symptom Score (IPSS) between the treatment group and the placebo group. Table 2 shows the baseline IPSS scores and reveals no difference between the treatment and control group at the beginning of the trial (95% C.I, P = 0.31). At the end of the trial (Table 3) there were no significant differences in the proportion of patients who reported improvements in IPSS between the treatment group and the placebo group (95% C.I, P=0.47).

QoL score	Drug	Placebo	Total
4	14 (43.8%)	18 (56.3%)	32
5	18 (52.9%)	16 (47.1%)	34
6	8 (66.7%)	4 (33.3%)	12
Total	40	38	78

Table 4: Baseline score in quality of life (QoL) among the study population by group allocation. There were no significant differences in quality of life baseline scores between the two groups at baseline (95% C.I, P = 0.38).

QoL:	Treatment allocation		Total
	Drug	Placebo	
Improved	21 (48.8%)	22 (51.2%)	43
Not improved	19 (54.3%)	16 (45.7%)	35
Total	40	38	78

Table 5: Comparison in improvement in quality of life (QoL) between the treatment group and the placebo. By the end of the trial, there were no significant differences in the proportion of patients who reported improvements in QoL between the treatment group and the placebo groups (95% C.I, P=0.66).

No adverse drug reactions were reported in either group and there were no reported deaths.

Discussion

Although patients in both treatment and placebo groups improved their IPSS scores, there was no significant difference between the groups. However, the improvements of IPSS in this study were not different from those found by Breza *et al.* (1998), which was 31-40% reduction at a dosage of *Prunus africana* 50 mg twice daily for a two-month duration. The findings in this study were also comparable to those of Chatelain *et al.* (1999), who compared *P. africana* at dosing once daily to 50 mg twice daily dosing and found more than 40% reduction in the mean IPSS from the baseline. Likewise, there were no significant differences in improvement in the QoL between the treatment group 21 (48.8%) patients as compared to 22 (51.2%) patients in the placebo group (Table 5). However, improvement in QoL in this study was greater than the 31-40% reported by Breza *J. et al.*

The proportion of patients who had improvements in IPSS and QoL showed satisfactory outcome with the drug under trial. In addition, three patients in the treatment group and one patient in the placebo group reported improvement in their sexual function. However, improvement in sexual function was not among the primary outcomes measure in this study.

The results show that, although a certain number of men with clinical BPH may improve their IPSS without intervention, the authors encourage phytotherapy in selected cases since the drug showed an acceptable degree of efficacy.

Partnerships

The study was conducted in collaboration between Muhimbili University of Health and Allied sciences and the Institute of Traditional Medicine of the Muhimbili University of Health and Allied Sciences, Dar-es-Salaam, Tanzania.

Impact

The results of the study showed satisfactory outcome in improvement in IPSS and QoL. There are now more patients with BPH consulting the Institute of Traditional Medicine to source the product. However, a need for long term and multi-centre clinical trial is recommended. The product is relatively cheap, affordable and accessible to a larger population. The source of the *materia medica*, *P. africana*, is a globally threatened plant species, hence conservation programmes are paramount (Maximillian and O’Laughlin, 2009). These results adds value to the existing policy and legislation that promote the contribution of traditional medicine in healthcare delivery in Tanzania (United Republic of Tanzania, 2002).

Replicability

A large number of patients are in demand for the product (drug). The drug should be available and accessible to patients. The drug need approval by Food and Drug Administration Authority. Applications for registration have been filed through the Institute of Traditional Medicine and are being processed.

Lessons Learned

It was not possible for most patients to undergo ultrasound estimation for prostate size as most of them could not afford to do so. Among those who came with their reports, most reports were not specific on prostate parameters and therefore it was not feasible to evaluate changes in prostate size. The increased use of the herbal product should be consistent with availability of the respective *materia medica*. It is likely, however that strictly selected cases can do better with phytotherapeutic agents.

Future Plans

A long term multi-centre clinical trial is recommended for further evaluation of the product. Conservation of *P. africana* plant species in the wild and through cultivation is needed to scale up production to meet increasing demand for the product.

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Traditional Chinese Medicine in the Treatment of Influenza

China

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Duration: July 2009 to November 2009

Total cost: USD 471,275

Summary

First noted in Mexico in 2009, the highly-damaging influenza A (H1N1) spread rapidly to other regions of the world. Effective and low-cost medicine was urgently needed, but in short supply in China. Traditional Chinese Medicine (TCM) has been used to treat seasonal influenza for thousands of years. During the early days of the 2009 H1N1 pandemic, the herbal formula Maxingshigan-Yinqiaosan (MY) was used widely by TCM practitioners to reduce symptoms but with no exact clinical evidence with regard to its efficacy. Supported by the Beijing Municipal Science and Technology Commission and the Beijing Administration of Traditional Chinese Medicine, during the H1N1 epidemic we conducted a prospective, randomized, controlled, non-blinded, multicentre trial that ran between July and November 2009 at 11 sites in four Chinese provinces.

Registered on the most authoritative website, ClinicalTrials.gov, the study used standard,

strict modern evidence-based methods. 410 participants were randomly assigned to a control group, an oseltamivir (Tamiflu™) group, an MY group and an oseltamivir plus MY group. Patients were prescribed following the principle of same symptom, same disease, same regimen, and same dose.

Oseltamivir and MY, both alone and in combination, reduced time to fever resolution in patients with H1N1 suggesting MY may represent an alternative H1N1 treatment. No side effects were observed.

This is the first research to study the efficacy and safety of TCM in the treatment of H1N1 using modern assessment methods with results published in the internationally-renowned *Annals of Internal Medicine*. The methods used were accorded a high ranking and the study was listed by five international authoritative medical databases, signifying that TCM treatment for H1N1 had won international recognition.

Background and Justification

In April 2009, cases of human infection with H1N1 influenza A virus were identified in the United States and Mexico and spread rapidly to other regions of the world, resulting in the first influenza pandemic since 1968. By March 2010 almost all countries had reported cases, and more than 17,700 deaths among laboratory-confirmed cases had been reported to the World Health Organization (WHO).

The antiviral agent oseltamivir (Tamiflu™) was widely used during the H1N1 influenza A pandemic, as recommended by the WHO. No direct comparative evidence on the role of oseltamivir in the H1N1 influenza A pandemic was reported, but isolates of pandemic H1N1 influenza A virus with resistance to oseltamivir were detected.

During that period, oseltamivir was expensive and the supply was insufficient. Thus, cheaper alternative drugs to oseltamivir were urgently needed.

Traditional Chinese Medicine (TCM) has been used to treat seasonal influenza for thousands of years. In a meta-analysis of 31 randomized clinical trials including 5,514 cases of influenza, the authors concluded that TCM had significantly increased clinical efficacy compared with placebo or no intervention (93.46% vs. 79.03%, respectively; $P < 0.001$), and no serious adverse effects were reported.

Modern pharmacological studies have demonstrated that some TCM formulae have antiviral and immunomodulating effects. During the early days of the 2009 H1N1 influenza A pandemic, the popular herbal formula Maxingshigan-Yinqiaosan (MY) was used widely by TCM practitioners to reduce symptoms.

Here we report on a prospective, randomized, controlled, non-blinded, multicentre trial carried out during the H1N1 influenza A epidemic between July and November 2009 at 11 medical sites in four provinces in China.

Patient enrolment

Patients aged 15 to 70 years who presented within 72 hours of onset of H1N1 influenza A symptoms were enrolled in the trial. All patients (410) were admitted to hospitals where they could be quarantined and observed. Patients who fulfilled all of the following criteria were included: documented body temperature 37.5°C or greater; one or more respiratory symptoms (cough, sore throat or rhinorrhea); and a positive result for H1N1 influenza A virus via a real-time reverse transcriptase polymerase chain reaction (RT-PCR). Patients were excluded if they had received an influenza vaccination in the 12 months before the start of the study; had active, clinically significant chronic illness or HIV/AIDS; were receiving systemic steroids or other immunosuppressants; had taken Chinese medicinal herbs or antiviral drugs; were pregnant; or had new infiltrate of the lungs on chest radiography.

Drug administration

The TCM formula used was Maxingshigan-Yinqiaosan (MY), which is composed of 12 herbs. The criteria for the quality of the herbs used were in accordance with the 2005 Chinese pharmacopoeia. Herbs from the same source were distributed to the 11 study sites. Before the start of the trial, the herbs were tested for heavy metals, microbial contamination and residual pesticides, and all results met Chinese safety standards.

The 12 herbs formula included: zhimahuang (honey-fried *Herba Ephedrae*), 6g; zhimu (*Rhizoma Anemarrhenae*), 10g; qinghao (*Herba Artemisiae Annuae*), 15g; shigao (*Gypsum Fibrosum*), 30g; yin-hua (*Flos Lonicerae Japonicae*), 15g; huangqin (*Radix Scutellariae*),

15g; chaoxingren (stir-baked Semen Armeniacae Amarum), 15g; lianqiao (Fructus Forsythiae), 15g; bohe (Fructus Forsythiae), 6g; zhebeimu (Bulbus Fritillariae Thunbergii), 10g; niubangzi (Fructus Arctii Tosum), 15g; and gancao (Radix Et Rhizoma Glycyrrhizae), 10g.

At each study site, a trained technician prepared the decoction according to a standardized procedure; each unit of formula yielded 800ml of decoction. Oseltamivir was given as capsules, and the TCM intervention was given as a decoction. Placebo capsules were not used; the control group received no intervention.

After agreeing to participate, signing the informed consent form and completing the baseline visit, all patients were randomly assigned to one of the three active treatment groups or the control group by using random-number tables. Randomization was stratified by the four study centres, located in Beijing, Yantai, Chengdu and Wuhan. These centres were selected to ensure broad geographic spread and representation of H1N1 influenza A epidemic areas in mainland China. A statistician who was not involved in data collection or analysis produced the randomization list. A coordinator at each site who was blinded to the participants' characteristics assigned the participants to treatment by telephoning a contact at the study-coordinating centre in Beijing Chao-Yang Hospital. The contact was not involved in the number generation and recruitment process. Participants were then randomly allocated to the control group or one of the intervention groups: oral oseltamivir, 75mg daily for 5 days; MY decoction, 200ml orally 4 times daily for 5 days, or oseltamivir plus MY.

All participants were hospitalized so that they could be quarantined and closely observed and were followed until discharge. Adherence to therapy was assessed by nurses who were blinded to the study. On the basis of the attending physician's judgment, participants were allowed to use acetaminophen if their body temperature was greater than 39°C. Likewise, the need for antibiotics was determined by the attending physicians. Any use of acetaminophen or antibiotics was recorded on the case record forms.

Assessment

During hospitalization, nurses who were blinded to the study measured participants' body temperatures daily at 02:00-06:00, 06:00-10:00, 10:00-14:00, 14:00-18:00, 18:00-22:00 and 22:00-02:00. The presence and severity of influenza symptoms (cough, sore throat, rhinorrhoea, headache and fatigue) and drug-associated side effects were also recorded daily. Symptom scores (0 = absent, 1 = mild, 2 = moderate, and 3 = severe) were recorded and compared with baseline scores until five days after treatment in all groups.

The primary efficacy end-point was designated as the time from randomization to fever resolution. Secondary outcomes were the proportion of patients who became afebrile,

improvement in symptom scores during the study period, side effects associated with the interventions, and incidence of secondary complications of influenza such as otitis, bronchitis, sinusitis or pneumonia.

Throat swab specimens were collected from all participants and sent to local branches of the Chinese Centre for Disease Control and Prevention for H1N1 influenza A RNA testing by using the protocol from the US Centres for Disease Control and Prevention. Serial real-time RT-PCR for viral RNA titres was performed daily from enrolment until discharge.

Full details of the numbers of patients involved and the steps undertaken during the study are presented in Fig. 1.

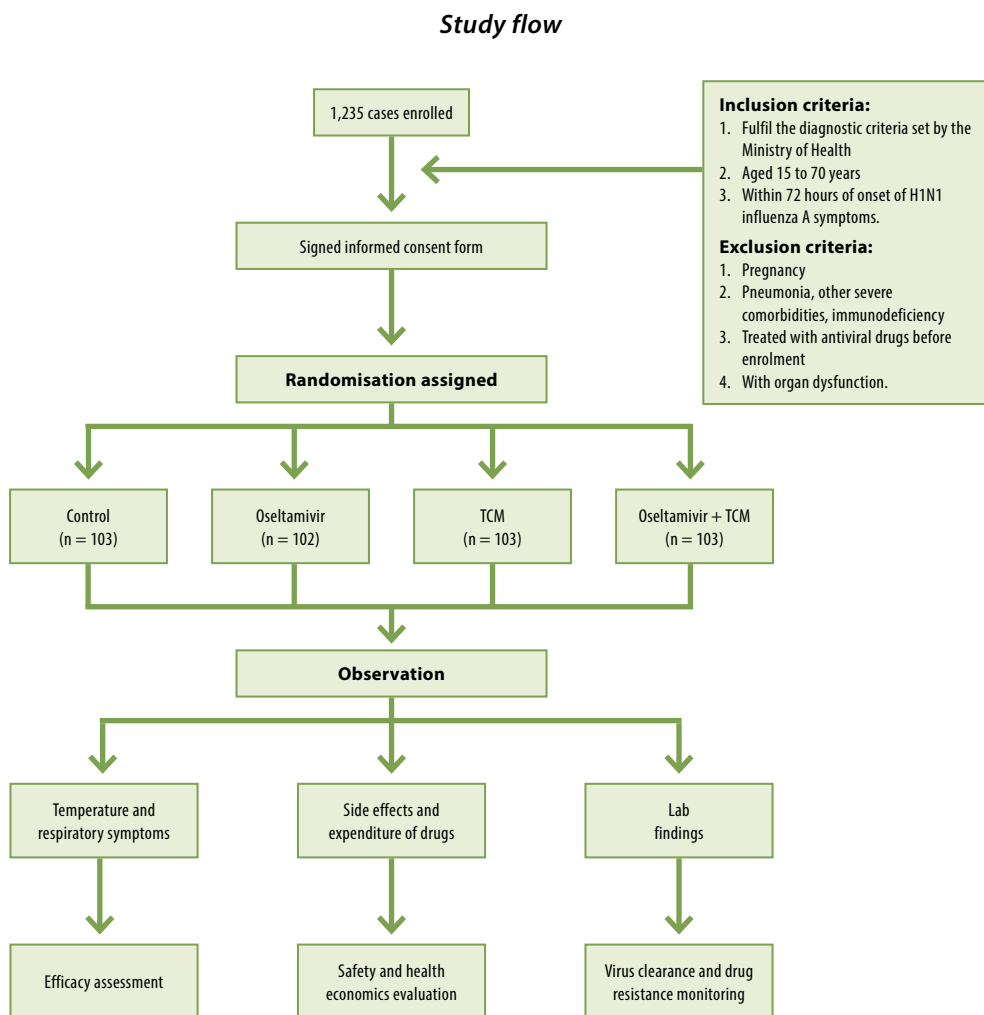


Figure 1: The study flow protocol assigning participants to the various drug and treatment regimes and their subsequent management.

Baseline characteristics did not differ among the 4 groups

Characteristic	Control Group (n = 103)	Oseltamivir Group (n = 102)	MY Group (n = 103)	Oseltamivir Plus MY Group (n = 102)
Men, n (%)	58 (56.3)	58 (57.8)	65 (63.1)	52 (51.0)
Mean age (SD), y	18.7 (5.3)	19.0 (6.2)	19.6 (7.1)	19.2 (6.5)
Received vaccine, n (%)	2 (2.0)	2 (2.0)	0 (0.0)	2 (2.1)
Comorbid conditions, n (%)	1 (1.0)	0 (0.0)	0 (0.0)	0 (0.0)
Temperature, n (%)				
37.5–38.0 °C	28 (27.2)	26 (25.5)	22 (21.4)	24 (23.5)
38.1–39.0 °C	60 (58.3)	52 (51.0)	59 (57.3)	53 (52.0)
>39.0 °C	15 (14.6)	24 (23.5)	22 (21.4)	25 (24.5)
Median symptom score (IQR)*	3.0 (2.0–3.0)	3.0 (2.0–3.0)	3.0 (2.0–3.0)	3.0 (2.0–3.0)
Symptom, n (%)				
Cough	78 (75.7)	75 (73.5)	76 (73.8)	73 (71.6)
Sore throat	66 (64.1)	57 (55.9)	61 (59.2)	63 (61.8)
Rhinorrhea	19 (18.4)	25 (24.5)	25 (24.3)	23 (22.5)
Headache	45 (43.7)	46 (45.1)	40 (38.8)	47 (46.1)
Fatigue	43 (41.7)	36 (35.3)	33 (32.1)	41 (40.2)
Median leukocyte count (IQR), × 10 ⁹ cells/L	5.8 (4.6–6.8)	5.7 (4.4–6.9)	5.1 (4.0–6.3)	5.5 (4.5–7.3)
Median interval between onset of illness and randomization (IQR), h	30.0 (11.0–47.0)	35 (17–40)	35 (25–49)	32 (16–53)
Days hospitalized				
Median (IQR)	6 (5–7)	6 (5–7)	6 (5–7)	6 (5–7)
Range	4–14	3–13	3–11	2–11

Characteristics:

- young, rare comorbidities and three-quarters of the participants with temperature above 38 °C
- interval between onset of illness and enrollment between 30 and 35 hrs

Table 1: Patients' baseline characteristics with the primary and secondary outcomes of the measures undertaken. (SD = standard deviation; IQR = interquartile range).

Participant characteristics

410 participants aged 15 to 69 years from 11 sites were included in the trial. Of the 410 participants, 102, 103, and 102 were randomly assigned to receive oseltamivir, MY, and combination therapy, respectively, while the remainder were assigned to a control group (Fig. 1). Baseline demographic characteristics, clinical features and laboratory findings were similar among the four groups (Table 1). Three-quarters of the patients had temperatures above 38°C, co-morbidities were rare (one person), and most patients were younger than 20 years old. The interval between onset of illness and enrolment in the study was between 30 and 35 hours.

Virological outcomes

Both baseline swab specimens and specimens collected on days 1 to 5 for evaluation of virus shedding were available for 148 of the 410 participants.

Changes in virus shedding from baseline to day 5 did not differ by treatment group ($p=0.69$ for time-by-treatment interaction).

However, oseltamir and MY, both alone and in combination, reduced time to fever resolution in patients with H1N1, compared to controls. These results suggest that MY may represent an alternative treatment for H1N1 infections (Table 2).

Fever duration (hr)				
Kaplan-Meier Estimate	Control Group (n=103)	Oseltamivir Group (n=102)	MY Group (n=103)	Oseltamivir Plus MY Group (n=102)
Median time to alleviation of fever (95% CI), h	26.0 (24.0 to 33.0)	20.0 (17.0 to 24.0)	16.0 (14.0 to 17.0)	15.0 (12.0 to 18.0)
Difference in median time to fever resolution (95% CI), %*	-	-34 (-46 to -20); P<0.001	- 37 (-49 to -23); P<0.001	-47 (-56 to -35); -P<0.001
Relative to control group				
Relative to oseltamivir group	-	-	- 5.0 (-22 to 17); P=0.65	- 19 (34 to -0.3); P=0.047
Relative to MY group	-	-	-	-15 (-30 to 4); P=0.122
Results				
<ul style="list-style-type: none"> • Oseltamivir and MY had similar effects on alleviating fever • Compared with oseltamivir, the combination therapy group was better in fever resolution ($p=0.05$) 				

Table 2: Comparison of times taken to alleviate fever in the control group and three treatment groups. (CI = confidence interval).

	Oseltamivir group		MY group	
Daily treatment costs	55.2 RMB	8.06 USD	16 RMB	2.33 USD
Cost over duration of treatment	276 RMB	40.31 USD	80 RMB	11.68 USD

Table 3: Comparison of average costs per patient of treatment of the oseltamivir and MY patient groups.

Safety

Two patients in the MY group had nausea and vomiting. No side effects were observed in the control, oseltamivir, or combination therapy group. No differences were observed in complications after treatment among the four groups.

Cost

Although treatment by both oseltamivir and MY was effective, treatment costs in the MY group averaged 16 RMB (2.33 USD) per day (80 RMB or 11.68 USD over the duration of treatment), compared to 55.2 RMB (8.06 USD) per day in the oseltamivir group (or 276 RMB or 40.31 USD over the course of treatment). This equates to savings of over 70% (Table 3).

Partnerships

This was a multicentre research project and was completed through the collaboration of 11 medical centres, including Beijing Chao-Yang Hospital, Beijing Institute of Respiratory Medicine; Beijing Hospital, Ministry of Health; Dongzhimen Hospital affiliated to Beijing University of Chinese Medicine; Yantai Infectious Disease Hospital, Shan-dong; Chengdu Infectious Disease Hospital, Sichuan; Beijing Haidian Hospital, Beijing; Beijing Ditan Hospital, Institute of Infectious Diseases, Capital Medical University, Beijing; Renmin Hospital of Wuhan University; Changxindian Hospital of Fengtai District of Beijing; Second Hospital of Chaoyang District of Beijing, Beijing; West China Medical School, West China Hospital, Sichuan University, Sichuan.

In addition, serial real-time RT-PCR for viral RNA titres was carried out with assistance of local branches of the Chinese Centre for Disease Control and Prevention.

Impact

The research results were acknowledged by renowned international institutions, and were published in the Annals of Internal Medicine as original research. The project made up an indispensable part of a set of research achievements that won the First Class Prize of the State Scientific and Technological Progress Award, and the First Prize of the Beijing Science and Technology Awards.

The research results were reported many websites, such as the New York Times and Reuters. The journal article was included in five international authoritative medical databases, including PIER (Physician's Information and Education Resource), Science-Based Medicine, NHS, EBSCO and Tripdatabase.

The project also won an international honour for the medical response work done by China, and also helped to promote TCM on the international market.

Replicability

We have shown that MY could be an effective, more economic solution to the widespread use of oseltamivir.

Policy Implications

The project innovated the "Fever Theory" of TCM, and the research results provide strong evidence for guidelines for the use of TCM in the treatment of influenza, as well as a basis for the further exploration of TCM that has been used for the treatment of seasonal influenza for thousands of years. Therefore, the treatment was well accepted by the public.

Future Plans

Based on the work done, we will further explore other TCMs for treating pneumonia. We also aim to explore the mechanism of action of MY using animal models and in vitro experiments. In addition, we will collaborate with other medical centres to perform multicentre clinical trials, as well as strengthening the collaboration with basic research teams to understand the mechanisms, by which TCMs achieve their protective effect in patients.

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Non-clinical and Clinical Evaluation of *Alternanthera sessilis* for Haemoglobin Augmentation in Anaemia

Philippines

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Duration: 2008-2010

Total cost: USD 11,000

Summary

The major health consequences of iron deficiency anaemia (IDA) include poor pregnancy outcomes, impaired physical and cognitive development, increased risk of morbidity in children and 20% of maternal deaths. This condition also diminishes the work capacity of individuals and entire populations, leading to serious economic problems and obstacles to national development. Early treatment of this disease is necessary to restore personal health and raise national productivity levels by as much as 20% (World Health Organization, 2012). In serious cases of anaemia, blood transfusion is recommended to save the life of the patient.

Previous studies have shown that *Alternanthera sessilis* has the potential to be an alternative treatment for iron deficiency anaemia based on the results of a Phase 1 Clinical Trial (Arollado, 2010). Analysis revealed the presence of iron and vitamins A, C, B1 and B6. Its safety was confirmed since heavy metals were not detected. Further evaluation of the plant extract, when administered to the subjects in the clinical trial, revealed that there were no side

effects experienced from the formulation. This is especially significant since commercial iron preparations are often associated with unwanted effects such as gastrointestinal upsets, nausea and vomiting. In addition, biochemical parameters such as liver profile, creatinine and complete blood count of the volunteers in the Phase 1 trial appeared normal.

Since the plant is a common edible weed that grows in Philippine farmlands throughout the year, it is a convenient and readily available natural source. Propagation of the weed for the eventual commercial production of the drug requires low cost and low maintenance. This thus translates to a natural treatment which is less expensive than commercial drugs.

Based on our studies, propagation of this plant has to be initiated to provide enough material for the formulation to be used for further investigation and production. Due to its potential value as a drug, there is a need to collaborate with other agencies to commercialize and market this product.

Background and Justification

In 2008, the World Health Organization (WHO) reported iron deficiency as the most common and widespread nutritional disorder in the world, affecting 30% of the world's population, specifically children and women in developing countries. It is also the only nutrient deficiency that is significantly prevalent in industrialized countries. The prevalence of anaemia in the Philippines is about 19.5% based on a survey of the Food and Nutrition Research Institute of the Philippines in 2008.

Herbal medicines have now been recognized as natural alternative drugs for synthetic medicines because they are often cheaper and safer than the latter. *Alternanthera sessilis* (L.) R.Br. (Family Amaranthaceae) also known as sessile joyweed or dwarf copperleaf is a common edible weed that grows widely in Philippine farmlands (Fig. 1). Earlier investigations showed that 100 g of this weed contain minerals such as iron (1.84 mg), magnesium (314 mg), calcium (299 mg), copper (0.89 mg), zinc (2.05 mg), sodium (168 mg) and potassium (620 mg). Based on this, it could be a potential source of iron for those suffering from iron deficiency anaemia (IDA).

The aims of the project were to:

- Provide scientific proof that *A. sessilis* is a potential drug for effective haemoglobin augmentation in the clinical setting; and
- Provide a cheaper alternative treatment for patients suffering from IDA.

Description



Figure 1: *Alternanthera sessilis* (L.) R. Brown, a common weed in the Philippines, is being investigated as a treatment for anaemia.

The steps taken during the investigation and implementation process are outlined in Fig. 2.

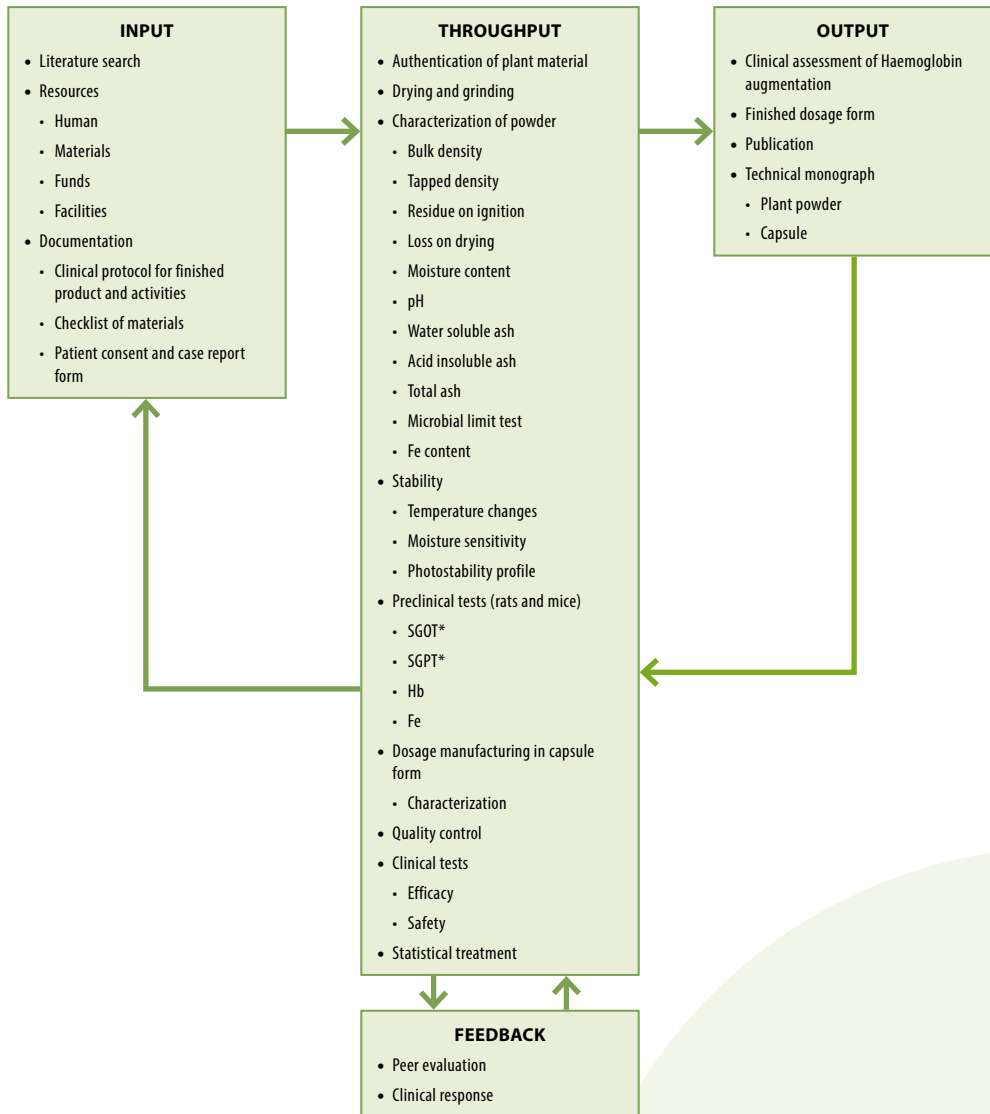


Figure 2: Conceptual framework for the research.

*SGOT = Serum glutamic oxaloacetic transaminase and SGPT = serum glutamic-pyruvic transaminase, both of which are liver enzymes.

Samples of *A. sessilis* were collected from the island of Panay, often being bought at local markets during the main growing season. These were dried and powdered and subjected to various tests of their physico-chemical properties (Table 1) and to determine their iron content (Table 2). Results were consistent between samples and the powder shown to be stable over several months, allowing the project to move to preclinical studies.

Results

Preclinical trials tested levels of *A. sessilis* administration to rats and mice at 100% of the dose of the standard drug (ferrous sulphate) as well as at 80, 60 and 40%. Their effects on levels of iron and haemoglobin, as well as targeted liver enzymes were all positive with no adverse effects detected.

Tests	Trial		
	First	Second	Average
Bulk density	0.3333 g/mL	0.3448 g/mL	0.3391 g/mL
Tapped density	0.4167 g/mL	0.4237 g/mL	0.4202 g/mL
Residue on ignition	0.3168%	0.3161%	0.3164%
Loss on drying	7.40%	7.43%	7.415 %
Moisture content	7.765%	6.62%	7.192%
pH	6.56	6.56	6.56
Water soluble ash	4.22%	3.59%	3.905%
Acid insoluble ash	5.425%	3.95%	4.685%
Total ash	20.11%	20.13%	20.125%

Table 1: Physico-chemical characteristics of powdered *A. sessilis*.

Trial	Parts per million (ppm)
1	52.15
2	51.59
3	50.57
Average	51.43

Table 2: Assay of powdered *A. sessilis* for iron content.

We then proceeded to the trials with human subjects. Firstly, powdered *A. sessilis* was prepared in standardised 'Lupo' capsules (Table 3) with an average iron content of 0.024mg/capsule.

SUMMARY OF EVALUATION			
Criteria	Specifications	Result	Remarks
Average weight	500 mg	465.035 mg	93.007% of target
Range	450-500 mg (+ 10% of label claim)	451.2 mg - 501.7mg	96.70 – 107.88% of average weight
Standard deviation	To be established	11.404	-
% relative standard deviation (calculated to assess the manufacturing precision)	2% maximum	2.45%	Slightly above limit (Usual for small lots)
Average iron content	Average weight x % Fe	0.024 mg/ capsule	Clinical trial : dose three times a day 3 capsules ≈ 0.072 mg 6 capsules ≈ 0.144 mg

Table 3: Summary evaluation of Lupo capsules.

Criteria	Specifications (Normal values)	Change (0 → 90 days)	Statistical Evaluation
Haemoglobin (Hgb)	12-14 g/dL (F) 14-16 g/dL (M)	↑ Increase	Significant F=13.300, p=0.000
Haematocrit (Hct)	41-50% (whole blood)	↑ Increase	Significant F=12.370, p=0.000
Red blood cells (RBC)	3.9 -5.5 x 10 ⁶ /μL	↑ Increase	Significant F=0.431, p=0.007
Mean corpuscular volume (MCV)	80-100 femtolitres/ cell	↔ Change	Not Significant
Mean corpuscular haemoglobin (MCH)	27-32 picogrammess/ cell or 1,68 - 1.92 femtomol/cell (SI-units)	↔ Change	Not Significant
Mean corpuscular haemoglobin conc. (MCHC)	25 g/dL	↔ Change	Not Significant
Blood iron concentration (serum ferritin)	15-200 ng/mL (F) 30-300 ng/mL (M)	↑ Increase	Significant F=13.158, p=0.000

Table 4: Effect of *A. sessilis* capsules on blood characteristics after 90 days.

Phase 1 clinical trials compared two groups, each consisting of 15 volunteer patients aged 18-65 years. Blood characteristics were monitored during the trial. Results after 90 days showed significant positive effects upon haemoglobin, haemocrit, numbers of red blood cells and levels of iron in the blood (Table 4).

Although the amount of iron present in one capsule is low compared to the recommended daily intake (0.024 mg compared to 15-30 mg), an increase in haemoglobin was still observed. This could be due to synergistic effects of other compounds present in the *A. sessilis*.

In addition no adverse effects were detected during a series of tests on liver and kidney function demonstrating the safety of the product.

Partnerships

There were no regional or international collaborators in this project. The community that sold this vegetable in the market participated in this project by providing required raw materials for payment. Since the raw materials are seasonal, cultivation of this plant is needed to sustain the required volume of samples for the project

Impact

This project won second prize in the National Research Council of the Philippines during a poster contest in 2009. After the project was finished, many patients with leukaemia, low platelet count, high white blood cell counts and liver disease have been using of this product continuously. Laboratory tests have confirmed the drug's positive effects.

Farmers growing this plant will be encouraged to cultivate more of it due to high existing demand for the raw material.

Lessons Learned

The project has great potential as it answers a basic community need. This said, as it relied upon procuring the raw plant material from the market there were times when access to materials was limited, hence the need to promote cultivation of the plant.

Future Plans

The plan is to study the mechanism of action of this product and continue the clinical trial with the ultimate aim of formulating *A. sessilis* extract as a commercial drug.

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Construction of the Vessel-collateral Theory and its Guidance for Prevention and Treatment of Vasculopathy

China

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Summary

In traditional Chinese medicine, the vessel-collateral theory was constructed systematically and considered important in improving the prevention and treatment level of vasculopathy, or diseases of the blood vessels. The hypothesis of 'homeostasis' (*Cheng*), compensatory auto-adaptation (*Zhi*), regulation (*Tiao*) and equilibrium (*Ping*), based on the 'qi-yin-yang-five elements' coupled with the *ying* (nutrients)-*wei* (defence) theory, has become the core content of the vessel-collateral theory. Clinical and laboratory trials have been developed to further confirm the scientific connotations of the hypothesis and have resulted in a set of capsules for treating various vasculopathies.

For example, *Tong Xin Luo* capsules developed using vessel collateral theory showed good efficacy in protecting the vascular endothelium, stabilizing vulnerable plaques and reducing blood vessel spasms, helping in the treatment of acute myocardial infarction, cerebral infarction and the microvascular complications of diabetes. *Shen Song Yang Xin* capsules for the treatment of arrhythmia have also contributed an integrated adjustment advantage. Likewise, *Qi Li Qiang Xin* capsules have been developed for the treatment of both the manifestation and the root cause of chronic heart failure. Thus this research has improved both prevention and treatment of major vascular system diseases.

Background and Justification

According to WHO statistics from 2000, some 17 million people die of cardiovascular diseases each year. The Statistical Gazette on Healthcare Development in China, issued by the Ministry of Health of China, has indicated that cardiovascular diseases are the leading cause of mortality and proportional mortality since 1990, responsible for 40% of all deaths. Additionally, in 2006, the Annual Reports of Cardiovascular Diseases showed that, every year, two million people develop cerebral apoplexy, 500,000 people develop myocardial infarction and almost three million people die of cardiovascular diseases. In China, such figures are linked to the distinctive features of 'three highs and three lows' – a high incidence, high disability rate and high mortality combined with low awareness, low control rate and low recovery rate.

The explanations provided by reductionist thinking to explain disease have been increasingly challenged by the emergence of global complexity science. Subsequently, a return to holism has become the core driving force of the life sciences in the 21st century to explain or interpret living organisms using nonlinear science, complex systems and systems biology. Deeply rooted in clinical practice, early philosophical thinking of 'extending one's knowledge through investigation' (*Ge Wu Zhi Zhi*) and dissection,

traditional Chinese medicine contains ancient philosophical ideas of *qi-yin-yang*-five elements. It combines the meta-physical '*qi*' with anatomical 'blood' and understands the internal law of life activities and disease development and progression from a holistic, systematic and constantly changing perspective. In this regard, traditional Chinese medicine is of practical significance in the shift from reductionism to a holistic study system, thereby creating an opportunity for innovative advances in the prevention and treatment of cardiovascular diseases.

Description

Vessel-collateral theory studies the occurrence and development law, essential pathology, clinical signs and symptoms, pattern identification, and the treatment of 'vessel collateral-vascular system conditions'. Vessel-collateral conditions can result either from the functional or structural injury to blood vessels and collateral vessels (that direct blood around injured blood vessels), from pathogenic factors, or from secondary pathology of the *zang-fu* organs or tissues. They cover a wide range of cardio-cerebrovascular diseases, including arrhythmias, chronic heart failure, pulmonary heart conditions, rheumatic heart disease and peripheral angiopathy. Common examples are cardiac pain due to angina, stroke, palpitations, cardiac fullness, oedema due to heart *yang* deficiency, thoracic fluid retention, cardiac obstruction and gangrene. Unfortunately, the conceptual confusion between 'meridians and vessels' and 'meridians and collaterals' originating from the historical prejudice towards 'collaterals' and 'vessels' compromised the importance of vessel-collateral theory and its resultant guidance for the prevention and treatment of vasculopathy, or diseases of the blood vessels. Consequently, constructing a systemic vessel-collateral theory has helped improve the prevention and treatment of vasculopathy.

As pathways of circulating *qi* and blood, meridians are associated with the *zang-fu* organs and connect the upper body with lower body and the interior with the exterior. The *Nei Jing* (Inner Classic) states that "meridians are located in the deeper area, while collaterals are the transverse branches of the meridians and minute collaterals are subdivided branches of the collaterals". In other words, collaterals diverge from the meridians and contain different layers. They are extensively distributed over the *zang-fu* tissues, like a network system to maintain life activities and homeostasis of the organism.

Collaterals are further divided into meridian collaterals and vessel collaterals. The former transport *qi*, while the latter transport blood. Together they constitute two major inter-dependent but interactive networks that perform the physiological functions of "circulating *qi* and blood as well as nourishing *yin* and *yang*". With a combined vessel

collateral and meridian collateral theory, the academic system of Chinese medicine consists of the *zang xiang* (core), meridians (pivot) and *qi* and blood (foundation).

Other than a network for transporting blood, the 'vessels' are the pathways of the heart (lung)-blood-vessel circulation system and also an independent solid organ, an extraordinary fu-organ. Morphologically, vessels are empty cavities and associated with the heart and lung. Categorized into veins and arteries, vessels are distributed in a multi-layered network. Physiologically, vessels store and preserve essential *qi* to maintain a relative homeostasis of blood volume and quality. Kinetically, vessels dilate and contract following heart beats. Functionally, vessels transport blood to nurture the whole body, help metabolism and promote interchange between blood and other fluids. The *Shanghang Za Bing Lun* (Treatise on Cold Damage and Miscellaneous Diseases, 1976) recorded the concept of 'vessel collateral', discussed vessel-collateral conditions, initiated the collateral-unblocking formulae and thus laid the theoretical, diagnostic and therapeutic foundation of vessel-collateral theory.

Clinical and laboratory trials have confirmed the scientific connotations of vessel-collateral theory and have resulted in a set of capsules for treating various vasculopathies. *Tong Xin Luo* capsules showed good efficacy in protecting the vascular endothelium, stabilizing vulnerable plaques and reducing blood vessel spasms, helping in the treatment of acute myocardial infarction, cerebral infarction and the microvascular complications of diabetes. *Shen Song Yang Xin* capsules for the treatment of arrhythmia have contributed an integrated adjustment advantage. Likewise, *Qi Li Qiang Xin* capsules have been developed for the treatment of both the manifestation and the root cause of chronic heart failure. Thus this research has improved both prevention and treatment of major vascular system diseases.

Impact

The project developed the 'pattern identification and treatment of collateral disease' and helped establish a new discipline of collateral disease theory in practice. Groundbreaking treatment protocols were coupled with new national patent medicines: *Tong Xin Luo* capsules for cardio-cerebrovascular disease, *Shen Song Yang Xin* Capsules for cardiac arrhythmias, and *Qi Li Qiang Xin Jiao Nang* for chronic heart failure.

The development of these treatments has received various awards, including:

- Five first prizes in provincial/ministerial projects;
- Second prizes for national science & technology inventions, and for national science & technology progress;
- The Holeung Ho Lee Foundation prize.

In addition, an academic book prize, 'Collateral Disease Theory in Practice', was published by the China Association of Chinese Medicine.

Lessons Learned

All the above studies have proven the guidance, scientific value and future application potential of the homeostasis (*Cheng*), compensatory auto-adaptation (*Zhi*), regulation (*Tiao*) and equilibrium (*Ping*) hypothesis, which is the core of the vessel-collateral theory. In addition, the theory may also become a key idea for multidisciplinary studies on vasculopathy, which will guide our further understanding of complex vascular system diseases.

Future Plans

Research will continue to guide vascular disease prevention in chronic heart failure, arrhythmias and coronary heart disease.

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A Research Strategy for the Development of Clinical Evidence for Traditional Herbal Medicine

Zimbabwe

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Summary

Despite greater access to more efficacious and less toxic treatment options, patients still take herbal medicines for various reasons. The drumstick tree (*Moringa oleifera* Lam.) is commonly used for medicinal and nutritional purposes among HIV-positive patients in Zimbabwe. The World Health Organisation supports the appropriate use of herbal medicines and encourages the use of remedies that have been proven to be safe and effective. Clinical trials are considered the gold standard in terms of evidence to support therapy, yet very few clinical trials are ever conducted using herbal medicines.

We conducted a cross-sectional survey to determine the patterns of use of *M. oleifera* among HIV positive patients with a focus on plant part, dosage, prescribers and the associated medical conditions. We used the data to develop a clinical trial protocol which was reviewed by two institutional review boards as well as the national ethics

committee and drug regulatory authority. Upon approval, we implemented the protocol among HIV-positive adults to determine the safety and tolerability of *M. oleifera* when taken concomitantly with antiretroviral drugs.

Participants did not report any adverse events during the study. There were no significant differences in the proportion of pre- and post-treatment biochemistry and urinalysis readings falling into toxicity ranges. Interaction with patients attending the opportunistic infections clinic resulted in a more open practitioner-patient relationship in which participants were more willing to discuss herbal medicine use and developed a positive attitude towards clinical research. Successful dialogue with the various ethics and regulatory bodies, as well as clinical research sites and laboratories, established a regulatory and operational framework for future herbal medicine trials in Zimbabwe.

Background and Justification

The use of herbal medicines is widespread in Africa. In 2003 the World Health Organisation (WHO) estimated that 80% of people in Africa use traditional herbal medicines for some aspect of primary healthcare (WHO, 2003). Survey data from the last five years shows a sustained high prevalence of use in many African countries, particularly among HIV-positive patients. Rates range from 84% in South Africa, to 63% in Uganda and 98% in Zimbabwe (Babb *et al.*, Langlois-Klassen *et al.*, Mudzviti *et al.*, 2012). Further details from such surveys indicate that a wide range of herbs are used. A group of traditional healers in Ethiopia cited 155 different species they used in their treatments, the adult population in a rural district in Zimbabwe cited 93 species, while caregivers in Nigeria cited 40 different species for malaria alone (Maroyi *et al.*, 2013; Olorunnisola *et al.*, 2013; Abera, 2014).

Common reasons given for use of herbal medicine are related to inaccessibility of components of the conventional health system; issues such as high cost of conventional drugs, poor accessibility of healthcare facilities as well as perceived efficacy of herbal medicine are often brought up. Other users however, cite cultural reasons and management of adverse effects of conventional medicines (Olorunnisola *et al.*, 2013). In the latter case, many people still take herbal medicines even within adequate health systems with greater access to more efficacious and less toxic treatment options; and often take them together with conventional drugs. Between 61% and 91% of patients with various chronic conditions reported practising herb-drug co-administration in Jamaica (Olorunnisola *et al.*, 2013; Delgoda *et al.*, 2010).



Figure 1: Standardized capsules of dried *Moringa olifera* leaf powder.

Many users of herbal remedies believe all herbs are safe because they are natural and that the long history of traditional use has demonstrated that they are not associated with severe adverse effects. We know, however, that herbal medicines are often taken in a crude form containing a large number of compounds which the body treats in the same way as any conventional drug compound, and thus can interact with conventional drugs in the same ways drugs interact with each other (Cordier and Steenkamp, 2011; Müller and Kanfer, 2011).

Therefore, there is definitely a need for consideration of herb-drug interactions by prescribers and clinical pharmacists. We also know that good clinical decisions should be evidence-based. The World Health Organisation supports the appropriate use of herbal medicines and encourages the use of remedies that have been proven to be safe and effective (WHO, 2014). Clinical trials are considered the gold standard in terms of

evidence to support therapy. A crude review of medical research databases shows that a significant amount of work has been done around herb-drug interactions. But closer analysis shows that the data is skewed towards Western and Chinese herbal medicines rather than African ones. Furthermore, the majority of data available are from isolated organ systems, and *in vitro* and *in vivo* animal studies rather than clinical studies (van den Bout-van den Beukel et al., 2006). Data from these pre-clinical studies are inconclusive and cannot be used by clinicians to advise patients and/or inform policy. Many of the conclusions in fact recommend further research in the form of appropriately designed clinical pharmacokinetic studies (Monera *et al.*, 2008).

Description

We therefore set out to develop clinical evidence for traditional herbal medicines commonly used by HIV-positive patients in Zimbabwe. Local survey data had shown that *Moringa oleifera* Lam. is commonly used for medicinal and nutritional purposes among HIV-positive patients in Zimbabwe. We began by conducting a cross-sectional survey to determine the patterns of use of *M. oleifera*. A previously piloted researcher-administered questionnaire was used to interview patients who reported to an opportunistic infections clinic over three months about their use of herbal medicines. The questionnaire focused on *M. oleifera* plant part used, dosage, prescribers and the associated medical conditions.

The acceptance rate was 97% and in the course of the study 263 men and women were recruited. Our results also showed that *M. oleifera* supplementation is common among HIV-positive people. Sixty-eight percent (68%) of the study participants consumed *M. oleifera*. Of these, 81% had commenced antiretroviral drug treatment for HIV/AIDS. Friends or relatives were the most common source of a recommendation for use of the herb (69%). Most (80%) consumed *M. oleifera* to boost the immune system. The leaf powder was mainly used, either alone or in combination with the root and/or bark. This data has been published in the Journal of Public Health in Africa (Monera and Maponga, 2012).

We then used the survey data to design an intensive pharmacokinetic sampling study to compare pre- and post *M. oleifera* plasma concentration profiles of efavirenz and nevirapine (two commonly prescribed anti-HIV drugs) in HIV-positive, antiretroviral therapy experienced patients (the MOART study). The clinical trial protocol was reviewed by two institutional ethical review committees, as well as the national ethics committee and the national drug regulatory authority. This turned out to be a long process with a lot of deliberations given the absence of a regulatory framework for herbal trials in Zimbabwe.

A manuscript with full details of the ethical and regulatory deliberations is under review for publication.

Results

Upon approval, we implemented the clinical trial protocol among HIV positive adults to determine the safety and tolerability of *M. oleifera* when taken together with nevirapine or efavirenz. We enrolled a total of 19 participants with a mean age of 44 (± 8) years over 5 months; 13 were female and 6 were male. *M. oleifera* leaves were harvested from a rural district in Zimbabwe, processed and assessed for microbial and heavy metal contamination to assure quality. The *Moringa* was administered as a standardised dry leaf powder in a one sequence, open label, cross-over design. A range of clinical and pharmacokinetic endpoints were compared across the two phases of the trial to assess the clinical effects of *M. oleifera* in HIV-positive patients.

We have now completed the safety analysis. Participants were in good health upon clinical examination and did not report any adverse events during the 5-months study period. They reported an increase in appetite and some weight gain was observed.

There were no significant differences in the proportion of pre- and post-treatment biochemistry and urinalysis readings falling into toxicity ranges, all of which were grade 1. In addition, *M. oleifera* did not alter the pharmacokinetics of either, efavirenz or nevirapine to any clinically relevant extent. A full manuscript detailing the safety and pharmacokinetic endpoints is currently under review.

Partnerships

Through a collaborative Global Pharmacology Capacity Building Programme, with the School of Pharmacy at the State University of New York Buffalo, USA, we have established an International Pharmacology Speciality Laboratory in Zimbabwe where the drug assays for future clinical studies with pharmacokinetic endpoints will be conducted (Fig. 2). Before this study established this laboratory, facilities with expertise in the assay of drugs in biological samples in the country were limited. Specimens had to be shipped abroad for analysis, which involved a complicated and bureaucratic procedure of approval.

Efavirenz and nevirapine drug concentrations were determined through collaboration with two specialist pharmacology laboratories.



Figure 2: The newly-established International Pharmacology Speciality Laboratory – the first of its kind in Zimbabwe.

Impact

Prior to this study, no clinical trials with herbal interventions had been approved by a drug regulatory authority or conducted in Zimbabwe. In January 2013, our *M. oleifera* supplementation trial in HIV-positive patients became the first clinical trial with herbal intervention to successfully obtain both ethical and regulatory approval in Zimbabwe, thereby establishing a framework for the future conduct of similar trials. Our close interaction with patients attending the opportunistic infections clinic resulted in a more open practitioner-patient relationship.

Participants were more willing to discuss herbal medicine use and developed a positive attitude towards clinical research. The positive safety outcomes justified the 'WHO Alternative Clinical Observational Study' approach for herbal trials. Participants did not report any adverse events during the study and there were no significant differences in the proportion of pre- and post-Moringa biochemistry and urinalysis readings falling into toxicity ranges, all of which were grade one.

Replicability

Successful dialogue with the various ethics and regulatory bodies as well as clinical research sites and laboratories, established a regulatory and operational framework for future herbal trials in Zimbabwe.

Lessons Learned

Clinical trial protocols with herbal interventions can be successfully designed, approved and implemented. There is a need to streamline the ethical and regulatory processes to optimize review timelines and enhance the efficiency of clinical research.

Future Plans

Through this trial we have established an Herbal Trials Unit in the University of Zimbabwe College of Health Sciences. We are currently working on two new studies focusing on other traditional herbal medicines used by HIV/AIDS patients as well as a paediatric *Moringa* supplementation protocol. We are also in the process of establishing a long term surveillance programme to monitor clinical end points in HIV/AIDS patients using herbs together with their antiretroviral drugs. These studies are expected to progress more quickly as result of the regulatory frameworks and laboratory infrastructure established by the MOART study and therefore progress more quickly.

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From Zheng Classification to New Drug Discovery in Chinese Medicine

China

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Summary

Rheumatoid arthritis (RA) was adopted as a disease model to examine how Zheng classification, based on disease diagnosis, represents a novel approach to the research

and development of combinational drugs design based on *Fu Fang* (a Chinese herbal formula). Some unique research design and methods were introduced.

Background and Justification

Zheng classification, the core of traditional Chinese medicine (TCM) theory, promotes personalized medicine by modifying the clinical diagnosis into a more precise aggregate when integrating Zheng classification with disease diagnosis. The advantages of this aggregation can contribute to the discovery of new drugs.

Description

In TCM theory, Zheng, also termed “syndrome” or “pattern”, is the basic unit and a key concept. Zheng can be understood as a guideline for patient classification in clinical practice from a different viewpoint or dimension when compared with disease diagnosis in biomedicine. Over the past 30 years, an increasing number of researchers have focused their attention on developing evidence for Zheng and sought to identify its mechanism. As of August 2015, there were 711,299 papers on Zheng in Chinese lodged in the China National Knowledge Infrastructure (CNKI) together with 843 papers on the PubMed database. New trends are emerging in Zheng research in the integration of Zheng classification with biomedical disease diagnosis (Jiang *et al.*, 2012).

Zheng classification can provide deeper insight into the specific indications of drugs

The most important influence when blending Zheng classification and clinical diagnosis in biomedicine is the improvement of clinical diagnosis (Lu *et al.*, 2012; Jiang *et al.*, 2012).

Zheng classification uses phenotype-like clinical manifestations to classify patients, thus further assisting in their stratification for intervention to improve the efficacy of the treatment based on a Zheng classification-related clinical trial strategy.

A previous study showed that patients with rheumatoid arthritis (RA) ‘cold’ Zheng had a significantly higher response rate to the biomedical therapy than ‘hot’ Zheng patients with RA (He *et al.*, 2007; He *et al.*, 2008). The differences between the two Zheng have been outlined (Lu *et al.*, 2012a; Lu *et al.*, 2012b). Consequently, it was hypothesized that a comparison analysis between the responsive and non-responsive cases might help detect

the effectiveness-related signs and symptoms, after which a further round of clinical trials could be conducted, focusing on the patient subgroup with positively related signs and symptoms as part of inclusion criteria. It could therefore be anticipated that a higher effective rate would be obtained in the second round of clinical trials as the patients would have been stratified further in relation to their response-related factors. Early in 2015, a two-stage clinical trial of TCM therapy (*Tripterygium wilfordii* polyglycoside tablets and *Yi Shen Juan Bi* pills) for the management of RA was published (Jiang *et al.*, 2015). The stage one trial was an open-label trial and aimed to explore which groups of TCM information correlate with better efficacy whilst the stage two trial was a randomized, controlled, double-blind trial that incorporated the efficacy-related information identified in the stage-one trial into the inclusion criteria. The results conclusively demonstrated the role of Zheng classification in efficacy improvement.

This innovative clinical trial design can also be used for the clinical efficacy evaluation of an “old” drug to “renew” its indication (Zhang *et al.*, 2014). Moreover, the Zheng classification concept may also assist with the assessment of drug safety. A 2013 study provides a new paradigm for better understanding the risks and limitations when using potentially toxic herbs in clinical applications (Tan *et al.*, 2013).

The incorporation of the Zheng classification into biomedical disease diagnosis will lead to a new era in the development of personalized medicine, very much a trend for the future, involving medication tailored to individual patients and incorporating the use of multiple therapeutic agents and the assessment of nutritional, psychological and lifestyle factors when deciding the best course of treatment. These early investigations suggest that Zheng classification could be of use in moving forward with improving clinical diagnosis improvement, and provide momentum for the move towards therapeutics and pharmacology. Improving treatment efficacy with specific therapeutic indications may identify specific indications that provide research opportunities for the development of new drugs.

Zheng classification used to help choose a single appropriate drug for a range of diseases

Another important concept in TCM, termed “Treating Different Diseases with the Same Therapy” (TDDST) has been applied in practice. Some patients with RA and others with coronary heart disease (CHD) can be treated with similar therapies (e.g. activation of blood stasis for RA patients with a Zheng of blood stasis). This suggests that there might be some connection between the conditions of RA and CHD in line with a TCM diagnosis within the context of an imbalance in bodily functions, biological networks or biological bases. In order to substantiate such concepts, there exists a need to track down

any interlinking data from reliable databases (Zhang *et al.*, 2011). Our study proposed a hierarchical analysis algorithm termed “discrete derivatives” based on the frequencies of concurrent Medical Subject Headings (MeSH) terms, providing some significant results which support the concept of TDDST, together with the biological markers and biological networks existing in RA and CHD (Zhang *et al.*, 2014). These networks can be affected by herbs widely used in TCM therapies for both RA and CHD (Niu *et al.*, 2014). Another example to is to explore commonalities in different diseases using mass spectrometry-based metabolic phenotyping studies to identify any generalized metabolic defects associated with arthritis, together with the metabolic signatures of four major types of arthritis (Bi Zheng in CM): RA, osteoarthritis, ankylosing spondylitis, and gout (Jiang *et al.*, 2013). A global metabolic profile has been identified for all arthritic patients, suggesting that there are common metabolic defects resulting from joint inflammation and lesion. Meanwhile, differentially expressed serum metabolites have been identified, constituting a unique metabolic signature for each type of arthritis that can be used as biomarkers for diagnosis and patient stratification. Similar symptoms shared by the different types of arthritis have continued to confound clinical diagnoses and represent a clinical dilemma when deciding treatment choices with a more personalized or generalized approach. Therefore, Zheng classification, as an approach to identifying relevant diseases in patients within different Zheng categories can certainly contribute important concepts in the innovation of treatments for different diseases with the same drug and this concept offers a broad vision for improving drug discovery.

Zheng classification offers a clue to the development of combination therapeutics

The paradigm shift in new drug discovery is via the modulation of multiple proteins rather than a single target. Some successful drugs now in the marketplace have, by chance, wound up hitting several targets, which is perhaps why they are effective. Active Chinese herbal ingredients and thousands of traditional herbal formulae have long been viewed as a rich source of therapeutic leads in drug discovery. Combinatory drugs or health products focus on multi-target drugs and this has emerged as a new paradigm in drug discovery. Therefore, the intention of our research was to purposefully aim at multiple targets with a combination of drugs. Taking new drug discovery in rheumatology as an example, we reported results from text mining and mapped the biological network of RA and integrated it into a Cytoscape network (Zheng *et al.*, 2011). We established a comprehensive platform covering a large number of public molecular databases including SinoMed, PubMed, TCMD, DrugBank, ChEMBL, GAD, GO, IntAct and PharmGKB. The state-of-the-art use of protein structure, protein–protein interaction, signalling, genetic interaction, metabolic networks and chemical similarity in the discovery of drug targets was summarized. The prediction method consisted of two main steps: (i) prediction of

the pharmacological effects from chemical structures of 100 compound combinations (from text-mining results), and (ii) the inference of unknown combination drug-target interactions based on the similarity of their pharmacological effects. Ten combination drug candidates were finally arrived at. Experimental and clinical validations are now necessary, but the originality of the method lies in the prediction of their potential pharmacological similarity for any combination drug candidates and in the integration of the available chemical, proteomic, genomic and pharmacological data within a unified framework. We anticipate that this type of data might streamline the re-targeting of drugs.

Partnerships

Among the partners of the various projects were the China Academy of Chinese Medical Sciences, Shanghai Jiao Tong University Affiliated Sixth People's Hospital and the China Astronaut Research and Training Centre.

Impact

Among the awards won by the research team was a one-year studentship provided by the Mr. Kwok Yat Wai and Madam Kwok Chung Bo Fun Graduate School Development Fund to C. Liang in 2015.

The special circumstances: Institute for Advancing Translational Medicine in Bone & Joint Diseases provide multidisciplinary scientists from Faculty of Science and School of Chinese Medicine a synergistic collaboration platform which efficiently and effectively translate basic scientific findings into knowledge that benefits patients with bone and joint diseases, which is often described as an effort to carry scientific knowledge 'from bench to bedside'.

Replicability

Two patents have been awarded for the team's products, while another four patent applications were under consideration.

Lessons Learned

In the past few years, the pharmacy industry has seen a shift from the search for 'magic bullets' that specifically target a single disease-causing molecule towards the pursuit

of combination therapies that comprise more than one active ingredient. While some researchers have focused on studying combinations of conventional drugs, others, with good reason, have turned to Chinese herbal medicines.

Future Plans

Zheng classification shows great promise for outlining drug indications that may have important benefits from both a patient and an economic perspective. Moreover, the goal of using old drugs in new ways is becoming more efficient through the use of a Zheng classification approach. To accelerate intelligent drug discovery, we propose combination drug strategies in which the new drugs target illnesses on the Zheng disease classification subnetworks. Nevertheless, the emergence of validated Zheng classification applications looks ever more certain, bringing with it the hope of a new therapeutic era.

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Section 2: Physical Methods

Two traditional techniques that derive particularly from traditional Chinese medicine are acupuncture and wet-cupping. Both techniques are gaining in popularity around the world, with acupuncture especially becoming more and more widely used.

In the four case studies presented in this section – three from China and one from Turkey – modern scientific analyses are applied to these ancient practices.

08	The Clinical Efficacy, Evaluation and Central Mechanism Study on Acupuncture for Treating Functional Dyspepsia	China	76
09	Wet-cupping Removes Oxidants and Decreases Oxidative Stress	Turkey	85
10	Electroacupuncture for Moderate and Severe Benign Prostatic Hyperplasia: A randomized controlled trial	China	91
11	The Efficacy of Acupuncture for Migraine Prophylaxis	China	102

The Clinical Efficacy, Evaluation and Central Mechanism Study on Acupuncture for Treating Functional Dyspepsia

China

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Total cost: USD 800,000 - State Key Programme for Basic Research of China (973 Programme, No. 2006CB504500, No. 2012CB518501)

Summary

Using two randomized, controlled clinical trials and two neuroimaging studies, we found that, compared with fictitious acupoints, acupoints on the gallbladder meridians, and non-specific acupoints on the stomach meridian, the specific acupoints on the stomach meridian significantly improved the symptoms and quality of life of functional dyspepsia patients, and elicited more significant cerebral responses, especially in the symptom-related brain regions, the anterior cingulate cortex (ACC), insula, thalamus and cerebellum.

The results indicated that acupuncture is effective and safe for functional dyspepsia, and puncturing at acupoints provided better efficacy than fictitious ones.

The results also demonstrated the existence of acupoint specificity for the specific acupoints at the stomach meridian for treating functional gastrointestinal disorders.

Finally, the results suggested that dynamics and targeting are the important characteristics of the central mechanism of acupoint specificity. Targeting implies the central mechanism of acupoint specificity is disease-oriented, exhibiting more significant modulation on the disease-associated brain regions. The dynamics imply the central mechanism of acupoint specificity is one of dynamic variability depending upon the various physical states, treatment duration and acupoint combination.

Background and Justification

Functional dyspepsia (FD), a major gastrointestinal disorder, is characterized by persistent and recurrent postprandial upper abdominal discomfort after meals, early satiety, abdominal distension or burning without any organic or biochemical abnormality and although FD is not life-threatening, it has long been an important health issue and social problem for its high prevalence, uncertain pathogenesis, low response rate and great influence on quality of life. Therefore, effective complementary and alternative therapies are needed.

Acupuncture has been used effectively to treat FD, but lacks high quality clinical evidence to support it.

According to acupuncture theory and clinical experience, acupoints on the stomach meridian are the most commonly used points in FD treatment, but the mechanism of acupoint specificity remains unclear.

Description

We carried out a systematic review to evaluate the quality of clinical evidence and conducted two randomized control trials to verify the clinical efficacy and acupoint specificity, plus two neuroimaging studies to investigate the mechanism on acupoint specificity in FD treatment.

The clinical efficacy evaluation included three steps: a systematic database review; a randomized controlled trial with 700 participants that tested at different acupoints for FD (Fig. 1); and another randomized controlled trial for acupuncture at acupoints selected according to the meridian distribution and syndrome differentiation for FD with 200 participants (Fig. 2).

Two neuroimaging studies attempted to determine the central mechanism of acupoint specificity and the factors that influence acupoint specificity in FD treatment, respectively (Figs. 3 and 4).

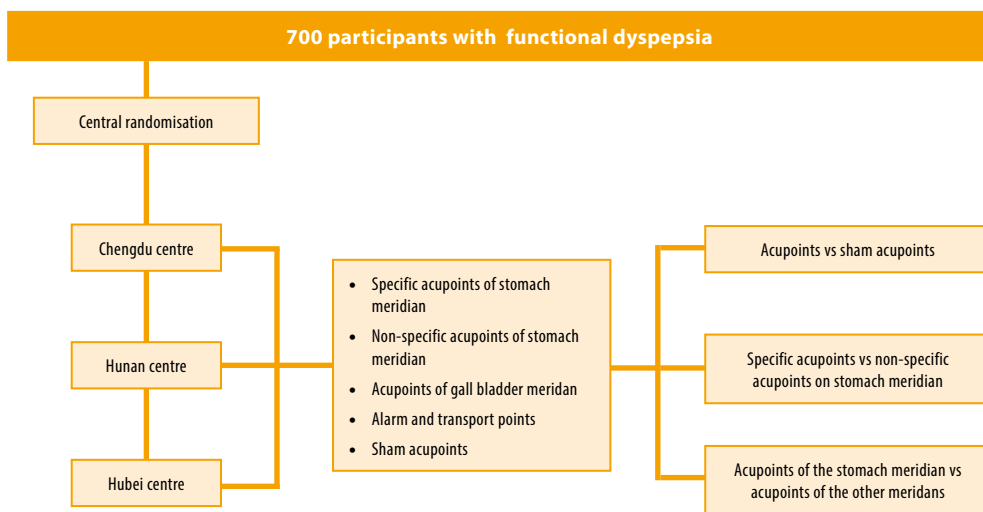


Figure 1: Flow chart of a randomized controlled trial: Acupuncture at different acupoints for functional dyspepsia (FD).

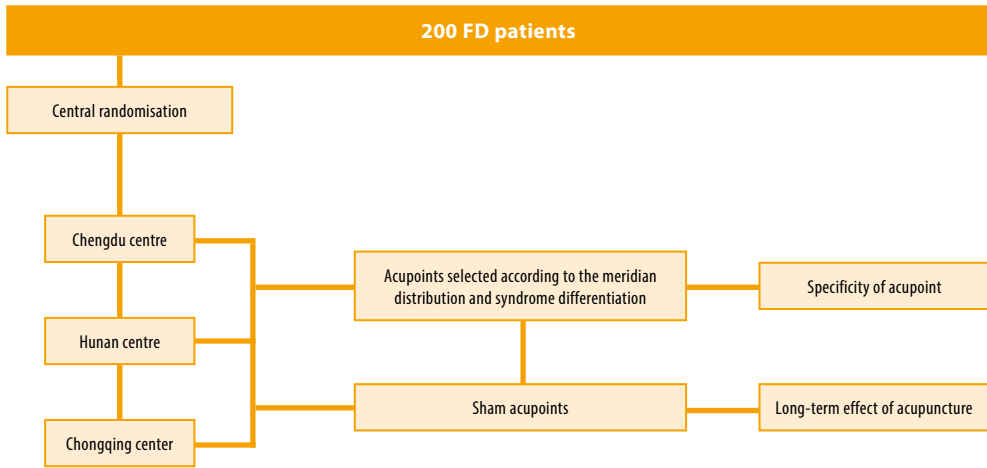


Figure 2: Flow chart of a randomized controlled trial: Acupuncture at acupoints selected according to the meridian distribution and syndrome differentiation for functional dyspepsia (FD).

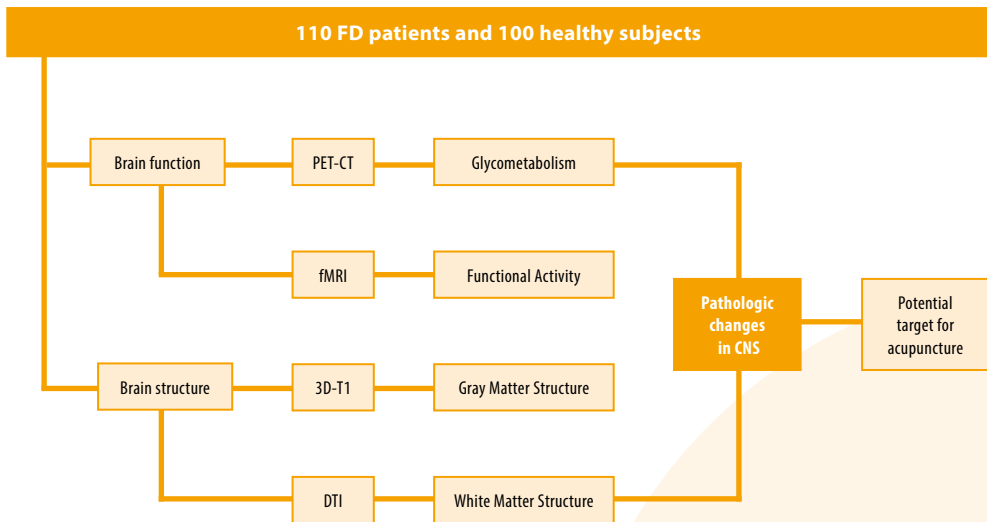


Figure 3: Flow chart of first neuroimaging study: cerebral structural and functional alterations of functional dyspepsia (FD). CNS: central nervous system.

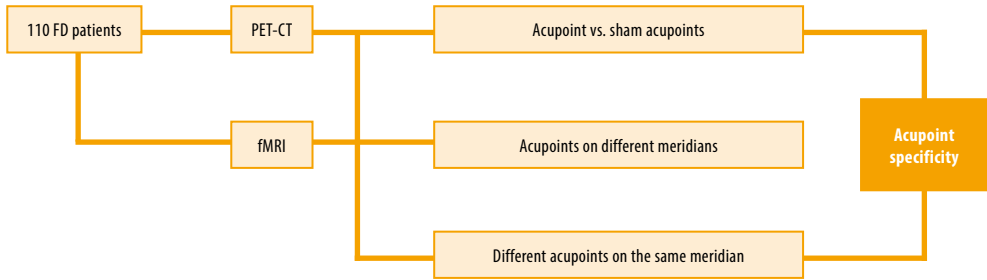


Figure 4: Flow chart of second neuroimaging study: cerebral mechanism of acupuncture for treating functional dyspepsia (FD).

Results

The evidence from the literature review provided limited information on how to improve the benefit and safety of acupuncture for treating FD. Better-designed clinical trials with larger sample sizes were therefore needed.

The trials reported here showed that acupuncture was effective for treating FD. Specific acupoints on the Stomach meridian gave superior results compared to sham acupoints, acupoints on the gallbladder meridians, and non-specific acupoints on the stomach meridian, alarm and transport points in improving the symptoms and quality of life of FD patients (Figs. 5 and 6).

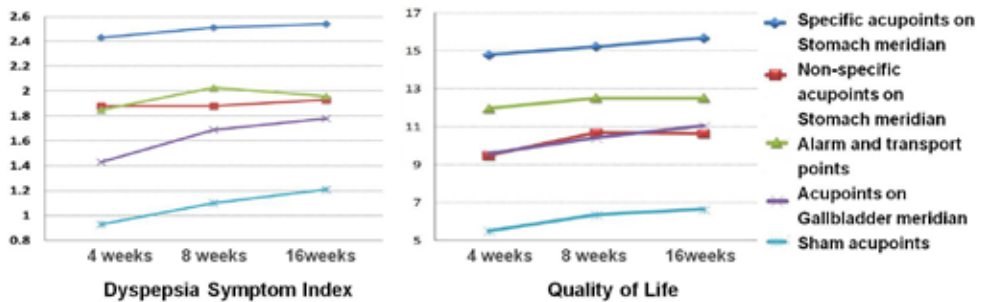


Figure 5: Improvement of Dyspepsia Symptom Index (left), and quality of life score (right) after acupuncture interventions.

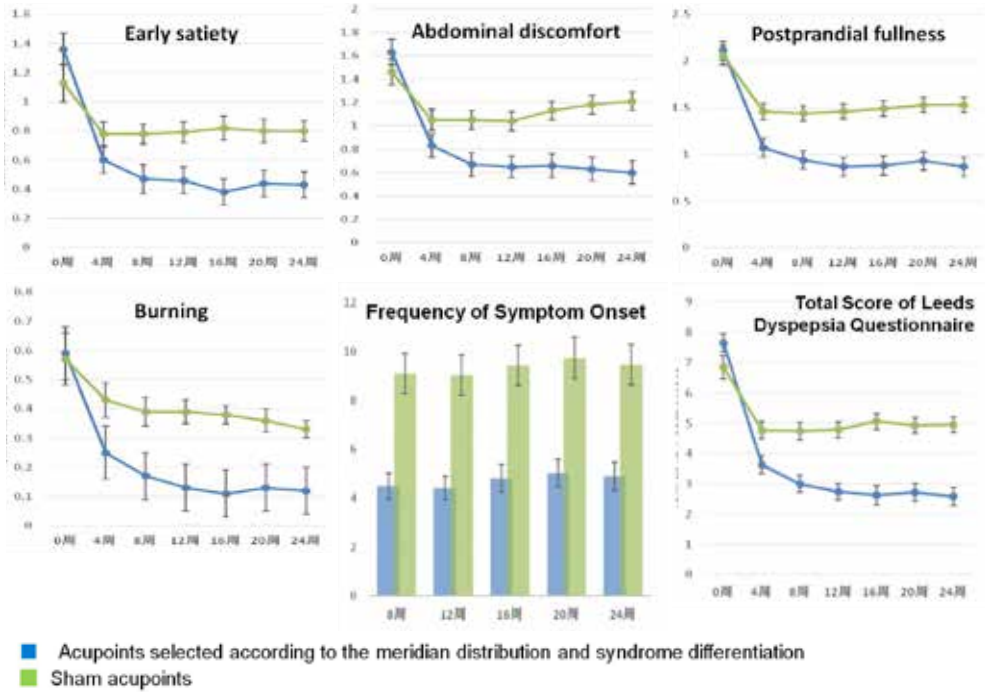


Figure 6: Change in Leeds dyspepsia questionnaire scores after interventions.

The neuroimaging studies showed that FD patients exhibited functional and structural abnormality in the central nervous system. The specific acupoints on the stomach meridian elicited more significant cerebral responses, especially in symptom-related brain regions. The central integrated mechanism of acupoint specificity is dynamically variable according to different physical states, duration of treatment and compatibility of acupoints (Figs. 7, 8 and 9).

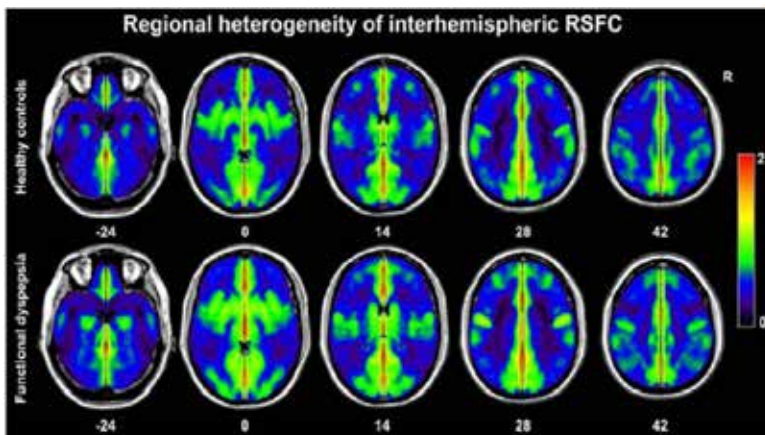


Figure 7. Cerebral structural and functional alterations of FD patients and healthy subjects.

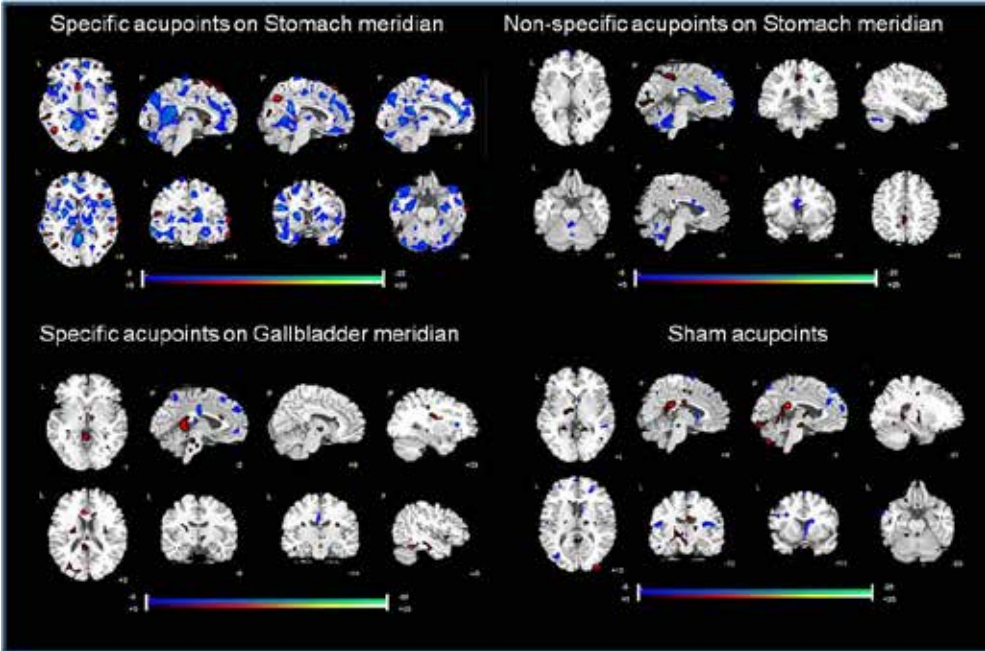


Figure 8: Cerebral responses on puncturing at the specific acupoints on stomach meridian (top left) were significantly different from those at sham acupoints (bottom right), acupoints on other meridians (bottom left) or non-specific acupoints on the stomach meridian (top right).

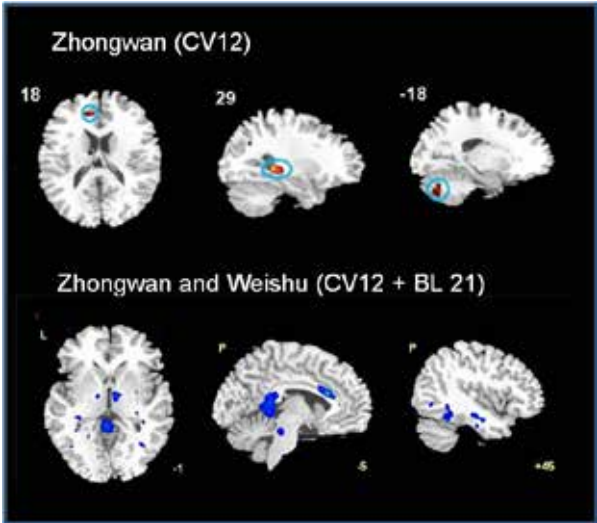


Figure 9: Demonstration that 20 acupuncture treatment sessions gave improved results compared to 5 sessions with regard to regulating brain function and improving clinical manifestations.

In addition, qualitative assessments of patients' quality of life before and after the acupuncture treatments demonstrated improvements (Fig. 10).

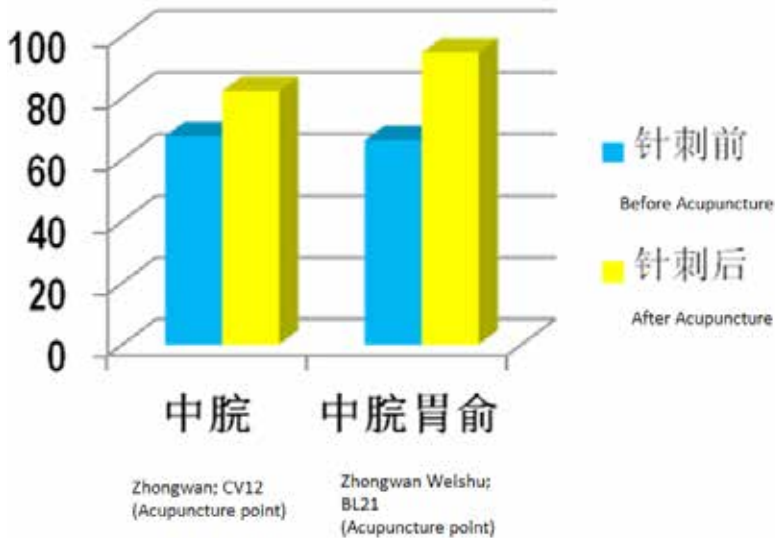


Figure 10: Improvement of quality of life before and after acupuncture treatment for functional dyspepsia (FD).

Partnerships

Among the partners involved in these studies were:

- Hunan University of Traditional Chinese Medicine,
- Hubei University of Traditional Chinese Medicine,
- School of Life Sciences and Technology, Xidian University,
- Huaxi MR Research Centre at the West China Hospital of Sichuan University,
- Sichuan Academy of Medical Sciences, and
- Sichuan Provincial People's Hospital.

Impact

- In total, 45 research papers have been published in Science Citation Index (SCI) journals.
- Following these reports, methods of neuroimaging studies proposed in this project have been adopted in eleven scientific research institutions nationwide, and accepted by 37 organizations outside China.

The research presented here was awarded the 2012 National Science and Technology Progress Second Prize, and the 2014 Sichuan province scientific and technological progress award.

Future Plans

We intend to investigate the mechanism of acupoint specificity using connectomics, i.e. comprehensive maps of the connections within the nervous system.

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Section 2

09

Physical Methods

Wet-cupping Removes Oxidants and Decreases Oxidative Stress

Turkey

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Summary

Wet-cupping therapy (WCT) is one of the oldest known medical techniques. Although it is widely used in various conditions such as acute or chronic inflammation, infectious diseases and immune system disorders, its mechanism of action is not fully understood. In this study, we investigated the oxidative status as the first step to elucidate its possible mechanisms of action.

Oxidative stress reflects an imbalance between the systemic manifestation of reactive oxygen species and a biological system's ability to readily detoxify the reactive intermediates or to repair the resulting damage. Disturbances in the normal redox state of cells can cause toxic effects through the production of peroxides and free

radicals that damage all components of the cell, including proteins, lipids and DNA.

The study population consisted of 31 healthy volunteers who undertook WCT. Venous blood samples and wet cupping (WC) blood samples were taken concurrently to assess oxidative stress, serum nitric oxide, malondialdehyde levels, and superoxide dismutase and myeloperoxidase activities were measured spectrophotometrically. WC blood had higher activity of myeloperoxidase, lower activity of superoxide dismutase, and higher levels of malondialdehyde and nitric oxide compared to venous blood. We can conclude that WC removes oxidants and decreases oxidative stress.

Background and Justification

The originality of our study is that we simultaneously measured all these parameters in wet cupping (WC) and venous blood samples of healthy people for the first time in the literature.

Cupping is a traditional therapy dating back at least 2000 years. There are many different cupping applications in practice such as needle cupping, moving cupping, retained cupping, medicinal (herbal) cupping and bleeding cupping (wet cupping). The latter is the most commonly used cupping procedures. Each type of cupping therapy may be used for different diseases or different treatment aims. In general, a glass cup is applied on the skin over an acupuncture point, painful area, or a reflex zone. This treatment creates a vacuum over certain points on the skin. Some researchers hypothesize that implementation of cups on selected acupoints on the skin provides a therapeutic effect by hyperaemia.

Wet-cupping has been claimed to drain excess fluids and toxins, loosen adhesions and lift connective tissue, bringing blood flow to skin and muscles, and stimulating the peripheral

nervous system. In addition, cupping is said to reduce pain and high blood pressure as well as to modulate neurohormones and the immune system. Cupping therapy is also used to improve subcutaneous blood flow and to stimulate the autonomic nervous system.

Free oxygen radicals formed during physiological and pathophysiological metabolism are balanced by a similar rate of their consumption by antioxidants. Although their excess production may cause oxidative damage of biological molecules, cell membranes and tissues, their generation is inevitable during certain metabolic processes.

Free radical-mediated oxidative damage has been implicated in the pathogenesis of a large number of diseases, including autoimmune diseases of endocrine glands, cancer, inflammatory diseases, cardiovascular disease (including atherosclerosis, hypertension, ischaemia/reperfusion injury), diabetes mellitus, neurodegenerative diseases (Alzheimer's disease and Parkinson's disease), rheumatoid arthritis, and ageing. A recent study showed that cupping had therapeutic effects on myocardial infarctions and cardiac arrhythmias in rats. Another recent study also investigated the possible useful effects of cupping therapy on cardiac rhythm in terms of heart rate variability (HRV). All HRV parameters in healthy persons improved after cupping therapy compared to those measured before cupping therapy, suggesting that cupping might be cardio-protective. It is suggested that cupping therapy restored sympathovagal imbalances by stimulating the peripheral nervous system.

These examples demonstrate the wide application of WC in cases associated with oxidative damage.

Description

For this study, WC therapy was applied to 31 healthy volunteers, 15 females and 16 males; aged 21-40 years (mean age 30.24 ± 9.53 years). Written informed consent was obtained from each participant and the study protocol was accepted by the local ethics committee.

To assess oxidative stress, serum nitric oxide, malondialdehyde levels as well as activity of superoxide dismutase and myeloperoxidase were measured spectrophotometrically. WC blood exhibited higher myeloperoxidase activity, lower superoxide dismutase activity, higher levels of malondialdehyde and nitric oxide compared to venous blood. WC removes oxidants and decreases oxidative stress.

For the cupping therapy, sterile 5 cm diameter disposable cups were used. Five points of the posterior neck, bilateral perispinal areas of the neck and thoracic spine were selected for treatment (Fig. 1). The same points were cupped in all participants. The application

areas were first cleaned with antiseptic solution. Cups were then placed on these points and negative pressure applied using a cupping pump. The cups were removed after 2-3 minutes. Then, the skin was then punctured to a depth of 2mm within the cupping sites using 26-gauge disposable lancets. After this, vacuum pumping was re-applied and 3-5 cm³ of blood was drained per cupping site. The application sites were then covered with sterile pads. No adverse reaction was experienced but as fainting due to intolerance to pain was possible, a doctor, a nurse and emergency response kit and stretcher were kept ready in the treatment room.

Venous blood samples were collected after overnight fasting and just before WC implementation. WC blood samples were taken from the cups after bleeding and vacuum applications. The serum fraction was obtained by centrifugation and stored at -80 °C until analysis.



Figure 1: Points chosen for wet- cupping (WC) therapy.

Results

Significant differences were found between the levels of malondialdehyde and nitrite/nitrates in WC compared to venous blood (Table 1). Likewise, myeloperoxidase activity was significantly increased in WCT, but no differences were detected in superoxide dismutase activity.

	Venous	Wet cupping	t	p
MDA level (nmol/L)	16.31±3.78	21.34±8.8	2.81	0.009
NO _x level (μmol/L)	25.55±9.65	30.01±11.9	2.88	0.007
MPO activity (U/mL)	33.01±22.41	68.93±40.7	4.9	0.000
SOD activity (U/mL)	45.66±5.46	41.14±12.8	2.15	0.04

Results are expressed as mean ± SEM

MDA, malondialdehyde; NO_x, nitrite + nitrate; MPO, myeloperoxidase; SOD, superoxide dismutase

This graph shows positive correlation between venous and wet cupping blood NO levels

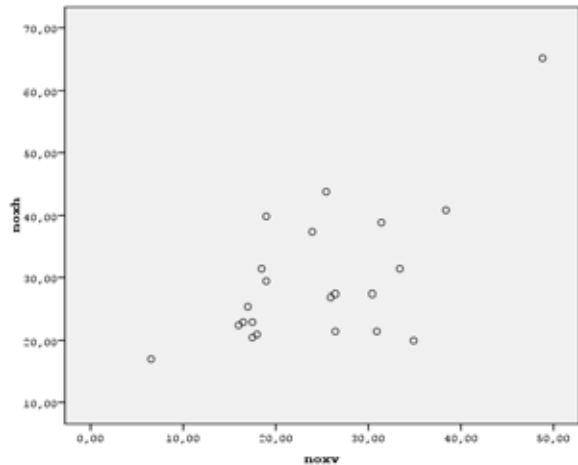


Table 1: Concentrations of oxidative status markers in blood taken from veins and wet cupping (WC) sites (n = 31).

Impact

Although our research is at an early stage, preliminary results have been published in a peer-reviewed journal.

Lessons Learned

We could not evaluate the levels of oxidative stress parameters in venous blood following WCT employment because second venous samples were not taken. Furthermore, all volunteers in our study group were healthy so we could not assess the effect of WCT on any diseases related to oxidative stress. We emphasize that more extensive studies with a broader study population should be carried out to further determine the effect of WCT on oxidative status.

Future Plans

In order to continue our work more efficiently within Turgut Ozal University, the establishment of a Complementary Medicine Institute has been proposed and the university is making preparations for its establishment.

Our academic research is ongoing and we are working on publishing our findings, although we have not yet begun cooperating with researchers in other countries.

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Publication

- Tagil, S.M., Celik, H.T., Ciftci, S., Kazanci, F.H., Arslan, M., Erdamar, N., Kesik, Y., Erdamar, H. and Dane, S. (2014). Wet-cupping removes oxidants and decreases oxidative stress. *Complementary Therapies in Medicine* 22(6): 1032-1036. DOI: <http://dx.doi.org/10.1016/j.ctim.2014.10.008>.

Section 2

10

Physical Methods

Electroacupuncture for Moderate and Severe Benign Prostatic Hyperplasia: A randomized controlled trial

China

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Summary

A randomized controlled trial was carried out to evaluate the effects of electroacupuncture (EA) on moderate to severe benign prostate hyperplasia (BPH), and to explore the therapeutic effective difference between EA at the recommended acupoint BL33, and a non-acupoint for BPH.

Men with BPH and an International Prostate Symptom Score (IPSS) ≥ 8 were enrolled in the trial. One hundred participants were randomly allocated to receive EA at acupoint BL33 (treatment group) and EA at a non-acupoint (control group). The primary outcome was the change of IPSS at the sixth week. Secondary outcomes included

changes of post-void residual (PVR) urine and of urinary flow rate (Qmax) at the sixth week and change of IPSS at the eighteenth week. Treatment group patients had 4.51 points and 3.2 points greater decline in IPSS than the control group at week 6 and week 18 respectively ($p < 0.001$, $p = 0.001$). No significant differences were found in Qmax and PVR between two groups.

The results indicate that EA is effective in improving patient's quality of life and acupoint BL33 may have better therapeutic effects than non-acupoints in acupuncture treatments of BPH.

Background and Justification

Benign prostate hyperplasia (BPH) is an enlargement of the prostate gland due to progressive hyperplasia of the stromal and glandular cells of the prostate. The prevalence of this disease is as high as 40% in men in their fifties and 90% in men in their eighties (Nickel, 2006). BPH is one of the most common causes of lower urinary tract symptoms (LUTS). Current treatments for BPH include watchful waiting, lifestyle modifications, alpha blockers, 5 alpha-reductase inhibitors, phytochemicals, and BPH-related surgery (Tanguay *et al.*, 2009). Although most of the aforementioned therapies have various degrees of documented effectiveness in the management of BPH, the use of these interventions are limited to specific patient populations or have certain side effects that interfere with patients' quality of life.

Acupuncture is a traditional Chinese medicine treatment that has been commonly used in the management of LUTS in China for thousands of years. The effects of acupuncture on LUTS were well documented in Chinese medicine textbooks and are well-supported by modern research studies (Wang, 2003). Ricci *et al.* (2004) found that electroacupuncture (EA) had better effects in decreasing number of voiding times of urinary urgency that persisted after transurethral resection of the prostate. Kubista *et al.* (1976) found that EA could significantly increase the closing pressure in women with stress incontinence

compared with a placebo, and Philp *et al.* (1988) found that acupuncture increased the bladder capacity in patients with bladder instability. Besides effects on urinary storage problems, acupuncture was also found effective in the prevention of recurrent lower urinary tract infections in adult women (Aune *et al.*, 1998; Alraek *et al.*, 2002), and in improving the quality of life in patients with chronic prostatitis (Capodice *et al.* 2007).

BPH is clinically characterized by various LUTS which may include or be similar to urinary urgency, stress incontinence, bladder instability, and urinary tract infection (UTI). Therefore, we hypothesize that acupuncture may be effective in the management of BPH.

This hypothesis is supported by our previous studies in which we found that acupuncture at BL33 had better effects than terazosin in improving the International Prostate Symptom Score (IPSS), post-void residual urine (PVR), and maximum urinary flow rate (Qmax) on patients diagnosed with mild to moderate BPH (Yang *et al.*, 2008; Yang *et al.*, 2008a).

Theories of traditional Chinese medicine and results from modern studies indicate that acupoints of the fourteen meridians have specific functional regulatory effect on *zang-fu* organs (Shuran and Zhongsuan, 1987; Cheng and Han 2004; Xu *et al.*, 2010). However, studies in western countries found that dry needling, an acupuncture procedure at trigger points (including non-acupoints that do not belong to the meridian system), were effective in the management of various diseases (Diraçoğlu *et al.*, 2012; Kalichman and Vulfsons, 2010). Both dry needling and traditional acupuncture treat diseases via inserting stainless needles into the human body. However, differences between acupuncture at acupoints and acupuncture at non-acupoints have not been fully investigated. In the present study, we aimed to compare the therapeutic effectiveness of EA at bilateral acupoints of BL33 with EA at non-acupoints (2 cun [around 6.7 cm] lateral to BL33) in a randomized controlled pilot study. The results showed that correct acupoint EA was more effective than non-acupoint EA in reducing IPSS (Ding Y, 2011).

Description

Participants who met the trial criteria (Table 1, 2) were given a sequentially-numbered, opaque, sealed envelope. These envelopes were distributed to patients by an investigator who was not involved in acupuncture procedures and data analyses. Based on odd or even numbers assigned in the envelope, 50 participants were randomly allocated to receive either EA at acupoint BL33 (treatment group) and another 50 to receive EA at non-acupoint (control group).

<i>Inclusion criteria</i>	<i>Exclusion criteria</i>
<ul style="list-style-type: none"> • 50–70 years old; • Moderate to severe BPH evaluated by IPSS; Patients having urinary dysfunction more than 3 months; • Patients with stable life signs; • Not on any α1 receptor blocker, 5α-reductase inhibitor or traditional Chinese medicine for over 1 week; • Volunteer to join this research and give informed consent prior to receiving treatment. • For safety reasons, patients were instructed of possible emergency conditions and were told to seek appropriate medical help if they should occur. 	<ul style="list-style-type: none"> • Urinary dysfunction caused by gonorrhoea or urinary tract infection; • Oliguria and anuria caused by urinary calculi, prostate cancer, bladder tumor and acute/chronic renal failure; • Urinary dysfunction caused by neurogenic bladder, bladder neck fibrosis and urethral stricture; • Failure of invasive therapy for prostatic obstruction; • Injured local organs, muscle and nerve caused by pelvic operation or trauma; • Upper urinary obstruction and hydrocoele combined with damaged renal function due to BPH diagnosed by B-ultrasound; • Patients unable to commit to treatment because of commuting problems to the hospital.

Table 1: The inclusion and the exclusion criteria for the clinical trial.

We used Huatuo brand needles (size 0.30 x 100 mm, manufactured by Suzhou Medical Appliance, Suzhou, Jiangsu Province, China) together with GB6805-2 Electro-Acu Stimulators (Huayi Medical Supply & Equipment Co., Ltd, Shanghai, China). For the treatment group, we needled at bilateral BL33 with a 45° angle. A feeling of soreness and distension is felt when needling into the third posterior sacral foramina (S3) with eventual radiation of the sensation to the perineum. Needles were inserted 60–80 mm without lifting, thrusting or rotating and, once in position, connected to the electric stimulator with a disperse-dense wave of 20 Hz. The current intensity was increased to the patients' maximum tolerance and then slightly reduced to a bearable level. For the control group, we took the site 2 cun lateral BL33 as the non-point. Manipulation methods and electric stimulator parameters were the same with those of the treatment group.

There were 16 sessions for all patients (five sessions in the first and second weeks and three sessions in the third and fourth weeks). Each session lasted 30 minutes. Acupuncture for the two groups was operated by the same acupuncturist who has more than ten years experience. This acupuncturist was blinded to the outcome assessment at baseline, week 6 and week 18.

All patients were evaluated during the first week for baseline values which included IPSS, PVR and Qmax. The primary outcome involved the change of IPSS from baseline at the 6th week; secondary outcomes included changes of PVR and Qmax at the sixth week and change of IPSS at the 18th week. Safety evaluation included haematoma, fainting, severe pain, and local infection during and after acupuncture. In addition, emergency conditions which require catheterization were also recorded if any.

The statistical analysis was performed by a statistician blinded to treatment allocation in the Clinical Evaluation Centre of China Academy of Chinese Medical Sciences.

Results

From September 2010 to May 2012, a total of 192 patients with LUTS visited the Acupuncture Department at Guang'anmen Hospital in Beijing. 92 patients were excluded from the present study because they either did not meet the inclusion criteria or met one or more of the exclusion criteria (Table 1).

One hundred of them were included and randomized to receive either acupoint acupuncture or non-acupoint acupuncture treatments (Figure 1). Figure 2 details the time frames of recruitment, treatment and follow-up periods.

Study flow

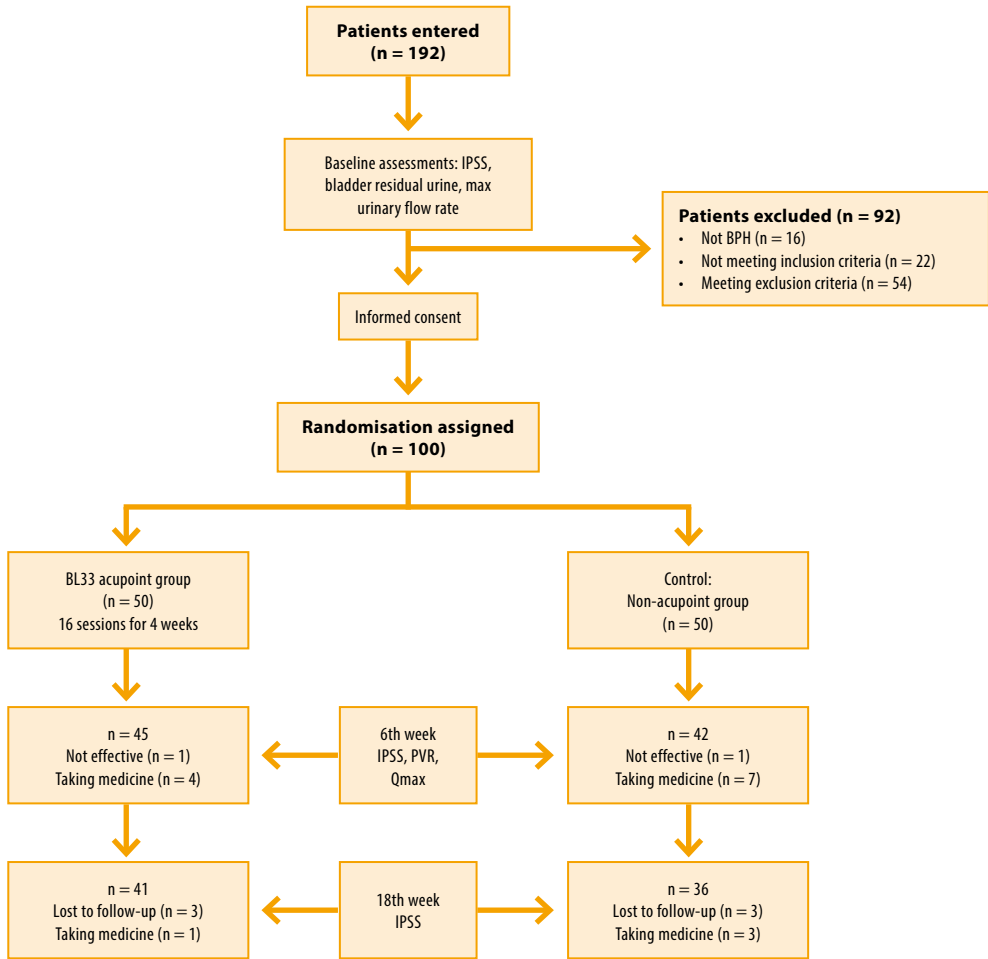


Figure 1: Flowchart for study participation.

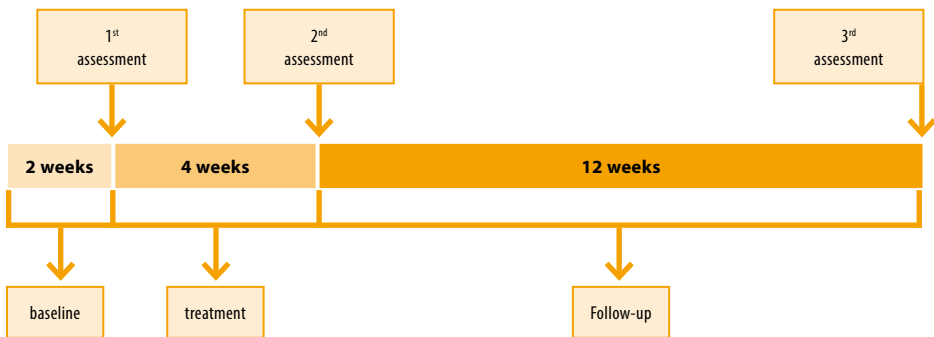


Figure 2: Time frame of each period of the trial.

Demographic characteristics and baseline information of the 100 participants are shown in Table 2. No statistically significant differences were found between the two groups in age, gender, and baseline values. The mean age of all participants was 65 years old.

	Acupoint group (n=50)	Non-point group (n=50)	P-value (two-tailed)
Age	64.80±7.05	65.94±6.74	0.411
Course of disease	76.08±57.59	73.66±57.72	0.834
IPSS	20.10±6.52	18.76±6.06	0.289
Qmax	13.04±6.73	15.93±7.33	0.051
PVR(ml)	20 (0,128)	16 (0,128)	0.260

Table 2: Demographic information and baseline characteristics.

Analyses of IPSS at the sixth week were based on both the intention-to-treat population (ITT) and the per protocol (PP) population (i.e. those who successfully completed the trial) (Table 3 and Table 4). At the sixth week, the ITT analysis indicated that IPSS reduced from 20.10±6.52 at baseline to 12.84±5.87 for the acupoint treatment group, and from 18.76±6.06 at baseline to 16.42±6.80 for non-point control group. With the PP analysis, IPSS of the two groups reduced to 12.60±5.85 and 16.05±6.83, respectively. At the sixth week, acupoint group patients had a 4.51 ($p<0.001$) and 4.12 ($p<0.001$) points greater decline than the non-acupoint control group in the ITT and PP populations, respectively (Table 5). At the 18th week, a 3.2 points ($p=0.001$) greater decline was found for the acupoint treatment group compared to the non-acupoint control group.

		Acupoint group (n=50)	Non-point group (n=50)
IPSS	Baseline	20.10±6.52	18.76±6.06
	Week 6	12.84±5.87	16.42±6.80
	Change in IPSS	7.26±5.12	2.34±4.85
	Week 18	14.62±5.76	16.96±6.47
	Change in IPSS	5.28±5.16	1.8±5.06
PVR (ml)	Baseline	20 (0,128)	16 (0,128)
	Week 6	20 (0,300)	15 (0,180)
	Change in PVR	0 (-172,84)	0 (-120,80)
Qmax	Baseline	13.04±6.73	15.93±7.33
	Week 6	12.63±6.11	15.00±6.50
	Change in Qmax	0.36±4.51	0.98±4.05

Table 3: Descriptive statistics of ITT population.

		Acupoint group (n=45)	Non-point group (n=42)
IPSS	Baseline	19.84±6.46	18.83±6.00
	Week 6	12.60±5.87	16.05±6.83
	Change in IPSS	7.24±5.23	2.79±5.17

Table 4: Descriptive statistics of the per protocol (PP) population.

		Treatment effect estimate (Mean difference)	Standard error	P-value
IPSS (ITT)	Week 6	4.51	0.93	0.0000
	Week 18	3.20	0.93	0.0001
IPSS (PP)	Week 6	4.12	1.03	0.0000
Qmax (ITT)	Week 6	0.18	0.80	0.819

Table 5: Statistical analysis of the trial outcomes: ANCOVA test results. P-values less than 0.05 are considered significant.

Qmax and PVR. No significant differences were found between the two groups in Qmax at the sixth week ($p=0.819$, Table 5). PVR data followed a non-normal distribution and no significant difference was found ($P=0.35$).

Adverse events. No serious adverse events happened in either group. Two cases of mild haematoma were reported in the non-acupoint control group during study. The patients were told to apply ice and compression within 24 hours and heat compression after 24 hours to the acupuncture treatment areas. The haematomas disappeared in about two weeks.

Impact

In this study, EA at BL33 had better effects on IPSS, but no difference on PVR and Qmax as compared with non-acupoint EA. The results indicate that EA is effective in improving patients' quality of life and acupoint BL33 may have better therapeutic effects than non-acupoints in acupuncture treatment of BPH.

Lessons Learned

Blinding is difficult in acupuncture studies, so real randomized placebo-controlled trials may seem impossible. In this randomised controlled trial, enrolled patients were distributed via sequentially numbered, opaque, sealed envelopes by an investigator who was not involved in acupuncture procedures and data analyses. For the blinding of outcome assessors, the staff in charge of the assessment and the statistician were all blinded to the patients' allocation. Acupuncture operation (performed by an experienced acupuncturist) and filling of case report forms (by a postgraduate) were done under strict supervision. Phone calls and e-mails were used to inform the patients for follow-up assessments. Any medicine taken and other treatment conducted during this time were recorded in detail. Although non-acupoint EA procedures were used as a control in the present study, they are still acupuncture procedures; thus we could not rule out the confounding factor of needling and placebo effects in the present study.

Future Plans

This randomised controlled trial was performed in only one hospital rather than multi-centres. Therefore, the results of the present study may not well-characterize the general response of patients with BPH around the world. In future, to further test the therapeutic effects of EA on BPH, additional large scale, multi-centre, international cooperative studies are warranted.

Publications

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The Efficacy of Acupuncture for Migraine Prophylaxis

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Duration: June 2007 - February 2009.

Total cost: USD 70,000.

Summary

A multicentre, double-dummy, single-blinded, randomized controlled clinical trial was conducted at the acupuncture outpatient departments at five hospitals in China to evaluate the effectiveness of acupuncture. A total of 140 patients with migraine without aura were recruited and randomly assigned to two different groups: the acupuncture group treated with verum acupuncture plus placebo and the control group treated with sham acupuncture plus flunarizine. Patients

were treated using acupuncture three times per week and drugs every night; patients from both groups were evaluated at week 0 (baseline), week 4, and week 16. The results suggested that acupuncture was more effective than flunarizine in decreasing days with migraine attacks, whereas no significant differences were found between acupuncture and flunarizine in reduction of pain intensity and improvement in the quality of life.

Background and Justification

Migraine is a common recurrent headache disorder with characteristics of unilateral location, pulsating quality, moderate or severe in intensity with associated symptoms of photophobia, phonophobia, nausea, vomiting, etc. The one-year prevalence for migraine was 14.7% (19.2% women and 6.6% men) in the United States and 8–13% in Asia. Because patients with migraine usually have frequent, severe, and disabling headache attacks, medical treatment is often required. Based on the recommendations of the European Federation of Neurological Societies guidelines on drug treatment, oral non-steroidal anti-inflammatory drugs (NSAIDs), and triptans are recommended for acute migraine attacks. However, patients may experience some side effects from pharmacological therapies, such as gastrointestinal and cardiovascular disorders. Moreover, headaches from overuse of medication and increased headache frequency may be caused by regular use of analgesics or specific anti-migraine treatments.

As a major component of traditional Chinese medicine (TCM), acupuncture has been used to treat headache in China for thousands of years. Over the past few decades, acupuncture has also been used widely as a treatment for migraine in western countries as well. Because of the growing use of acupuncture, the US Headache Consortium has suggested that acupuncture might play an important role in managing migraine without any side effects.

The evidence for acupuncture's effect on migraine has been questioned with regard to the quality and methodology of the acupuncture clinical trials performed in China, such as inappropriate group setting control, inadequate outcome measurements,

unclear classification between acute and preventive treatment, and the lack of detailed randomization and blinding information. In addition, researchers have argued that results from trials with different forms of intervention between experimental groups and control groups (such as acupuncture vs. medicine) cannot be used to compare and evaluate the efficacy of acupuncture.

Description

The acupuncture treatment consisted of three 30-minute sessions per week, administered over 4 weeks. The acupoints, including both obligatory and additional points, were selected based on the consensus of clinical experiences of acupuncture experts. The obligatory points included DU20 (Baihui), DU24 (Shenting), GB13 (Benshen), GB8 (Shuaigu), and GB20 (Fengchi). Additional points were chosen individually depending on the syndromes presenting: SJ5 (Waiguan) and GB34 (Yan-glingquan) for Shaoyang headache (TE-GB); LI4 (Hegu) and ST44 (Neiting) for Yangming headache (LI-ST); BL60 (Kunlun) and SI3 (Houxi) for Taiyang headache (SI-BL); LR3 (Taichong) and GB40 (Qixu) for Jueyin headache (PC-LR); PC6 (Neiguan) for nausea and vomiting; and LR3 (Taichong) for dysphoria and susceptibility to rage.

In light of our clinical experience and previous studies, for traditional acupuncture providing long lasting effects in decreasing both pain intensity and medication intake and in line with theories of TCM, it is important to choose acupoints on the basis of syndrome differentiation. In our trial, the acupoints were selected according to the national clinical guideline and late masters' experiences complying with the methodology of syndrome differentiation of meridians.

Acupoints were punctured perpendicularly with lifting, thrusting, and twirling for obtaining *DeQi*. The sensation of *DeQi* was defined as numbness, distension, soreness and heaviness around the point felt by patients. As exceptions, acupoints of DU20 (Baihui), DU24 (Shenting), GB13 (Benshen), and GB8 (Shuaigu) were punctured horizontally: the needle being inserted obliquely under the galea aponeurotica and then turned horizontally with twirling.

Results

The prophylactic effect with acupuncture being as good as flunarizine persisted from the end of the treatment (week 4) through the next three months, with acupuncture performing slightly better than flunarizine. The proportion of responders and migraine days of the acupuncture group was significantly improved. Additionally, the number

of patients that required acute medication in the acupuncture group was significantly reduced compared to the control group. However, no statistically significant differences were encountered in visual analogue scale (VAS) scores and the physical and mental components summary scores for SF-36 during the trial (Table 1).

During the follow-up period, several patients in the acupuncture group reported that the attacks seemed to be a prodrome of a migraine episode, such as tense paresthesias in the head, and lasted from several seconds to a few minutes. These attacks failed to develop into a typical migraine and required no medication. These symptoms perhaps demonstrate that acupuncture might reduce the chances of a migraine attack after a prodrome occurs.

Group	Time point	Acupuncture group n = 70		Control group n = 70		P [†]
		Mean ± SD	95% CI	Mean ± SD	95% CI	
Responder rate [‡] (ITT)	Week 4	41 (59%)	–	28 (40%)	–	.043
	Week 16	39 (56%)	–	26 (37%)	–	.042
Difference from baseline in days of migraine (ITT) [‡]	Week 4	4.1 ± 3.5	(3.2–4.9)	1.9 ± 2.3	(1.4–2.5)	<.001
	Week 16	4.1 ± 3.5	(3.3–5.0)	2.0 ± 2.7	(1.4–2.6)	<.001
Visual Analogue Scale (ITT) [‡]	Baseline	6.9 ± 1.7	(6.5–7.3)	6.7 ± 1.9	(6.2–7.1)	.143
	Week 4	4.3 ± 2.7	(3.7–5.0)	5.2 ± 2.0	(4.7–5.7)	
	Week 16	4.6 ± 2.6	(3.9–5.2)	5.4 ± 2.3	(4.9–6.0)	
	P [‡]	<.001		<.001		
SF-36 (ITT), Physical [§]	Baseline	49.7 ± 7.3	(48.0–51.5)	50.6 ± 6.4	(49.1–52.1)	.249
	Week 4	55.9 ± 7.7	(54.1–57.7)	53.6 ± 7.1	(51.9–55.3)	
	Week 16	57.0 ± 7.5	(55.2–58.8)	54.8 ± 7.2	(53.0–56.5)	
SF-36 (ITT), Mental [§]	Baseline	49.7 ± 9.0	(47.5–51.8)	51.0 ± 7.5	(49.2–52.8)	.213
	Week 4	56.0 ± 7.1	(54.3–57.7)	52.6 ± 7.4	(50.8–54.3)	
	Week 16	55.5 ± 6.9	(53.9–57.2)	53.6 ± 7.0	(52.0–55.3)	
Reduction in the number of people with acute medication, n (%) (ITT)	Week 4	20 (61%)	–	4 (14%)	–	.001
	Week 16	17 (52%)	–	5 (18%)	–	.014

CI, confidence interval; ITT, intention-to-treat; SF-36, 36-item short-form health survey.
 Significant difference, P < .05. Data presented as mean ± SD, number (percentage) and 95% CI.
[†] P for comparison with control group.
[‡] P values based on chi-square test.
[§] P values based on analysis of covariance analysis.
^{||} P values based on repeated measures.
[¶] P for comparison within each group.

Table 1: The primary and secondary outcomes of the measures undertaken.

Partnerships

This study was designed and carried out cooperatively by a group of experienced acupuncture experts, acupuncture practitioners, neurologists, methodologists, and statisticians from the following participating institutions: the Acupuncture Department of Beijing Traditional Chinese Medical Hospital, the Neurology Department and Pain Department of the Third Hospital of Peking University, the Neurology Department of Beijing Tiantan Hospital, the Acupuncture Department of Huguosi Hospital, and the Acupuncture Department of Dongzhimen Hospital.

Impact

This study is the first acupuncture clinical research paper in China included and recommended by the Faculty of 1000 (F1000). The acupuncture intervention applied in this trial was embodied in the new edition of TCM Clinical Guidelines for Headache.

Our department was the first one to systematically evaluate the effects of acupuncture on different categories of headache according to ICHD-3, published in a report in 2013. After this study, we conducted clinical research to demonstrate the effect of acupuncture on menstrual-related migraine and acute migraine attacks, with experimental studies explore the mechanism underlying its effect.

The success of this experience mainly relied on the knowledge of syndrome-meridians differentiation by the participating acupuncture experts, the manipulation techniques of the acupuncture practitioners and migraine-related neurological knowledge of the neurologists. The methodology of syndrome differentiation of meridians and the selection of acupoints played key roles in the beneficial effects of acupuncture.

Replicability

To apply this experience successfully, an acupuncturist with clinical experience of acupuncture of no less than 5 years and a basic knowledge of TCM's differentiation of syndromes and meridians together with headache-related neurology is necessary.

Lessons Learned

The clinical trials described here have added to the gradual accumulation of knowledge surrounding migraine and its understanding in terms of both traditional Chinese medicine theory and neurology.

Migraine has certain trigger factors, which are not often known by patients. A lack of migraine-related education means our patients cooperate poorly with the treatment, exposing themselves to various trigger factors in their daily lives. In addition, the population susceptible to migraine – largely young females – is less careful in regard to therapy regulation and trigger factor avoidance. These factors all represent major disadvantages in achieving a good clinical outcome.

Future Plans

The effect of acupuncture on acute migraine attack and menstrual-related migraine is due to be evaluated by further randomized clinical trials. In addition, basic research is currently being conducted on the mechanisms of acupuncture involved in migraine prophylaxis.

Potential collaborations with researchers from Germany, the UK and the USA are being developed with a view to conducting joint clinical trials. Further acupuncture training based on the present experience for European and American practitioners will be provided at that time.

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Section 3: Public Health

Traditional medical practices are widely used in many developing countries and are often more accessible and affordable than western medicine.

The following two case studies – from South Africa and Sri Lanka – describe outreach efforts that aim to use the advantages of both systems for improved public health outcomes.

12	Incorporating Traditional Health Practitioners into Community-based Healthcare in KwaZulu-Natal, South Africa	South Africa	110
13	Integrating Principles of Modern Epidemiology, Health Promotion and Public Health Practices to Establish a Health Unit System Based on Ayurveda Health Promotion	Sri Lanka	124

Incorporating Traditional Health Practitioners into Community-based Healthcare in KwaZulu-Natal, South Africa

South Africa



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Duration: 15 May 2013 to 31 July 2015

Total cost: USD 418,456 from US AID/University Research Co. LLC

Summary

The extensive networks of Traditional Health Practitioners (THPs) are potentially capable of expanding / simplifying the prevention, care, treatment and support access for tuberculosis and HIV. Using USAID TB Program funding, the Durban University of Technology conducted a project on the engagement of THPs in improving access to HIV/AIDS and tuberculosis (TB) services, building on previous collaboration between THPs and biomedical practitioners on sexually transmitted infections (STIs).

A pictorial HIV/TB Screening Tool and a Referral Form were developed for use by the THPs when referring their clients to health facilities. Operating in two municipalities of Amajuba District and four municipalities of Zululand, 800 THPs were trained in their wards and linked with their organizational bases (the nerve centres), clinics and community healthcare workers. Training included, referral, HIV and TB screening, infection control, and HIV testing.

From October 2013 to June 2015, 379 trained THPs consulted with 35,445 clients, screening them for HIV and TB. Of these, 2,047

were presumed infected with 1,194 who consented referred to local clinics for testing. Twenty percent (160) of the THPs voluntarily undertook HIV testing. More than 600,000 community members were reached using media, community dialogues and health talks. To promote TB infection control, 7,630 N95 (particle filtering) masks, were handed to THPs.

The KwaZulu-Natal Department of Health has incorporated the project's referral system, as launched by the KwaZulu-Natal Member of the Executive Council for Health, Sibongiseni Dhlomo, into its Referral Policy in Amajuba, while South Africa's Deputy Minister of Health, Mathume Joseph Phaahla, also noted the importance of this referral system.

We have demonstrated the potential role that can be played by THPs in enhancing community-based TB prevention, care and support programmes, which may now be scaled up. Collaboration between the Department of Health and THPs is essential to ensure access and continuity of care in TB and HIV/AIDS programmes.

Background and Justification

KwaZulu-Natal has an estimated 15,000 traditional health practitioners located in every district, local municipality and, perhaps, every ward in a similar manner to faith-based organisations. THPs form an important sector of the KwaZulu-Natal Provincial Council on AIDS (PCA) and all District AIDS Councils (DACs), Local Municipal AIDS Councils (LACs) and most Ward AIDS Councils (WACs). More than 3,000 of these have received basic to advanced training on HIV/AIDS and tuberculosis. However, without co-ordination and

empowerment at community level, this sector may not play an effectively meaningful role in the fight against HIV and AIDS, sexually-transmitted infections and tuberculosis. It is important that collaborative relationships between THPs, government, policy makers and donors are developed, nurtured and enhanced. Strong referral networks and alliances between public health centres, THPs, non-governmental organizations, and other volunteers in the community must be developed and strengthened (Abdool Karim *et al.*, 1994).

To achieve this, strong government involvement is necessary. However, the roles of all involved need to be clarified in such partnerships. In essence this calls for the creation of social capital at the community level as demonstrated by the African National Congress election manifesto of 2009 with the slogan “working together we can do more” (ANC, 2009). Social capital is defined by some as those specific processes between people and organisations, working collaboratively and in an atmosphere of mutual trust, that lead to the goals of mutual social benefit.

This project created such a social capital at community grassroots levels in KwaZulu-Natal that will lead to reduction in new HIV/AIDS cases, STIs and TB infections, together with a reduction in preventable deaths due to HIV/AIDS, STIs and TB, whilst ensuring a high quality of life for the infected and affected people of KwaZulu-Natal, through, among others:

- Developing an ongoing dialogue with community members regarding health issues;
- Creating or strengthening community organisations aimed at improving health;
- Assisting in creating an environment in which individuals can empower themselves to address their own and their community’s health needs;
- Promoting community members’ participation in ways that recognise diversity and equity, particularly those most affected by the health issues in question;
- Working in partnership with community members in all phases of a project to create locally appropriate responses to health needs.

The project strengthened the Operation *Sukuma Sakhe* model of service delivery launched by the Premier of the KwaZulu-Natal Government, Zweli Mkhize, designed to address, among other things, issues of community participation, integrated services delivery, and behavioural change. In this model, the ‘war room’ (the project’s organisational base or nerve centre) represents the basis for coordination at ward level. In each war room is a dedicated team including, but not limited to, community members, community care givers, youth ambassadors, extension officers, sports volunteers, social crime prevention volunteers, community development workers together with all government departmental

officials, and civil society organisations (KwaZulu-Natal, 2012).

The main issues involved were:

- To increase the capacity of THPs to support the management of HIV/AIDS, STI and TB;
- To optimise the participation of THPs in the Multi-Sectoral Provincial Strategic Plan for HIV and AIDS, STIs and TB, 2012-2016, for KwaZulu-Natal;
- To strengthen the implementation of the Provincial *Masisukume Sakhe* ('Lets build together') Programme by empowering THPs to function within the Ward AIDS Committees and war rooms;
- To strengthen relationships between public health and the traditional health care workers of KwaZulu-Natal through the development of a uniform referral system for adoption and use by the KwaZulu-Natal Department of Health, Municipal Health Units and THPs across the province.

Description

The project was rolled out through six activities:

Activity 1: To mobilise THPs and their target communities to be proactive in addressing issues of TB and HIV/AIDS prevention and management whilst at the same time highlighting the role of THPs and traditional healthcare clinics within the primary health care setting.

Activity 2: To position THPs as strategic stakeholders and advocates in the promotion of primary health care within their respective communities.

Activity 3: To improve the target audience community's positive behavioural health-seeking practices (i.e. improved attitudes, knowledge and beliefs surrounding TB/HIV and STI).

Activity 4: A strengthened referral system including the capacity of clinic nursing staff and THPs to work collaboratively in the management of HIV/AIDS, TB and STIs.

Activity 5: To strengthen the implementation of the Provincial *Sukuma Sakhe* Programme through empowering THPs to function effectively within the Ward AIDS Committees and war rooms.

Activity 6: To strengthen coordination, monitoring and evaluation, and reporting of project activities.

To achieve these goals, we developed a training manual consisting of the following modules:

Module 1: HIV/AIDS

Transmission
Treatment

Module 2: HIV/AIDS Prevention

HIV/AIDS Counselling and Testing
Abstain - Be faithful - Condomise
Cultural approaches of prevention
Medical Male Circumcision
Prevention of Mother-to-Child-Transmission

Module 3: Key populations

Module 4: TB (Based on National TB Guidelines)

Transmission
Screening
Infection control

Module 5: TB Treatment

Directly observed treatment, short-course
Adherence

Module 6: STI/opportunistic infections (OI)

Module 7: Monitoring, evaluation and reporting

Module 8: Palliative care

Training was facilitated by a team of conventional healthcare professionals and THPs.

The approach used was based on adult-learning principles of active learner participation. In addition, we believe that THPs are experts in traditional healthcare practices and hold particular views on issues discussed under different training modules and that they had valuable knowledge and insights to share and learn during the course.


In this regard facilitators:

- respected and valued diverse opinion;
- delivered and shared training content in the home language of the participants (IsiZulu);

- made an effort to translate most of written training materials and/or handouts for distribution into isiZulu; and
- ensured training staff played mostly a facilitative role in the active learning exercises.

Among the tools developed were a referral form (Fig. 1), patient record form (Fig. 2) and a reporting flow chart (Fig. 3).

**KWAZULU-NATAL
TRADITIONAL
HEALTH
PRACTITIONERS**



health
Department
of
Health
PROVINCE OF KWAZULU-NATAL


INCWADI YOKWEDLULISELA YABELAPHI BENDABUKO TRADITIONAL HEALTH PRACTITIONERS REFERRAL LETTER

**ISIGABA 1: IMININGWANE YESIGULI
SECTION 1: PATIENT DETAILS**


Igama Lesiguli
Name of Patient: _____

Ikheli Lesiguli
Address of Patient: _____


**ISIGABA 2: UDLULISELWA:
SECTION 2: REFERRED TO:**




Isibhedlela
Hospital




I-Kliniki/Isikhungo
sezempilo
Clinic/Health Facility



HCT
BY
COUNSELLING
& TESTING



Ezenhlalakahle
Social Services
and welfare



Izinhlangano zomphakathi
okungezona ezakHulumeni
NGO/CBO

**ISIGABA 3: ISIZATHU SOKUDLULISA
Isizathu somelaphi wendabuko
SECTION 3: REASON FOR REFERRAL
Traditional Health Practitioner's Reason**

<p><input type="checkbox"/> Ukusoka Male Medical Circumcision</p> <p><input type="checkbox"/> Ukukwehlela Coughing</p> <p><input type="checkbox"/> Ukuyimbela ukutholeleka Komuntwana ngegciwane Prevention of Mother to Child Transmission</p> <p><input type="checkbox"/> Isifuba esibuhlungu Chest Pain</p> <p><input type="checkbox"/> Ukujuluka obusuku Sweating at Night</p> <p><input type="checkbox"/> Ukukhishwa isisu Diarrhoea</p> <p><input type="checkbox"/> Inkinga yesikhumba Skin Problem</p> <p><input type="checkbox"/> Ukungakuthandi ukudla Loss of Appetite</p> <p><input type="checkbox"/> Ukuncipha emzimbeni Loss of Weight</p>	<p><input type="checkbox"/> Usokwe MMC Done</p> <p><input type="checkbox"/> Ukukholelwa isandulela-nguolazi HIV Test Done</p> <p><input type="checkbox"/> Ukuyimbela ukutholeleka komuntwana ngegciwane Prevention of Mother to Child Transmission</p> <p><input type="checkbox"/> Ukukholelwa izifo zocansi STI Test Done</p> <p><input type="checkbox"/> Ukukholelwa i-TB TB Test Done</p> <p><input type="checkbox"/> Ukukholelwa okanye nje (chaza ngezansi) Other Tests Done (indicate below)</p> <p><input type="checkbox"/> Imithi enikaziwe Treatment Given</p> <p><input type="checkbox"/> _____</p> <p><input type="checkbox"/> _____</p> <p><input type="checkbox"/> _____</p>
---	---

**ISIGABA 4: ISITEMBU SOSUKU
SECTION 4: DATE STAMPS**

THP Date Stamp

Clinic/Doctor Date Stamp

Figure 1: Referral form developed during the project.

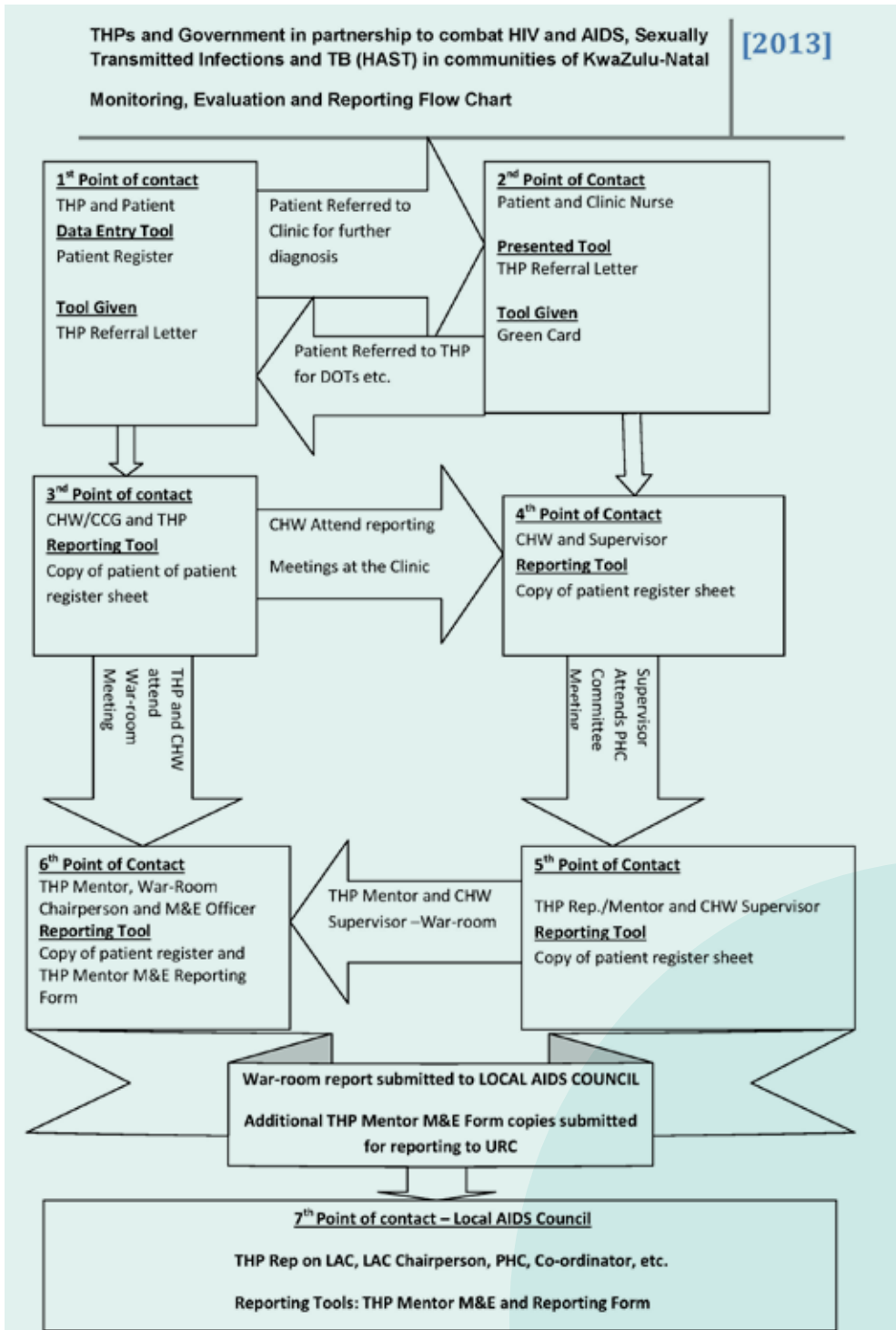


Figure 3: Reporting and information flow chart for monitoring and evaluation purposes.

Results

From October 2013 to June 2015, 800 THPs were trained. Of these, 379 (47%) consulted with 35,445 clients, screening them for TB and HIV/AIDS. Of these, 2,047 were presumptive of TB or HIV/AIDS while 1,194 who consented were referred to the local clinics for testing. Twenty percent (160) of the THPs also voluntarily undertook HIV testing (Fig. 4). As part of promoting infection control, 7,630 N95 (particle filtering) masks, were handed to THPs. The main reason behind 53% of THPs not reporting was due to their illiteracy (inability to read or write in isiZulu). This was not made a precondition for participating in this project. The project also presented its work at the 4th South African TB Conference (2014), and the 7th AIDS Conference (2015).

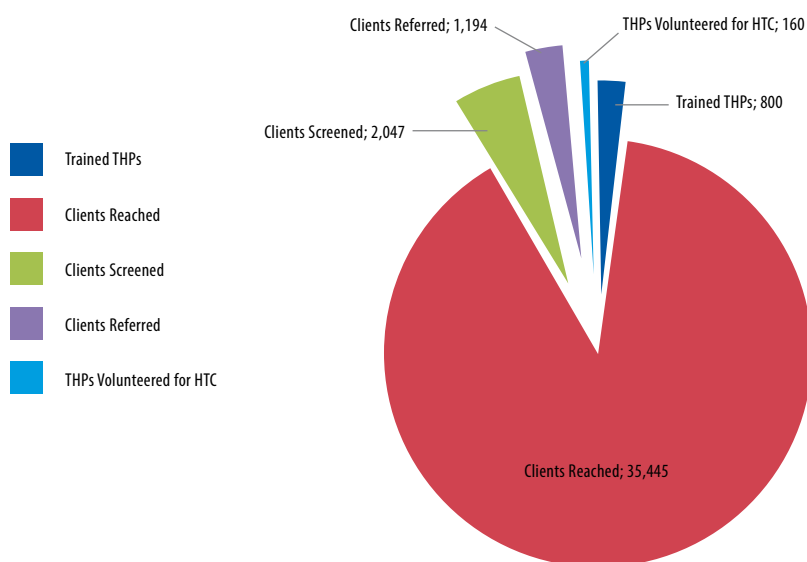


Figure 4: Results of traditional health practitioners (THPs) work with their clients.

The KwaZulu-Natal Department of Health has incorporated the project's referral system into its Referral Policy in Amajuba as launched by the KwaZulu-Natal Member of the Executive Council for Health, Sibongiseni Dhlomo (Fig. 5). South Africa's Deputy Minister of Health, Mathume Joseph Phaahla, opening a National Workshop of the Department of Health on THPs, also noted the importance of this referral system.

By means of newspapers, radio, community dialogues and health talks we were able to reach 622,088 people (Fig. 6).



Figure 5: KwaZulu-Natal Health Member of the Executive Council, Dr. Sibongiseni Dhlomo (seated second from right) with other dignitaries at the THP graduation and launch of the referral policy in Newcastle, South Africa, 8 July 2014.

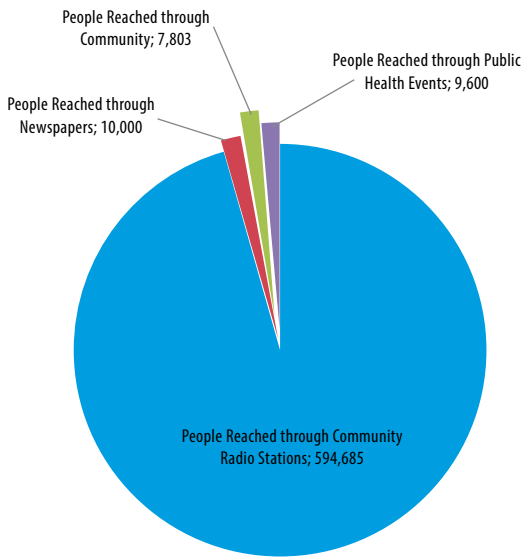


Figure 6: Indication of the reach of the advocacy communication and social mobilisation aspect of the project.

Partnerships

We have formed partnerships with the Hospice Palliative Care Association of South Africa (HPCA) and the CaSIPO (Care and Support to Improve Patient Outcomes) project to support and strengthen the integration of care and support services within the broader health system, and to strengthen community systems and organisations to ensure the provision of a continuum of comprehensive care and support services (palliative care). Public/community/stakeholders and private sector involvements and weaknesses between partners were strengthened by conducting regular (every six months) project review meetings.

Impact

Tables 1 and 2 give an indication of the state of affairs in specific areas related to this project in Amajuba Health District. Though it could not be said to be a direct proof of the impact of the project.

The data indicate that during the period the project was implemented there was improvement in testing, disease detection and condom provision rates.

Indicator	Jan-Mar 2014	Oct-Dec 2014	% Change
No of individuals tested for HIV	33,598	35,761	+6
No of individuals with TB detected	395	499	+26
Male condom distribution			
• Health facilities	668,147 (51%)	593 102 (28%)	-11
• Non health	663,000 (49%)	1 545 000 (72%)	+43

Table 1: Report from Amajuba Health District during the period covered by the project.

Indicator	Apr-June 2014	Apr-Jun 2015	% Change
HIV testing	49 896	43 080	-11
TB detection	453	363	-12
Male condom distribution (unspecified)	2,025,808	4,394,928	46

Table 2: Report from Zululand Health District during the period covered by the project.

Our poster was nominated for 'best poster' in the 'best practice' category of the South African AIDS Conference in June 2015.

Sustainability

During our end-of-project review meeting it was agreed that all stakeholders (Department of Health, THPs and Durban University of Technology) will work together in putting together a project sustainability resource mobilization plan.

The fact that THPs are willing to support communities in improving their health even if it means making referrals to the public health system and that the KwaZulu-Natal Department of Health has dedicated staff to co-ordinate activities involving collaboration with THPs made it easier to implement this project. THPs are now integral members of Ward, Municipal, District and Provincial AIDS Council members. They are also members of clinic committees and hospital boards.

Replicability

South Africa has adopted a Policy of Primary Health Care Re-engineering which is strengthened by the implementation of this project. The policy has two basic principles:

- It should be sustained by integrated, functional and mutually supportive referral systems, leading to the progressive improvement of comprehensive healthcare for all, and giving priority to those most in need;
- It relies, at local and referral levels, on health workers, including physicians, nurses, midwives, auxiliaries and community workers as applicable, as well as traditional health practitioners as needed, suitably socially- and technically-trained to work as a health team and to respond to the expressed health needs of the community.

Three other provinces (Eastern Cape, Limpopo and Mpumalanga) in South Africa have adopted and adapted the referral system we have developed and put it into use. This is about strengthening the primary healthcare system and improving access.

This work is of relevance to those countries and regions where THPs have no formal links with the public health system. In Malawi, an earlier version of this work was put into use with positive results.

Lessons Learned

Since they began referring patients to these facilities under this project, there has been a notable challenge with regard to THP-referred patients' outcomes, i.e. getting to know exactly what happened to patients referred by THPs to either hospital or clinic, there having been very little feedback. As a result, we have agreed with the Department of Health's District Coordinators to have a common mechanism to collect this data from the clinics and a tool has been designed to obtain this particular data requirement (Fig. 7)

During the end-of-project review meeting it was agreed that THP leaders and District Health Coordinators should work together in ensuring that the referral system works and report areas of malfunction in order to work out strategies for timely troubleshooting.

The tool for ongoing referral record-keeping in clinics has been revised and which Department of Health Coordinators now need to circulate it to all participating clinics for the collection of monthly statistics.

1	How many patients were referred by THPs to your facility for symptoms related to :	No.
1.1	TB	
1.2	HIV / AIDS	
1.3	STI	
1.4	MMC (Medical male circumcisions)	
1.5	PMTCT (Prevention of mother-to-child transmission)	
1.6	Other (specify)	
2	How many clients received positive results on:	No.
2.1	TB	
2.2	HIV	
3	How many clients were referred back to THPs for :	No.
3.1	DOT support	
3.2	Treatment support / adherence	
4	How many condoms were distributed to THPs for :	No.
4.1	Males	
4.2	Females	

Figure 7: THP patient referral outcomes form – developed to improve tracing of patient outcomes.

The success of the project was also assured by using the power of media (community radio stations) to mobilize communities to access HIV/AIDS, STI and TB (HAST) services. Social mobilisation for HAST was aimed at providing the community with adequate information and support about all aspects of these services and, in particular, the role of THPs with, above all, the creation of a space for ongoing dialogue with community members regarding health issues.

Future Plans

This project was conceived to address issues relating to improving HAST services through the involvement of THPs. It is possible to include other medical conditions. There are also plans for collaboration and sharing of the results with other organizations/countries.

Additional contributors

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Integrating Principles of Modern Epidemiology, Health Promotion and Public Health Practices to Establish a Health Unit System based on Ayurveda Health Promotion

Sri Lanka

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Duration: 2013 to present

Total cost: USD 50,000

Summary

Complimentary and alternative medicine (CAM), long an integral part of Sri Lanka's health system, includes a range of different practices including Ayurveda, Siddha, Unani and Yakka vedakama, this latter preserved among aboriginal peoples and is a traditional/indigenous medicine unique to Sri Lanka.

In 2001, an Ayurveda Community Health Promotion Service (ACHPS) was established by the Ministry of Indigenous Medicine in Anuradhapura district targeting health promotion and community health activities based on the health unit system of the conventional medicine system in Sri Lanka, where the country is divided into more than 300 health units known as Medical Officer of Health (MOH) divisions.

A collaborative programme was started between the Anuradhapura district ACHPS and the Centre for Education and Research into Complementary and Alternative Medicine (CERCAM) of the Faculty of Medicine and Allied Sciences, Rajarata University of Sri Lanka. The activity involved an integrated training programme for all Ayurveda health

promotion officers to train them in Ayurveda health promotion concepts and to use modern epidemiological principles, clinical research, health programme planning and bases for public health practice to facilitate the implementation process. The idea was to specifically identify the principals and proven concepts in Ayurveda for community-level health promotion activities, which was done by experts in Ayurveda, and then use epidemiological and public health practice principles for programme implementation, monitoring and evaluation.

This programme, which was launched in 2013 and saw 55 Ayurveda health promotion officers trained, was subject to quantitative and qualitative evaluation after one year.

The training sessions were shown to be very effective as the mindset of all those involved, as well as the programme as a whole, changed from being one that duplicated the health promotion activities of the conventional healthcare system to that of a programme initiating new approaches in community health.

Background and Justification

Ayurveda is a system of medicine that has a rich knowledge base for health promotion. It has a high cultural acceptability and could play a significant role in health promotion and disease prevention in countries such as Sri Lanka.

Being accepted and regulated by the Government of Sri Lanka and run as a parallel system to conventional medicine, its effective implementation, while integrating with modern practices of health promotion, could deliver good results to the community.

The Centre for Education and Research in Complementary and Alternative Medicine (CERCAM) is a centre in the Faculty of Medicine and Allied Sciences of Sri Lanka's Rajarata University, established with the purpose of looking into other systems of medicine with an open mind and carrying out research and educational activities in complementary and alternative medicines.

This is the first and only such centre established in a faculty of medicine in Sri Lanka although traditional, complementary and alternative medicines are in wide use even today alongside conventional medicine. The centre has expertise in conventional medicine as well as that of Ayurveda and indigenous medicine. Understanding the value of such integration the CERCAM entered into a three-year collaboration with the Anuradhapura district Ayurveda Community Health Promotion Service (ACHPS). The ACHPS is under the direct supervision of the Ministry of Indigenous Medicine. The Anuradhapura district ACHPS was launched in 2001 as a pilot project by the Ministry of Indigenous Medicine, with the intention of creating a similar unit system to that practiced in the conventional medicine sector under the Ministry of Health.

Description

Through the collaboration between CERCAM and ACHPS, several programmes were planned to apply Ayurvedic and indigenous medicine in health promotion, while preserving their principles and holistic approach, which differ from the usual reductionist approach of conventional medicine. The intention of the pilot project was to explore the effectiveness of an Ayurveda community health promotion service in relation to the promotion of health at a community level so that, if successful, it could be implemented throughout the country.

The interventions were combined with time-tested epidemiological and public health tools for effective implementation. In order to do this, it was paramount to educate and train a number of Health Promotion Officers (HPOs) working for the ACHPS together with doctors.

The ACHPS is headed by a director and has 22 Community Medical Officers (CMOs), each being appointed to a division of the district. The director and the CMOs are Ayurvedic doctors who have graduated from university-affiliated Ayurveda and indigenous medicine institutes and who are registered with the Ayurveda medical council.

In addition, the ACHPS has 210 HPOs who function as field officers. There are several HPOs for each division working under the supervision of a Community Medical Officer (CMO).

The HPOs' educational qualifications include the passing of the Advanced National Level examination, an exam held at the end of school education.

The absence of formal training for HPOs was a strongly-sensed deficit within the ACHPS and we took the opportunity to educate them both on the principles of health promotion in Ayurveda and indigenous medicine as well as modern epidemiological principals, clinical research, health programme planning and public health practice principles, designing a certificate course through Rajarata University that the officers could add to their educational and professional training qualifications. Training of doctors was done through workshops and designing and carrying out different activities together and by planning research within the programme.

The training of CMOs on modern principles was also carried out.



Figure 1: A group of Health Promotion Officers (HPOs) during one of their training programmes.

Fifty-four HPOs completed their training (Fig. 1) and were awarded their certificates by Rajarata University. They are now working within the ACHPS and the feedback from CMOs is very encouraging. Another batch of 50 HPOs is currently undergoing training.

Though there were many expectations at the start of the project, a lack of understanding on organizing and structuring such community-based health programmes together with the vacuum of knowledge in relation to some epidemiological and public health practice principles for programme implementation, monitoring and evaluation made the project less convincing. Nearly 12 years after its inception it remains confined to the

Anuradhapura district and its true effectiveness remains to be validated. Thus, despite its strengths, weaknesses in the delivery system have undermined the programme's role as an effective system that could be utilized to promote the health of the community.

Partnerships

The project was undertaken in a partnership with the Ministry of Indigenous Medicine, the Government of Sri Lanka and other leading indigenous medicine institutions. In addition, volunteers in the geriatric care programme with non-governmental organizations such as the Lions' Clubs assisted in the geriatric healthcare programme.

Impact

It was shown that a properly and carefully thought-out professional association among medical professionals belonging to different domains could deliver mutually-beneficial results while improving the state's healthcare delivery. Currently 56 trained HPOs are working in the community.

In addition, 21 CMOs are better trained in the use of modern epidemiological principles, health programme planning and public health practice principles in their routine work. They are also trained in the education of community health volunteers.

The training of HPOs by officially getting them enrolled to follow a certificate course for community health in complementary and alternative medicine had many benefits apart from providing the necessary training. The academics that participated in training became aware of how such integration could be brought about without violating the principles of each domain and this became a novel experience for them.

It was also noted that professionals from conventional medicine started learning the principles involved in Ayurveda and were finding it interesting to explore it further. This led to more professional associations in relation to research and exploring treatment modalities for different ailments, some of which took place outside the programme.

The enthusiasm shown by the university in arranging this course showed the expectations of lay people in seeing the integration of medical systems in countries like Sri Lanka where traditional medicine still commands a considerable degree of affection and cultural acceptability.

Furthermore, this interaction was proven to be of great use when a breast cancer screening programme and an influenza outbreak response was commissioned in Anuradhapura

district with the help of the CMOs and HPOs. Though this was not among their usual duties their interaction with specialists in conventional community medicine made it feasible to use this readily available resource for preventive healthcare.

This collaboration, and the training provided during it, helped the doctors and director realise that it is important not to duplicate the work done by the Ministry of Health system and the similar structure of the conventional healthcare system which was more organized and well-established. This led the ACHPS to explore other, uncovered areas of health promotion where Ayurveda could play an important role and to the initiation of an integrated community-based geriatric healthcare programme within the collaboration programme. A pilot project has already been conducted and is proving to be effective.

Within this community-based geriatric care programme, volunteers from the community were trained on subjects such as how to prevent falls in the elderly, on what changes in the home environment need to be made in order to reduce falls and how to deliver home remedies such as the application of herbal oils and fomenting with herbal packs for aches and pains that are common in elderly people.

Sustainability

By entering into an official collaboration with ACHPS, we were able to build trust and mutual understanding among professionals of the mainstream and Ayurvedic/indigenous medicine sectors. This gave an assurance to the sustainability of the project such that it would not be affected by misunderstandings and incompatibilities of the two systems involved.

All the trainees were permanent government employees and the trained ones are already employed, helping to make the programme sustainable.

Replicability

Ayurveda CMOs are available in all districts of Sri Lanka. Therefore, based on the outcome assessment of this programme, the Ayurveda health promotion service can be implemented all over the country. Through this project patient referral among professionals in the two sectors has become acceptable to a certain extent. This can be improved at a national level. However, any country with a history of complementary and alternative medicines can follow a similar system as it is simple but effective.

Lessons Learned

Ayurveda and the indigenous system of medicine in Sri Lanka can be considered to be rich in health promotion and preventive aspects. Cultural acceptability is an added advantage that Ayurveda and indigenous medicine has in approaching the community for purposes of health promotion.

Though Ayurveda, Siddha, Unani and Sri Lankan indigenous medicine are regulated and accepted by the government, there are very minimal interactions between conventional medical professionals and providers of other forms of care. There is also hardly any coordination between the conventional healthcare system with the healthcare provided by the institutions that come under the Ministry of Indigenous Medicine.

This is further complicated by a clause of the Sri Lanka Medical Council that discourages its members from professionally associating with Ayurveda and other medical practitioners. In addition, conventional medicine professionals have a minimal knowledge of other systems of medicine and their principles. All of the above as well as some bad historical experiences in building the trust and confidence to work together have been among the main issues involved in such activities. Difficulty in understanding and appreciating different domains of medical practice is the other main issue in such interventions. In addition, the perceived fear of invasion or exploitation of indigenous medical resources by professionals and researchers in conventional medicine is another barrier that must be overcome in such collaborations.

Having CERCAM established in the Faculty of Medicine and Allied Sciences at Rajarata University of Sri Lanka was the main reason for being able to carry out this innovative programme. The mindset of the faculty to collaborate with the Ayurvedic and indigenous medicine sector while understanding the possible challenges they could face from their professional bodies added to the success of the project, as did having academics with expertise in both conventional and Ayurvedic medicine within the faculty and the freedom that was available within the faculty to take up challenges in order to improve healthcare delivery.

Having expertise in both conventional medicine and Ayurveda within CERCAM also gave the assurance that the collaborative work would not compromise the application of Ayurvedic principles at the design and implementation stages. Constant dialogue and respect for each other's principles and practices helped build the trust among the professionals belonging to the two sectors.

Through regular discussions and workshops we overcame the lack of understanding of modern epidemiological principles, clinical research, health programme planning and

public health practice principles among community medical officers. This also helped us realise that adult education was not a difficult task when this had a direct positive effect on their work.

However, dependence on total government funding created limitations. Some training programmes for doctors and HPOs had to be abandoned due to lack of funding and the geriatric care programme met with some limitations due to inadequate resources. To overcome this, locally available resources were used, for example consultancies by the faculty staff were offered free of charge. Public participation and acceptance was greater than usual in this project.

Thus, though integration of knowledge of different medical systems could deliver better results, it needs to be done carefully while respecting the fundamentals of each system. Integration in such situations also requires careful appreciation of each system's principles and being non-judgemental about their approaches to healthcare. Such an approach to integration requires expertise in both systems together with proper attitudes and skills. Lack of coordination and professional association among Ayurvedic and conventional medicine professionals has made it very difficult to forge an alliance for such an integration which could deliver many solid health outcomes to the community.

Future Plans

Plans for further expansion of the programme include:

- Completing the current training programme and upgrading the programme to diploma level;
- Follow-up monitoring and evaluation of the implementation of principles of epidemiology and public health in the Ayurveda community health practice;
- Training of Ayurveda doctors in public health and health promotion by offering an MSc. designed by the university (no such opportunity is currently available for Ayurveda doctors); and
- Implementing the integrated community-based geriatric care programme throughout all the districts in the area.

We have already shared the experiences with doctors of conventional medicine through discussions and presentations.

Section 4: Pharmacology

Modern medicine, especially the discovery of new lead compounds, relies heavily on a bank of pharmacological studies. Such studies are critical for determining modes of action of drugs, possible side-effects, and potential cross reactions with other compounds.

The following 11 case studies – from ten countries in Africa, Asia and Europe – examine the details and effects of studies on animal models such as *Drosophila* flies and rats, as well as on human subjects.

14	Management of Convulsion and Migraine by Inhalation Therapy	Madagascar	134
15	Ayurvedic <i>Amalaki Rasayana</i> and <i>Rasa-Sindoor</i> Suppress Neurodegeneration in Fly Models of Huntington's and Alzheimer's Diseases	India	147
16	A Naturally-occurring Antioxidant and Anti-inflammatory Bioflavonoid from the Seed of <i>Garcinia kola</i>	Nigeria	158
17	Uncovering potential of Indonesian Medicinal Plants for Dual Actions of Anti-diabetes and Anti-obesity	Japan/ Indonesia	167
18	From the Traditional Recipe to the Modern Remedy: The antitumor activity of the small burr parsley (<i>Caucalis platycarpos</i>)	Croatia	174
19	Antiplasmodial Activities of Fractions and Natural Compounds from <i>Icacina senegalensis</i> (Icacinaceae)	Senegal	184
20	Network Target: A novel approach to decipher traditional Chinese medicine	China	195
21	Zedupex, an Anti-herpes Herbal Medicine for Management of Human Herpes	Kenya	206
22	Exploring Medicinal Plants for Better Health: Antiplasmodial properties of <i>Clerodendrum myricoides</i> and <i>Dodonaea angustifolia</i>	Ethiopia	218
23	Exploring Cameroonian Medicinal Plants for a New Generation of Anti-malarial Compounds	Cameroon	227
24	Protective Effect from Ulcerative Colitis to Colitis-associated Cancer in Experimental Models with Huangqin Decoction	China	238

Management of Convulsion and Migraine by Inhalation Therapy

Madagascar

- **Philippe Rasoanaivo, Emmanuel Randrianarivo,
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Summary

The smoke of dry leaves of *Myrothamnus moschatus* is commonly used to treat convulsions and migraines in Madagascar. We have subjected this culturally-accepted empirical treatment to an examination of its scientific value and therapeutic applications. To this end, assuming that the active compounds are volatile constituents, we extracted the essential oil by steam distillation, determining its chemical composition using chromatography. We then evaluated its anticonvulsive activity, observing and quantifying the reduction and inhibition of seizures provoked by convulsing agents in rats. Administered subcutaneously at 0.4ml/kg, the essential oil completely inhibited pentylenetetrazole-induced convulsions, with neither toxicity nor sedative effects. Thereafter, we comparatively analyzed the chemical composition of the smoke and the dry leaves using a solid phase micro-extraction technique. The striking difference was the presence of significant quantities of limonene in the smoke although present in only small amounts in dry leaves and essential oil. *Citrus* species are good sources of limonene, which has

been reported to display anticonvulsant and neuroprotective effects, prompting us to evaluate the neuroregenerative effect of the essential oil of *M. moschatus* on an SHSY5Y neuroblastoma cell line culture. At higher concentrations, clear neuronal cell growth was observed. After basic toxicity and neurotoxicity evaluations, we successfully carried out clinical observational studies on a pharmaceutical composition including *M. moschatus* and *Citrus* sp. essential oil blends, with 31 convulsive patients and 38 migrainous patients being enrolled in the study. We mainly targeted patients unable to afford conventional drugs, and those refractory to, or complaining about toxicity of existing medications. Additionally three unexpected emergency convulsion cases and one very severe migraine case were treated with positive outcomes. Administration was by inhalation. Three therapeutic failures in convulsions and two in migraines were recorded. We manufactured two patented phytomedicines respectively under the tradename Fanalarofy® for convulsions and Fanalanendo® for migraines.

Background and Justification

Epilepsy, convulsive seizures and headaches are the most common chronic neurologic disorders affecting respectively 5 to 10 out of every 1,000 persons worldwide for epilepsy and 15 to 30 out of every 1,000 persons for migraines, with a heavy socioeconomic impact in terms of both lost productivity and burden to healthcare systems. Nearly 80% of people with these diseases reside in developing countries, where it remains a major

public health problem, not only because of the health implications but also for the social, cultural, psychological and economic consequences. The prevalence of epilepsy in sub-Saharan Africa seems to be higher than in other parts of the world, with 10 million people directly affected according to the World Health Organization (WHO) estimates. Patients are of all ages, but especially within childhood, adolescence and the ageing population. In the Central Highlands of Madagascar, an epidemiological study on epilepsy showed that the prevalence of the disease is estimated as 27 per 1,000 persons. In addition to environmental and genetic factors, the effects of central nervous system infections are the main cause of seizures and acquired epilepsy in Sub Saharan Africa. However, little public health attention has been paid to the neurological burdens associated with infectious diseases. Regarding migrainous headaches, this disease has been and continues to be underestimated in scope and scale, and headache disorders remain under-recognized and under-treated everywhere. Africa has an estimated 56 million people sufferers. In Madagascar, the prevalence of migraine was reported to be 19% with specific rates of 26.8% for women and 9.4% for men. By virtue of it mainly afflicting people of working age (22-55 years old), migraine not only causes high medical disability but also great socioeconomic consequences. One serious problem in low-income countries is the poor availability and high cost of medication. The epilepsy and convulsive seizures treatment gap are defined as the proportion of people who require but are not receiving treatments. With an average gap of approximately 75% for low-income countries and the poorest in Africa having a gap of more than 90%, the situation is quite alarming. In Madagascar, the treatment gap was estimated as 92%. However, given the sociological, economic and sanitary conditions of the country, the true treatment gap may be even higher.

Epilepsy is traditionally believed to be caused by a supernatural power, be it a god or a demon or an ancestral spirit, because of transgression of taboos or punishment for sins, or attributed to possession by evil spirits. It is also thought to be due to witchcraft, or poisoning, and often taken to be contagious. A fear of contagion results in isolation of the convulsive patients and unwillingness to intervene in preventing injury. Profound psychological and physical disability may thus occur. Headache is believed to result from excess blood in the brain. And to remove the excess blood, the healer hits gently the head until blood comes out through the nose. In Madagascar, and probably elsewhere Sub-Saharan Africa, traditional treatments for epilepsy are mainly prayers and exorcisms. The danger of such practices has been raised, but it is difficult to counteract deeply-rooted traditional beliefs. With the active participation of local populations, we learned from traditional healers that the smoke of *Myrothamnus moschatus* (Baillon) (family *Myrothamnaceae* as 'resurrection plant') is used to treat epilepsy and migraines. We postulated that volatile compounds might represent the active constituents of the plant.

A review paper reported the anticonvulsant effects of essential oils extracted from 30 plant species and 30 isolated constituents. Little has been done to bring this knowledge into drug/phytomedicine development and therapeutic use. As far as migraines are concerned, to the best of our knowledge, no review paper with scientific validation has been published on the use of essential oils for the management of migraines and headaches. However, many anecdotal reports have been found in current text, journal articles, Masters or PhD dissertations, and internet resources have been utilized as important sources of information about the successful use of essential oils in headache management. We therefore decided to investigate *Myrothamnus moschatus* with the aim of translating this culturally-accepted empirical treatment into a validated scientific finding with therapeutic applications.

Description

To translate the traditional recipe into therapeutic applications, we first set out to learn the learning ethnomedical uses and particular botanical features of *M. moschatus* in our bioprospecting programme. Dried leaves are smoked like a cigar and the smoke is gathered in a rice bag. The patient then inhales the fumes to expel bad spirits entering the body of a convulsive patient. We also learned that the dried leaves are used similarly for the treatment of migraines with nose bleeds. The Myrothamnaceae family comprises one genus, *Myrothamnus*, and two shrub-like species, *Myrothamnus flabellifolius* indigenous to Southern Africa and *Myrothamnus moschatus* endemic to Madagascar.



Figure 1: *Myrothamnus moschatus* in dry (left) and rainy (right) seasons.

We assumed that the bioactive substances would be volatile in nature. Consequently, the essential oil was extracted by steam distillation and its chemical composition determined by chromatographic techniques.

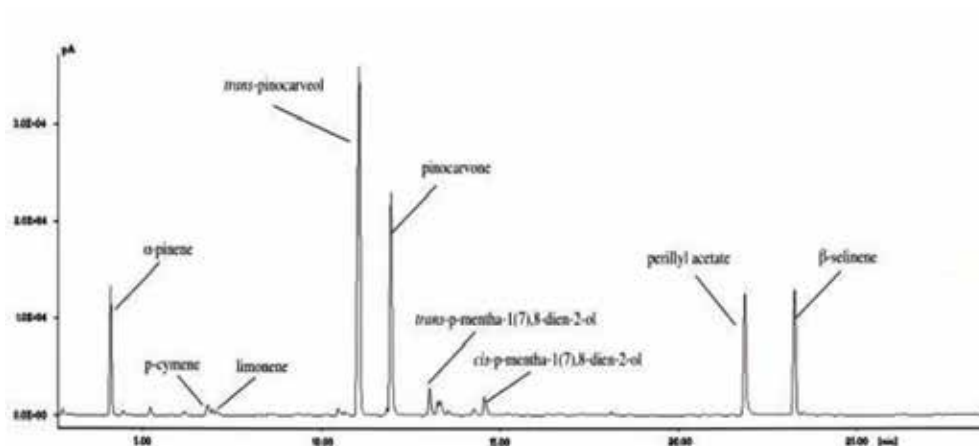


Figure 2: Chromatogram profile of the essential oils of *Myrothamnus moschatus*. Note the relatively low proportion of limonene present.

The chromatogram profile is shown in Fig. 2.

We then tested the anticonvulsant effects of the crude essential oil in rats against seizures induced by the chemo-convulsing agent pentylenetetrazole. We used subcutaneous injection using decreasing dosages of the oil (0.8 ml/kg, 0.4 ml/kg, 0.2 ml/kg). Diazepam at the dose of 1 mg/kg was used in intra-peritoneal injection as a positive control. Animals that did not convulse within 60 min were considered as protected. In unprotected animals, the latency to first convulsion and the durations of convulsions were recorded. The animals were observed for mortality for 24 hours after administration of pentylenetetrazole.

We observed a clear dose-response effect. At 0.8 ml/kg and 0.4 ml/kg, the convulsive effects induced by pentylenetetrazole were completely inhibited, without any toxicity or sedative effect. At lower doses, the essential oil demonstrated a significant increase in seizure latency and a significant reduction in seizure duration compared with the control group. No deaths were recorded in treated animals.

At this stage, we had to decide whether to proceed to the elucidation of the detailed mechanism(s) of action and the isolation of the active principle(s), or explore what else the traditional recipe might bring. To this end, we gained important points from a critical review (Loscher, 2011) that confirmed:

- the efficacy of new antiepileptic drugs (AEDs) is at best similar to that of older AEDs;
- the major goal should be more effective treatments for the AED-resistant epilepsy patients;
- new AEDs have clinically important usefulness for disorders other than epilepsy; and
- that current strategies of preclinical AED development need to be radically overhauled.

We therefore oriented our studies towards the analysis of the smoke of *Myrothamnus moschatus*.

We performed the analysis of the chemical composition of dry leaves and active smokes of *M. moschatus* using the solid phase micro-extraction (SPME) technique. The most striking difference between the chemical composition of smokes, dry leaves and extracted essential oil was the unexpected significant presence of limonene in the smoke. The comparative percentage of the main constituents from essential oils, dry leaves and active smoke is summarized in Table 1.

It was reported that limonene displayed anticonvulsant and neuroprotective effects. This prompted us to evaluate the neuroregeneration activity of the essential oil of *M. moschatus*.

Component	Essential oil (dry season)	Essential oil (rainy season)	Dry leaves at 37°C	Smoke
α -Pinene	5.8	10.5	1.3	4.8
p-Cymene	0.9	1.6	-	4.6
Limonene	0.4	2.3	0.5	6.6
trans-Pinocarveol	36.3	35.6	35.5	12
Pinocarpone	19.8	20.0	30.5	2
trans-p-Mentha-1(7),8-dien-2-ol	2.8	3.3	5.9	15.1
cis-p-Mentha-1(7),8-dien-2-ol	2.3	2.1	-	-
Perillyl acetate	12.7	6.0	2.6	0.6
β -Selinene	8.5	8.5	5.2	1.3

Table 1: Comparative percentage of the main constituents from essential oils, dry leaves and active smokes of *Myrothamnus moschatus*.

Smell reception occurs in the brain's limbic system, particularly the temporal lobe that is the site of temporal lobe epilepsy. Essential oils stimulate smell receptors that send chemical messages to the limbic system. The result is the rapid onset of action at very low dosages. Accordingly, the potential therapeutic applications of the essential oil by inhalation therapy may be:

- Prevention of oncoming convulsion or migraine as a simple self-help technique; or
- First management of emergency cases of convulsion.

Inhalation therapy could offer benefits such as less invasiveness, site-specificity, ease and speed to reach the brain, avoidance of the first hepatic passage, less systemic exposure and adverse effects.

We then proceeded to evaluate the neuroregeneration effect of the essential oil of *M. moschatus* by assessing the effect of the essential oil on the neurite outgrowth of neuronal culture of SHSY5Y neuroblastoma cell line (Fig. 3).

Neuronal regeneration was observed at different concentrations of the essential oil of *M. moschatus*. Untreated control cells clearly have a polygonal shape, with no or very few cell protrusions (neurites). By contrast, cells treated with the extract show a typical neuronal morphology with an increased number of protrusions. In fact, cells developed a huge number of neurites that connect cells to one and other, thus forming a neuronal network. This effect was dose-dependent and became most prominent with higher concentrations (1:40,000 and 1:80,000).

Based on this result, the potential therapeutic application of the essential oil may be the adjunct therapy to existing drugs for the chronic treatment of convulsion and migraine by neuro-protective effects.

Optimizing the anticonvulsant activity

The unexpected presence of limonene in the active smokes prompted us to explore the possibility of combining essential oil of *M. moschatus* with essential oils rich in limonene

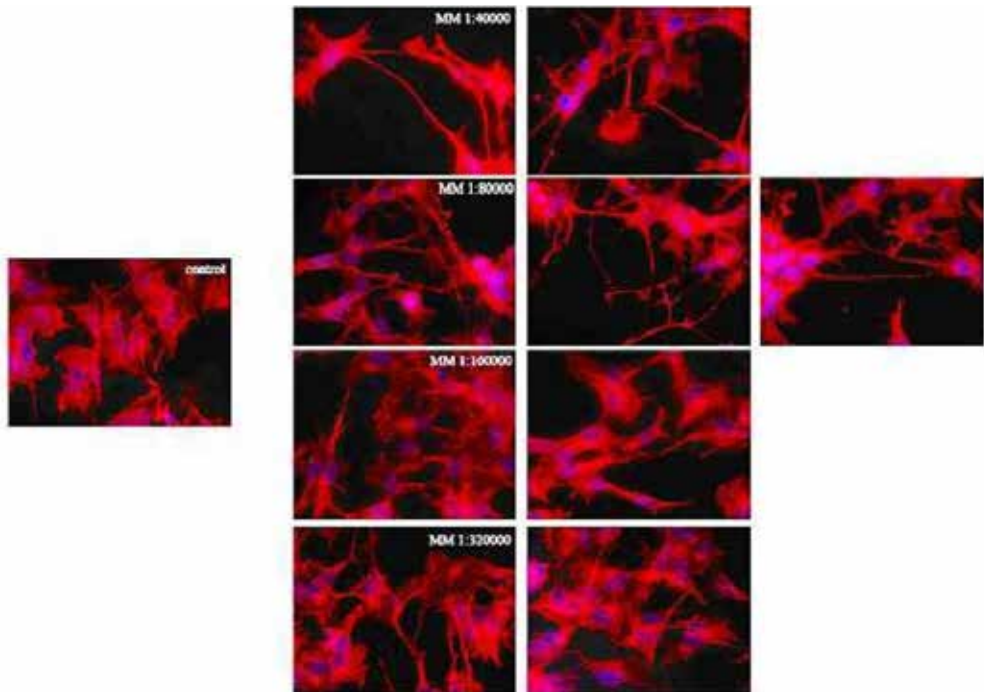


Figure 3: Neuronal regeneration observed at different concentrations of the essential oil of *M. moschatus*.

with the aim of optimizing the biological activity. *Citrus* species are good sources of limonene. Consequently, we extracted essential oils from different *Citrus* species and found that a wild species widely available had the highest amount in limonene (92% yield). Thereafter, we blended the two essential oils in different ratios and tested each combination in pentylenetetrazole-induced convulsions in rats. We found that the 50/50 blend had the highest comparative anticonvulsive effects.

Evaluating the natural abundance of *M. moschatus*

Prior to commercializing a new therapeutic agent, it is important to ensure a sufficient supply of the ingredients. Fig. 4 shows the geographical distribution of *M. moschatus*, and indicates that this species is found growing wild in different ecological regions of Madagascar. We therefore investigated the presence of chemical variability of the volatile composition of the species depending on phytogeographic origin of the samples (represented in red circles in the map), along with the traditional uses. Five distinct chemotypes were identified. Essential oil composition of *M. moschatus* is therefore very susceptible to ecological factors, with significant qualitative and quantitative variations. Interestingly, the variation of the chemical composition is followed by variations in the ethnobotanical uses of the samples.



Figure 4: (A): Map of Madagascar showing geographical distribution of *Myrothamnus moschatus* and locations where samples were collected (red circles). (source: MBG Vahinala project). (B) Collecting in dry season. (C) Collecting in rainy season.

Performing clinical observational studies

The diversity of anticonvulsant targets has been exploited for their utility in disorders other than epilepsy (Moch, 2010). This is called polypharmacology and drug repositioning. Interestingly, smoke of *M. moschatus* is also traditionally used to attenuate and arrest migraine. This prompted us to include migraine in our clinical observational studies. After general toxicity and neurotoxicity evaluations, we carried out clinical observational studies for patients suffering from convulsions and/or migraines with a pharmaceutical composition comprising essential oils of *M. moschatus* and *Citrus*. Each patient confirmed their informed consent.

We targeted two types of patients: patients unable to afford conventional drugs (essential oils as primary treatment), and those refractory to, or complaining about the toxicity of existing medications (essential oils as adjunctive treatment). Overall, 31 patients suffering from convulsions and 38 patients with migraines were enrolled in the trials. Additionally, unexpected emergency cases also received treatments. The route of administration was by simple inhalation. Results are outlined in Table 2.

CONVULSIONS: number of patients = 31; age: 18 months-55 years				
	Number of cases	Primary treatment	Adjunctive treatment	Therapeutic failure
Symptomatic epilepsy with generalized seizures	25	19	6	2 (cysticercosis)
Epilepsy with absence	2	0	2	0
Epilepsy with partial seizures	3	2	1	0
Generalized seizure accompanied by strange possessive behaviour	1	0	1	1 (unexplained)
MIGRAINES: number of patients = 38; age: 12-51 years				
Migraines without aura	31	19	12	2
Migraines with aura	2	0	2	0
Migraines with convulsions	5	3	2	0

Table 2: Clinical observational studies of a pharmaceutical composition comprising blend essential oils of *M. moschatus* and *Citrus* in convulsive and/or migraineous patients.

Twenty-one convulsive patients did not receive any conventional treatment. The seizures were successfully managed with the essential oil as a primary preventive treatment just before convulsions occurred. ten patients were refractory to, or complaining about the toxicity of existing conventional drugs. We progressively decreased the dose of conventional drugs while using the essential oil as adjunctive therapy. Overall, we observed a significant reduced frequency and duration of convulsions. Three therapeutic failures were observed, two with patients diagnosed with cysticercosis and one with unexplained causes despite repetitive EEG analysis and use of several conventional drugs. Some patients reported that the phytomedicines, once used, lost their effect after two months. As regards migraines, twenty-two patients did not receive any conventional treatment. They successfully used the essential oil as preventive medication. Sixteen patients were refractory to conventional drugs, or complained about their toxicity. In these cases, the essential oil was used as adjunctive therapy. Two treatment failures were observed.

In addition to these case studies, we used the essential oil in three emergency cases. On our way back home, we unexpectedly found a man of about 20 years old who presented severe convulsive seizures right on a public road, without nearly healthcare facilities. We immediately put the flask containing the essential oil into his nostril for one minute. The seizures stopped and he recovered consciousness. The second case concerned a baby aged 18 months who suddenly presented with a severe convulsive generalized tonic seizure. Her mother brought the baby to the clinical department of an institute. While waiting for diazepam that had to be bought from the closest pharmacy, we put the essential oil in her nostril. After a few minutes, the seizures ceased (in fact it took 45 minutes to get the diazepam). The third case concerned a 28-year old patient who came for consultation in a private clinic. She was in full convulsive crisis with hypotension and in very bad general state. Before transferring her to the hospital, the doctor applied a drop of the oil to her nostril. The doctor thought that she would die. Fortunately, her family said that her seizures ceased *en route* to the hospital. After intensive healthcare, she left the hospital in good condition. One case of very severe and unexpected migraine of a colleague was also successfully managed.

Patenting and commercialization

A national patent was granted: Philippe Rasoanaivo, Emmanuel Randrianarivo, Solofo Razafimahefa. *Composition pharmaceutique comprenant l'huile essentielle de Myrothamnus moschatus pour la prévention ou le traitement des désordres du cerveau*. OMAPI Patent N° 613, 20th October 2014.

Subsequently, two phytomedicines were manufactured, respectively, under the tradename Fanalarofy® (*fanala* = which remove, and *rofy* = chronic diseases) and Fanalanendo (anendo = headache) (Fig. 5). They are commercialized through the distribution channels of SOAMADINA, the commercial branch of the *Institut Malgache de Recherches Appliquées*.



Figure 5: Packaging of Fanalarofy® and Fanalanendo® - two products to emerge from this research now commercially available in Madagascar.

Partnerships

Internationally, we collaborated with colleagues at School of Pharmacy, University of Camerino, Camerino, Italy, and the Department of Environmental Biology, *La Sapienza* University, Rome, Italy.

Nationally, we partnered with public and private health care departments as well as SOAMADINA, the commercial branch of IMRA.

Impact

One significant indicator of the success of this experience that can be quantified is the good monthly sales revenue received from sales of the two products.

Local production is also sustainable thanks to available technical and commercial infrastructures.

Replicability

Madagascar has a very rich ethnomedical heritage. Nearly 4,000 plants possess medicinal uses. Most of research on medicinal plants in Madagascar has been focused on the isolation of the active constituents and little consideration has been put on the holistic approach. Many diseases are not caused by the dysregulation of a single molecular target but have a multi-factorial pathogenesis, and the observed effects often cannot be clearly assigned to specific chemical compounds. We therefore need a paradigm shift from the reductionist method to the holistic approach in investigating medicinal plants. At this point, our case study may serve as a model.

Lessons Learned

Some medical doctors have been reluctant to prescribe phytomedicines. To overcome this, a conference was delivered at the Malagasy Academy in April 2015 to explain the therapeutic usefulness of phytomedicines. A brochure has also been widely distributed.

Wild harvesting has limitations for the sustainable sourcing of the raw materials. Large scale *in situ* cultivation is needed for scaling up production.

Future Plans

Most of pharmacological tests used to validate the anticonvulsant activity of essential oils are phenotypic assays. It is necessary to move to the elucidation of the mechanism(s) of action of the essential oil of *M. moschatus* and some isolated compounds. This is under way.

While commercializing the phytomedicines, post-marketing surveillance will be carried out.

A big challenge is the large scale *in situ* cultivation of *M. moschatus*. The plant grows only in rocky areas, and its chemical composition is susceptible to variations following environmental factors. Work is being done to tackle this issue. The therapeutic usefulness of the pharmaceutical composition will be explored in other brain disorders, in the context of polypharmacology and drug repositioning.

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Ayurvedic *Amalaki Rasayana* and *Rasa-Sindoor* Suppress Neurodegeneration in Fly Models of Huntington's and Alzheimer's Diseases

India

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Duration: 2009-2014

Total cost: c. USD 55,000 from the Government of India

Summary

With a view to understanding the basic biology underlying the traditional Ayurvedic system of healthcare, we used the *Drosophila* fly model and examined the biological effects of two Ayurvedic *Rasayana* formulations, viz., *Amalaki Rasayana* (AR) and *Rasa-Sindoor* (RS), at organismal, cell and molecular levels. Rearing first instar larvae on food supplemented with 0.5% AR or RS affected biological parameters including development, life-span, fecundity and stress-tolerance in a formulation-specific manner, which generally agreed with the suggested therapeutic applications in Ayurveda. Both formulations significantly increased levels of several heterogeneous nuclear ribonucleoproteins (hnRNPs) and

cAMP response element binding protein (CBP) in different cell types in the fly model. We showed that dietary supplementation of either of these formulations during the larval period substantially suppressed neurodegeneration in fly models with polyglutamine neurodegenerative disorders and Alzheimer's disorder without any side-effects. Additionally, both of these dietary supplements substantially inhibited induced apoptosis (cell death), which adds to the survival of diseased neuronal cells that would otherwise have undergone apoptosis. Thus our studies suggest, for the first time, the potential of these Ayurvedic formulations in providing holistic relief from increasingly common neurodegenerative disorders.

Background and Justification

Ayurveda is the traditional Indian medicine system, widely practiced uninterruptedly at least for the past four thousand years. Classical Ayurvedic texts like the *Sushruta Samhita* divide Ayurveda into eight branches, of which the rejuvenating *Rasayana* therapy aims at promoting long life, enhancing physical and mental strength, and strengthening resistance against the infirmities and ailments of old age. An ethical lifestyle in conjunction with protocols involving diet, cleansing procedures and the intake of medicinal formulations are part of the *Rasayana* therapy. Under *Rasayana* therapy, orally-administered drugs are mostly based on plant products but may also include drugs derived from animal and mineral/metal sources. Etymologically, *Rasayana* implies the supply of the nutrient sap (*Rasa*) resulting from the digestion of food to the target (*Ayana*) body tissues. Classically, *Rasayanas* are believed to augment the transport and supply of *Rasa* to the tissues.

The available ancient Ayurvedic literature does not elaborate the mechanism/s and effect/s in terms of a contemporary understanding of biology or physiology. Although recent times have witnessed an increased interest in traditional and herbal medicine systems, most studies have used specific extracts or "active principles" derived from herbal

or other traditional drugs and formulations. However, since the Ayurvedic medicines and formulations are complex integrated derivatives involving specific preparatory steps, results of studies employing isolated active compounds may not provide full insight into the efficacy or mode of action of the traditional formulations. Therefore, it is necessary to undertake in-depth scientific investigations on the action/s of traditional Ayurvedic drugs or formulations using sound *in vivo* experimental model systems. Accordingly, we used the fruit fly, *Drosophila melanogaster*, as a model for understanding the cellular biological, biochemical and genetic bases of action for Ayurvedic formulations.

As a first approach, we used *Amalaki Rasayana*, a herbal derivative, and *Rasa-Sindoor*, an organo-metallic derivative of mercury. *Amalaki Rasayana* (AR) is a prominent drug in Ayurvedic classic texts such as the *Charak Samhita* and the *Ashtang Hridaya* and is claimed to enhance life expectancy, body strength, intellect and fertility, and to provide freedom from age-related illnesses. *Rasa-Sindoor* (RS) is indicated in a wide variety of disorders including chronic and recurrent infections (including pneumonia and bronchitis), anal fistulas, rheumatological diseases especially those of auto-immune origin, general and sexual debility as well as benign and malignant neoplasms. We found that these formulations do indeed affect some of the basic biological life parameters in the fly model, mirroring their expected usages in humans (Dwivedi *et al.*, 2012).

Increasing life span combined with life-style changes in recent decades has been associated with a significantly elevated incidence of a variety of neurodegenerative disorders. These include several polyglutamine (polyQ) expansion neurodegenerative disorders such as Huntington's disease (HD) and diverse spinocerebellar ataxias (SCA). Alzheimer's disease (AD), the other common form of senile dementia in humans, is associated with truncated A β peptides produced by aberrant proteolytic cleavage of the transmembrane receptor amyloid precursor protein (APP). A characteristic feature of these neurodegenerative diseases is the accumulation of polyQ inclusion bodies (IB) or the formation of amyloid plaques, respectively, by the repeat expanded or truncated protein. These inclusions and aggregates disrupt cellular homeostasis by sequestering a range of critical cellular proteins including molecular chaperones, transcription factors, proteasome subunits and cytoskeletal components resulting in cellular damage and consequent death of the affected neuronal cells. With a view to understanding the molecular and cellular pathophysiology of neurodegeneration and to discover potential drug targets for therapeutic applications, several human neurodegenerative diseases like HD, different SCAs and AD, among others, have been modelled in diverse organisms including *Drosophila*.

Several traditional Ayurvedic formulations are claimed to facilitate “healthy aging” (Singh, 2003) and thus have the potential to mitigate the suffering from neurodegenerative diseases (Lakhotia, 2013). We (Dwivedi *et al.*, 2012) found that feeding *Drosophila* larvae and adult flies on food supplemented with 0.5% (weight/volume) of AR or RS significantly improved their tolerance to thermal or starvation stresses and enhanced cellular levels of various heterogeneous RNA-binding proteins (hnRNPs) and CAMP response element binding protein/p300 histone-acetyl-transferase in wild-type larval tissues. These proteins have key roles in gene expression and RNA processing and transport. Several earlier studies in different model systems (Sofola *et al.*, 2007; Mallik and Lakhotia, 2010; Caccamo *et al.*, 2010; Berson *et al.*, 2012) have also shown that elevated levels of hnRNPs, CBP and better tolerance to thermal and/or oxidative stress suppress neurodegeneration. Therefore, we examined if dietary supplementation with AR or RS affects neurodegeneration in fly models of polyQ disorders or Alzheimer’s disease (AD). Interestingly, both the formulations notably suppressed pathogenesis in the fly models of human neurodegenerative disorders (Dwivedi *et al.*, 2013).

Description

Pilot experiments with food supplemented with 0.125%, 0.25%, 0.5%, 2%, 4% or 6% of the formulations were carried out and, based on the results, in subsequent experiments either of the two formulations were used at 0.5% (i.e., 500mg/100ml food) concentration for feeding *Drosophila* larvae and/or adult flies (Dwivedi *et al.*, 2012). Rearing on AR or RS supplemented diet resulted in substantial suppression of the different polyQ-dependent neurodegenerative phenotypes, *viz.*, eye morphology, differentiation and organization of rhabdomeres, apoptosis in developing eye discs, accumulation of inclusion bodies, induction of the chaperone-like Hsp70 and Hsp60, etc, clearly showing that these two formulations effectively suppress neurodegeneration in fly models of polyQ toxicity (Dwivedi *et al.*, 2013).

PolyQ transgene expression leads to an accumulation of polyQ inclusion bodies posterior to the morphogenetic furrow in differentiating late third instar larval eye discs (Fig. 1a,e). The polyQ inclusion bodies were significantly reduced in the eye discs of larvae reared on AR or RS supplemented food (Fig. 1b-d, f-h). Along with the reduction in polyQ inclusion bodies levels in eye discs in larvae reared on food supplemented with AR or RS, the differentiating ommatidial units posterior to the morphogenetic furrow also showed a remarkably improved organization (Fig. 1i-o). Measurement of the polyQ immunofluorescence intensity in eye discs confirmed that the accumulation of inclusion bodies in AR- and RS-fed larvae was significantly reduced when compared to those

reared on regular food (Fig. 1d, h). Since the levels of polyQ transcripts in formulation-fed larvae were similar to those observed in normally fed larvae (inset in Fig. 1c), the reduced accumulation of inclusion bodies may be the result of post-transcriptional events.

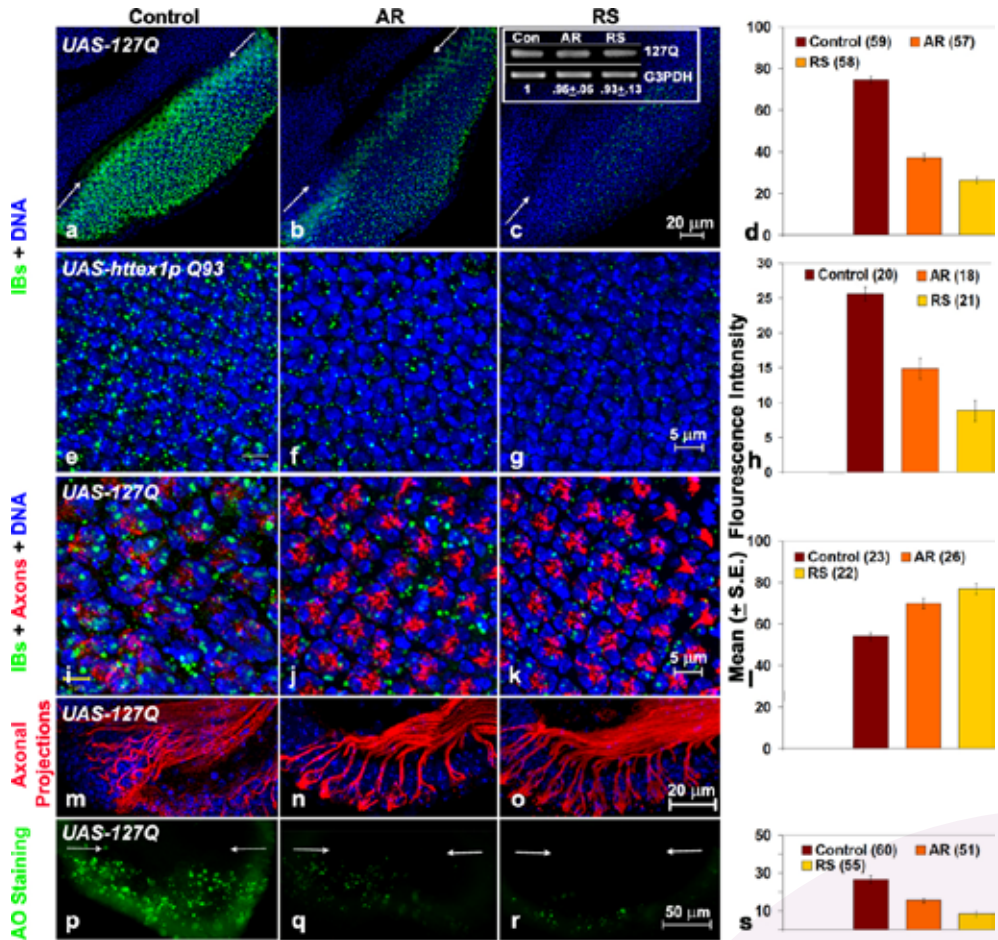


Figure 1: Eye discs expressing *GMR-GAL4>UAS-127Q* or *GMR-GAL4>UAS-htt1 p Q93* (indicated in left column of each row) from *Drosophila* larvae fed with Ayurvedic formulations. Left column (a,e,i,m): untreated control; Centre column (b,f,j,n), reared on *Amalaki Rasayana* (AR); Right column (c,g,k,o), reared on *Rasa-Sindoor* (RS).

(a-k) show reduced the accumulation of polyQ inclusion bodies (green); (i-k) show improved rhabdomere arrays; (m-o) show reduced damage to axonal projections in the optic stalk (red); and (p-r) show reduced cell death (green).

White arrows in (a-c and p-r) indicate position of the morphogenetic furrow in eye discs. The scale bars apply to all panels in the same row.

The inset in (c) shows the 127Q (upper row) and G3PDH (lower row) amplicons generated by semi-quantitative RT-PCR with total RNA from larval eye discs from *GMR-GAL4>127Q* larvae reared on control (Con), AR and RS supplemented food (indicated on top of the columns). The values below each lane indicate the mean (±SE, n = 3) levels of polyQ transcripts relative to that in the control sample, which was taken as 1.0.

Histograms (d,h,s) show the mean (±SE) fluorescence intensities (measured in arbitrary fluorescence units) of polyQ inclusion bodies (d,h), mab22C10 (l) and AO (s) staining, respectively, in *GMR-GAL4>UAS-127Q* expressing eye imaginal discs of late third instar larvae reared on different feeding regimes. Numbers in parentheses after the bar legends indicate the number of eye discs examined for each data point (Figure reproduced from Dwivedi et al., 2013).

We examined the cellular levels of two hnRNPs, viz. Hrb87F (hnRNP-A homologue) and Bancal (hnRNP K homologue) and CBP in *GMR-GAL4>UAS-127Q*-expressing eye discs in larvae reared on normal food and those reared on AR- or RS-supplemented food. Immunostaining with appropriate antibody and confocal microscopy showed that compared to normally-fed larvae (Fig. 2a,d,e and h), dietary supplement of either of the formulations resulted in significant increase in cellular levels of Hrb87F (Fig. 2b-d) and Bancal (Fig. 2f-h). The increase was more apparent in RS-fed larval eye discs.

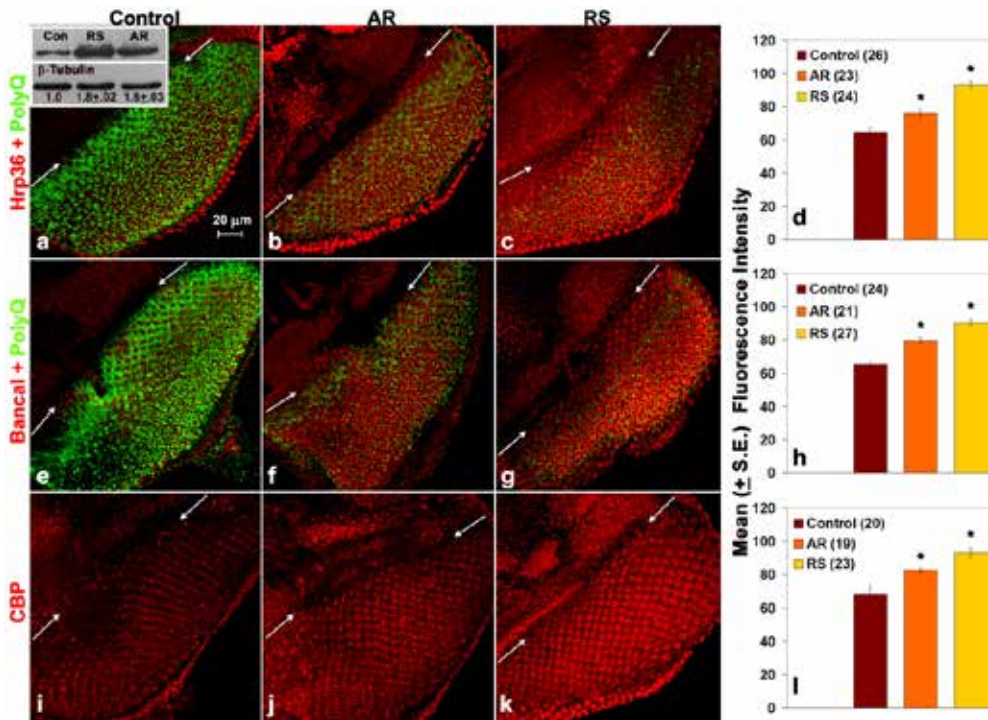


Figure 2: *Drosophila* larval eye discs expressing *GMR-GAL4>127Q* fed with Ayurvedic formulations. Left column (a,e,i): untreated control; Centre column (b,f,j), reared on *Amalaki Rasayana* (AR); Right column (c,g,k), reared on *Rasa-Sindoor* (RS).

(a-d) show elevated cellular levels of Hrb87F (red); (e-h) show elevated levels of Bancal (red); and (i-l) show elevated levels of CBP (red). Also, (a-c and e-g) show the reduction in accumulation of polyQ inclusion bodies (green).

White arrows indicate position of the morphogenetic furrow in the eye discs. The scale bar in (a) corresponds to 20 μ m and applies to all the panels.

The inset in (a) is a western blot of total protein from eye discs of 127Q-expressing larvae reared on normal (Con), RS- or AR-supplemented food to show the relative levels of Hrb87F (upper row); β -tubulin (lower row) was used as a loading control. The values below each column indicate the mean (\pm SE, n = 3) levels of Hrb87F relative to that in control sample, which was taken as 1.0.

Histograms (d,h,l) represent the mean (\pm SE) fluorescence intensities (in arbitrary fluorescence units) of Hrb87F (d), Bancal (h) and CBP (l), respectively, in 127Q-expressing larval eye imaginal discs following different feeding regimes while the numbers in parentheses after the bar legend indicate the number of eye discs examined (Figure reproduced from Dwivedi *et al.*, 2013).

Co-immunostaining with antibodies against polyQ also revealed that the increase in the cellular level of these hnRNPs following formulation feeding is associated with a reduction in the accumulation of inclusion bodies (Fig. 2a-g). An increase in levels of Hrb87F was also confirmed by western-blotting which also showed that RS-fed larval samples displayed a greater increase (inset in Fig. 2a). Immunostaining for CBP/p300 in *GMR-GAL4>UAS-127Q* expressing discs (Fig. 2i-l) showed that AR- or RS-feeding significantly enhanced the levels of CBP, more so in RS-fed samples (Fig. 2k,l). AR- or RS-feeding significantly improved proteasomal activity in formulation-fed larval tissues. The ubiquitin-proteasomal activity (UPS), involved in the degradation and clearance of unwanted proteins in cells, is compromised in the affected neuronal cells in polyQ/HD and AD, leading to enhanced accumulation of pathogenic proteins. Therefore, we examined the UPS activity in *GMR-GAL4>UAS-127Q*-expressing eye discs using the *UAS-Ub^{G76V}-GFP* transgenic line (Dantuma *et al.*, 2010) in which the green fluorescent protein (GFP) is tagged with ubiquitin so that under conditions of compromised UPS activity, GFP fluorescence persists. As expected, because of the compromised UPS activity, eye discs of normally-fed *GMR-GAL4>UAS-127Q*-expressing larvae showed high levels of GFP fluorescence (Fig. 3a). However, in AR- or RS-fed larval eye discs, the GFP fluorescence was significantly reduced, especially in RS-fed samples (Fig. 3b, c and d).

In order to further assess whether the improved UPS is indeed playing a role in reducing the accumulation of inclusion bodies and disappearance of *UAS-Ub^{G76V}-GFP* fluorescence, *GMR-GAL4>UAS-127Q*-expressing eye discs from differently fed late third instar larvae were incubated *in vitro* for two hours in medium containing 1 μ M clastolactacystin β -lactone, a proteasome inhibitor, prior to immunostaining for polyQ inclusion bodies. As expected, the accumulation of inclusion bodies was much higher in discs from normally fed larval eye discs in which the proteasomal activity was inhibited for two hours (Fig. 3i, compared with Fig. 3e). Interestingly, however, the accumulation of inclusion bodies even in the presence of proteasome inhibitor was much less in discs from AR- (Fig. 3j and l) or RS- (Fig. 3k and l) fed larvae, although they were slightly more abundant than in discs which were not exposed to the proteasomal inhibitor (Fig. 3f and g). Taken together, these results confirm that AR- or RS-feeding indeed improves proteasomal activity.

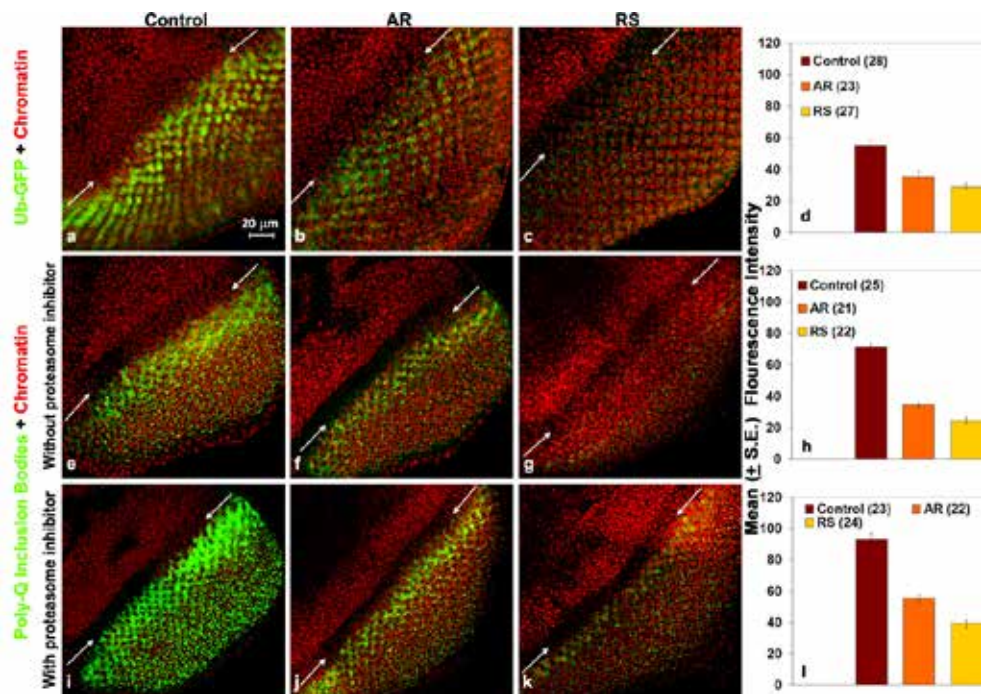


Figure 3: *Drosophila* larval eye discs expressing *GMR-GAL4>127Q* fed with Ayurvedic formulations. Left column (a,e,i): untreated control; Centre column (b,f,j), reared on *Amalaki Rasayana* (AR); Right column (c,g,k), reared on *Rasa-Sindoor* (RS).

(a-c) showing improved ubiquitin-proteasomal activity (UPS) activity through UB-green fluorescent protein (GFP) staining (green); showing polyQ inclusion bodies (green) and (a-k) and DAPI (4',6-diamidino-2-phenylindole) stained nuclei (red).

(e,f,g) are images of polyQ inclusion bodies in *127Q*-expressing larval eye discs without the 2-hour *in vitro* exposure to proteasome inhibitors while (h,i,j,k) are images of polyQ inclusion bodies in *127Q*-expressing larval eye discs exposed to the proteasome inhibitors.

White arrows indicate the position of the morphogenetic furrow in eye discs. The scale bar in (a) corresponds to 20 μ m and applies to all image panels.

Histograms (d,h,i) represent the mean (\pm SE) fluorescence intensities (in arbitrary fluorescence units) of Ub-GFP, polyQ inclusion bodies without (d, h) and after (i) treatment with a proteasome inhibitor, in *127Q*-expressing larval eye imaginal discs following different feeding regimes; numbers in parentheses after the bar legends indicate the number of eye discs examined for each data point. (Figure reproduced from Dwivedi *et al.*, 2013).

Thus the enhanced levels of hnRNPs and CBP together with improved proteasomal activity following formulation feeding seem to be some of the factors that suppress the polyQ-toxicity. The inhibition of induced apoptosis or cell death (Dwivedi *et al.*, 2015) by AR- or RS-feeding further adds to the suppression of neurodegeneration. A complete absence of AR- or RS-mediated suppression of neurodegeneration caused by *127Q* or Htt-ex1P Q93 toxic proteins in larvae that have reduced or complete absence of Hrb87F (data not shown) further confirmed that this hnRNP plays a pivotal role in bringing about the beneficial effects of both these formulations.

Impact

Our studies have established the fly model for understanding the “Science of Ayurveda”. In addition, they have indicated, for the first time, the potential of Ayurvedic formulations such as *Amalaki Rasayana* and *Rasa-Sindoor* to provide holistic relief from the increasing burden of neurodegenerative disorders in human populations.

Publication of our papers received wide coverage in popular media and *Nature* (India). See for example:

- www.telegraphindia.com/1131227/jsp/nation/story_17724324.jsp#.Urzk-dIW04k
- www.telegraphindia.com/1120518/jsp/nation/story_15501860.jsp
- www.nature.com/nindia/2012/120515/full/nindia.2012.73.html
- www.nature.com/nindia/2013/131230/full/nindia.2013.172.html

The community of Ayurvedic practitioners also appreciated these studies and the principal investigator of the project was invited to write guest editorials in Ayurvedic journals.

These studies show, contrary to unfounded but common misapprehensions, that the traditional mercury-containing formulation does not exhibit any toxicity when prepared following traditional practices.

Based on the outcome of this project, the principle investigator has (S.C. Lakhotia) been granted a new research project for extensive genomic and proteomic studies following administration of these *Rasayanas* to *Drosophila*. In addition, several other basic science investigators in India have initiated in-depth experimental studies on the biology of Ayurveda with a view to understanding the basic science underlying this traditional healthcare system which continues to be popular in India and many other countries.

Future Plans

Our ongoing research project aims to examine in detail the transcriptomic and proteomic changes brought about by these formulations in various cell types in different genotypes, including in fly and mammalian models of neurodegenerative disorders.

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A Naturally-occurring Antioxidant and Anti-inflammatory Bioflavonoid from the Seed of *Garcinia kola*

Nigeria

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Duration: 5 years

Total cost: USD 50,000

Summary

Garcinia kola is a medium-sized tree found in moist forests, is widely distributed throughout west and central Africa, and is highly valued in for its edible nuts. The seed, commonly known as bitter kola, is a masticatory and an important kola substitute at home and at social ceremonies, playing a major role in African ethno-medicine. Traditionally, the seeds are used in the treatment of inflammatory disorders, laryngitis, cough, hoarseness of voice and liver diseases. For instance, extracts of the seeds led to remarkable improvement in liver function in patients with chronic hepatitis and cholangitis after treatment for 14 days at a Nigerian herbal home (Iwu, 1982). Our studies show that *G. kola* possesses immunomodulatory, antiviral, anti-inflammatory and antioxidant activities.

Laboratory experiments conducted using animals show that Kolaviron, a bioflavonoid extracted from *G. kola*, is beneficial to the body's system by helping to combat free radicals, chelate dangerous metals, boost

the body's detoxification system, and reverse the noxious effects of inflammatory proteins and certain transcription factors that cause stress to the body's system. It also boosts sperm production, increases sperm motility and the sperms' antioxidant defence system and, as such, is useful in the male reproductive system, justifying its reputation in folklore as an aphrodisiac. Importantly, our recent data show that Kolaviron crosses the blood-brain barrier. It protected rats and human-derived brain cell cultures against drug-induced brain damage and as such has opened a new therapeutic window and application in neurodegenerative diseases such as Parkinson's and Alzheimer's diseases. A Phase 1 clinical trial with *G. kola* showed it has relevance in the management of osteoarthritis of the knee and other inflammation-related disorders. Overall, this compound is a promising candidate drug in cancer chemoprevention and management of other diseases in which inflammation and free radicals have been implicated.

Background and Justification

The African continent is home to a rich diversity of plants used as herbs, health foods and for therapeutic purposes. This is largely due to the geographical spread spanning a land mass of approximately 2,166,340 km² of closed forest. Over 5,000 different substances from plant species have been recognized as occurring in these areas (Iwu, 1993), many of which have been found useful in traditional medicine for prophylaxis and the cure of diseases. This great biodiversity offers economic promise, particularly in the rapidly-emerging biotechnology industry. In spite of the heterogeneous nature of the continent and a deluge of information on the composition and biological activity of many plant

substances, little effort has been devoted to the development of chemotherapeutic and prophylactic agents from these plants. In the case of *Garcinia kola* it has been recognised as playing a great role in traditional medicine in the management of various ailments but no in-depth scientific studies have been carried out to prove and to justify its wide range of medicinal properties.

Recognising the many traditional values and medicinal potential of the *G. kola* plant, such as its antibacterial properties, cough-relieving effect and the relevance of its seed in the management of liver diseases, it became imperative to systematically and scientifically evaluate its therapeutic uses.

The identification of *G. kola* seed as a naturally-occurring modulator of liver disease qualifies it as candidate that can be incorporated in the human diet at minimal cost since the majority of the population in developing countries are not yet sufficiently economically-buoyant to be able to purchase prophylactic drugs. Coincidentally, the edible seed occupies a prominent position in the social customs of the people in West Africa and elsewhere on the continent. In spite of its very bitter taste, this nut is consumed as a refreshing habit and plays an important role in social ceremonies.

The first step by our scientific working group was the isolation and identification of a bioflavonoid compound, termed Kolaviron, which was shown to elicit significant hepatoprotective properties in animal models.

Description

The risk of developing liver diseases is highest in developing countries, due to combined exposure to aflatoxin B1 and the hepatitis B virus. This deadly duo has been implicated as a major risk factor in the development of liver cancer, which has been shown to present with the poorest 5-year survival rates and accounts for 15% of total cancer deaths. However, we have identified a commonly consumed, locally available and accessible seed in the region, *G. kola*, from which an active compound, Kolaviron, is derived. From studies using experimental rats, we reported the chemopreventive effects of Kolaviron against several liver damaging chemical compounds, namely carbon tetrachloride, 2-acetylamino fluorine (2-AAF), acetaminophen, galactosamine, phalloidin, potassium bromate (a nephrotoxic compound) and, most notably, aflatoxin B1. This latter compound is produced by the fungus *Aspergillus flavus*, known as a contaminant of maize, peanuts and other food products in the tropics (Farombi *et al.*, 2005). Our recent study to unravel the molecular mechanisms of the hepatoprotective properties of Kolaviron also revealed its role in protection against dimethyl nitrosamine-mediated liver damage and the

downward regulation of certain transcription factors implicated in cancer (Farombi *et al.*, 2009). These findings indicate that Kolaviron may protect against carcinogen- and drug-induced oxidative and membrane damage and as such may be relevant in the chemotherapy of liver and kidney diseases.

In implementing the project, we investigated the mechanisms by which Kolaviron protects against hepatotoxicity and possibly human liver cancer, which hitherto had not been investigated by any researcher. One major mechanism of hepatoprotection is the ability of a compound to elicit antioxidative activity. Therefore, in several assays involving reactive oxygen species (ROS), we evaluated the antioxidant properties of Kolaviron. Kolaviron exhibited significant reducing power, a dose-dependent inhibition of oxidation of linoleic acid, and inhibition of hydrogen peroxide activity together with significant scavenging of superoxides and of hydroxyl radicals. The ability of Kolaviron to scavenge hydroxyl radicals by inhibiting the oxidation of deoxyribose may directly relate to its inhibitory action of lipid peroxidation and thus account for its hepatoprotective properties in animal models. In human lymphocytes and rat liver cells, our data demonstrate that Kolaviron prevented DNA damage by eliciting a concentration-dependent decrease in hydrogen peroxide-induced DNA strand breaks. Furthermore, in rats treated with Kolaviron for 7 days, lipoprotein resistance to copper-induced oxidation was greatly improved. In addition, Kolaviron inhibited the Cu^{2+} -induced oxidation of rat serum lipoprotein in a concentration-dependent manner, exhibiting a chelating effect on Fe^{2+} , thus demonstrating that Kolaviron protected against the oxidation of lipoprotein by mechanisms involving metal chelation and antioxidant activity, and, as such, might be a good candidate for preventing atherosclerosis and heart-related diseases (Farombi *et al.*, 2004).

Traditionally, *G. kola* seed plays important role as an aphrodisiac. We investigated this claim in a series of experiments in which rodents were exposed to Kolaviron as well as *G. kola* in the presence of environmental compounds known to cause testicular damage. Our data showed that Kolaviron treatment in rats ameliorated the toxicity posed to the testicular system by several environmental compounds that humans are occupationally and dietarily exposed to. In addition, in rats treated with *G. kola* at 0, 250, 500 and 1000 mg/kg for six consecutive weeks, there was an improvement in the spermatozoa characteristics and a boost in testicular antioxidant status. Furthermore, *G. kola* increased testosterone levels in these rats, reinforcing its reputation in enhancing reproductive capacity and thus justifying its folkloric use as aphrodisiac (Farombi *et al.*, 2013).

The contribution of environmental contaminants to the aetiology of neurodegenerative diseases, such as Parkinson's disease, has been broadly recognized. Atrazine (ATR), a widely used agricultural pesticide, has been reported to work as a potential contributory risk

factor for Parkinson's disease and other neurological disorders in which dopamine levels are affected. At the same time, neuroprotection to delay or halt progressive degeneration of specified neurons is now proposed as a causal therapeutic strategy for Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis (Abarikwu *et al.*, 2011).

We investigated the effects of Kolaviron on ATR-induced cytotoxicity on human neuroblastoma cells in order to find a possible therapeutic intervention by application of a natural compound against degenerative diseases. Kolaviron elicited significant protection against ATR-mediated toxicity in these brain cells. Our data therefore demonstrate that Kolaviron's antioxidative property and its ability to protect against programmed cell death (Fig. 1) makes this natural molecule potentially protective against ATR-induced cytotoxicity. The results may open up a new clinical perspective in progressive neurodegenerative diseases such as Parkinson's disease.

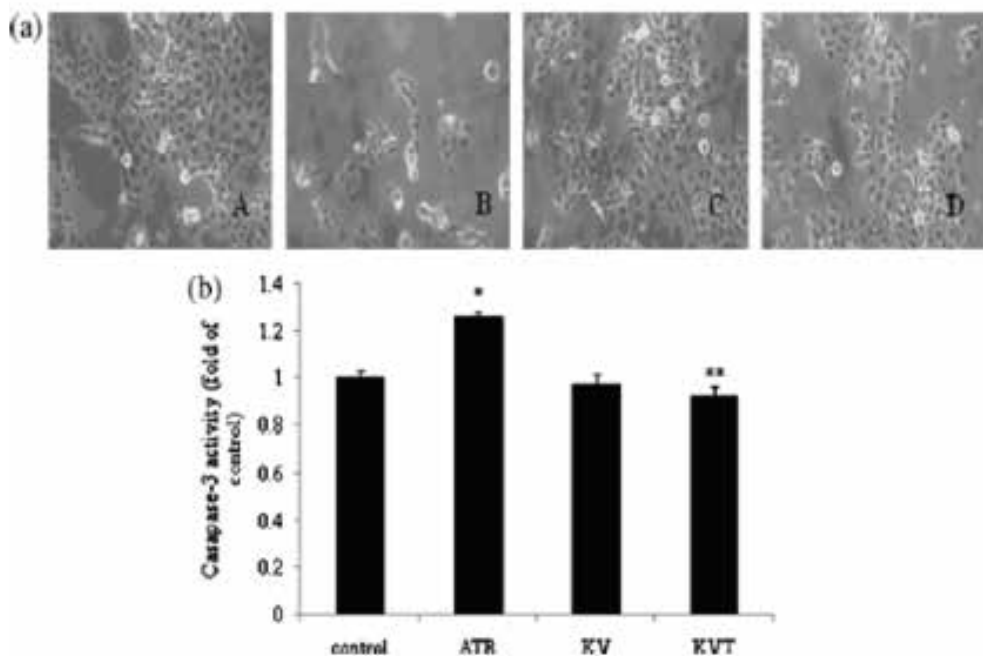


Figure 1: (a) Proliferation of SH-SY5Y cells treated with 0.3 mM atrazine (ATR) and/or Kolaviron (KV). A: control; B: 0.3 mM ATR; C: 60 IM KV; D: 0.3 mM ATR + 60 IM KV. (b) Caspase-3 enzymatic activity after 24 h exposure of SH-SY5Y cells to ATR in the presence or absence of KV. (Adapted from Abarikwu *et al.*, 2011).

Results obtained so far, many of which have been published in peer-reviewed journals, indicate that this naturally occurring bioflavonoid, Kolaviron, from the seed of *G. kola*, has positive modulating and chemopreventive/chemoprotective effects on the liver, brain and reproductive system as well as on the noxious effects of environmental contaminants through various mechanisms including antioxidant effects, the induction of xenobiotic detoxifying enzymes, metal chelation, scavenging of ROS, attenuation of ROS-induced

DNA damage and programmed cell death, the down-regulation of certain transcription factors and stress-response proteins and up-regulation of phase 2 antioxidant genes.

These results, derived from a series of innovative experiments, justify the important role the seed of *G. kola* plays in African traditional medicine in the treatment of coughs, colds, hoarseness of voice, liver diseases, accentuating longevity and as an aphrodisiac.

Partnerships

In the course of implementing this project, we collaborated with some international scientists in the field of chemoprevention with plant-based medicinal compounds. They included:

- Professor Lars O. Dragsted, Institute of Food Safety and Nutrition, Soborg, Denmark;
- Professor Young Joon Surh, Seoul National University, South Korea; and
- Professor Beatrice Pool Zobel (late), Institute of Nutrition, University of Jena, Germany.

Impact

The project has had a significant impact on the scientific community locally and internationally and has built capacity in the field of traditional medicine research. Notably:

We have published about 40 articles in peer-reviewed international and regional journals on the beneficial role of *G. kola* and Kolaviron.

- We have made several presentations at both regional and international conferences on the success of this innovative research on *G. kola*.
- Several MSc and 10 PhD students have been trained and mentored, and many of them are actively practicing in the field.
- Several prizes, honours and distinctions have been won by reason of the series of innovative work on *G. kola*. For instance the first author has recently been admitted as a Fellow of the Nigerian Academy of Science (FAS), which represents the apogee of scientific achievement in Nigeria. Also some of the works and recognition in the field led to his winning the Society of Toxicology Global Senior Scholarship (USA) and admittance to the Fellowship of the American Academy of Toxicological Sciences. Two of his PhD students received 'best poster presentation' prizes for their presentation on some aspects of this work.

Some sectors of the general public, especially among the elite, have reservations towards herbal products and have adopted a western diet and medicines, but over time this attitude is changing. Our experience has addressed this aspect significantly, as many have become better informed through seminars, publications in journals, periodicals and newspapers and conferences.

Sustainability

One way of sustaining the experience is to preserve the *G. kola* plant species in the forest. Deforestation is rife across large parts of Africa and many important medicinal plants are becoming endangered or extinct.

The experience has also impacted the Ministry of Environment in Nigeria, which organised a national summit on *G. kola*, with the principal author invited to act as chair. It is expected that through the Minister of Environment, legislators and policy makers in the country will be better informed. In particular, the summit discussed issues relating to forest loss and availability of the raw product.

Replicability

The success of this project has alerted policy makers and created a renewed awareness among the public on the beneficial role of this seed in Nigerian society.

The active compound Kolaviron isolated from *G. kola* seed was patented some years ago in the USA by another group that worked on it. New therapeutic compounds from the same seed have been isolated by our group and we are working on the patents.

Through international collaboration with scientists in Denmark, Germany, South Korea and the USA, the innovative experience has become relevant across different regions, especially as the whole world is in search of naturally occurring, non-toxic therapeutic agents.

Lessons Learned

The main circumstance for the successful implementation of this project is the availability, accessibility and affordability of the *G. kola* seeds, the obtaining of which did not pose problem, placing the population in the region at an advantage in having access to the material.

Some individuals in the community do not like to eat the seed because of its bitter taste but through publications and engaging the press we are making efforts to ensure that the public buys into this innovation.

The major obstacles faced in the successful implementation of the project are funds and availability of state-of-the-art equipment to conduct meaningful experiments. Self-funding, University of Ibadan Senate Research grants, as well as several international fellowships and grants helped significantly in conducting experiments that resulted in meaningful conclusions.

Future Plans

Future plans include carrying out additional experiments on the interaction of the seed and isolated compounds on the bioavailability of other therapeutic drugs that may be consumed simultaneously as well as carrying out clinical trials using the product.

There are also plans to continue scientific collaborations and to collaborate further with researchers in Asia, Europe and the USA.

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Uncovering the Potential of Indonesian Medicinal Plants for the Dual Actions of Anti-diabetes and Anti-obesity

Japan



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Summary

As obesity is a key factor in the development of type 2 diabetes, lowering lipid accumulation in adipose tissues is as important as increasing insulin sensitivity in diabetic patients. The selected plant extracts used in this screening have been traditionally used in Indonesian medicine for the treatment of diabetes and its complications. This study aimed to investigate the ability of the selected plants to increase both insulin sensitivity through the enhancement of glucose uptake after insulin induction in adipocytes and to suppress lipid production in the same target cells. For this purpose, dried Indonesian medicinal plants were screened accordingly. The screening platform consisted of insulin-induced glucose uptake, lipid accumulation, and cell viability. Out of 59 plants tested, 13 plants demonstrated their ability to increase glucose uptake in 3T3-L1 adipocytes after insulin induction, and four of these extracts

suppressed lipid production of the cells. The CCK-8 assay results of those four plant extracts suggest that the lipid inhibition activity of pasak bumi *Eurycoma longifolia* Jack (root) and black pepper *Piper nigrum* L. (fruits) extracts are not attributable to their cytotoxicity in the adipose cells. Both plant extracts increased glucose uptake by more than 200% at 50 µg/mL and suppressed lipid accumulation in a concentration-dependent manner. This indicates that screening of selected Indonesian medicinal plants has uncovered the potentials of pasak bumi root and black pepper fruits with dual active functions: increasing insulin sensitivity through the enhancement of glucose uptake, and reducing lipid accumulation in adipose cells. These findings suggest that both plants could provide additional benefits in the treatment of diabetes.

Background and Justification

Lowering lipid accumulation in adipose tissues (fat cells) is as important as enhancing insulin sensitivity in obesity-related diabetic patients due to the association between obesity and insulin resistance. This relationship presents major health hazards, including morbid obesity and cardiovascular complications. There is therefore a growing need for a medicine with two-fold properties.

For this reason, the study of the treatment of metabolic disorders using traditional forms of medicine has been intensified recently, as synthetic medicines for treatment of diabetes often cause undesirable side effects and certain drug groups even exacerbate obesity conditions. In addition, pharmacologists are now aware that the concept of 'one disease, one target, one drug' does not always lead to successful cures. To address the challenge of chronic and degenerative diseases, the holistic approach of traditional medicines represents a novel solution.

Indonesian traditional medicine, known as jamu, is used to treat various diseases, not only in rural areas, but also in urban settings. Unlike traditional Chinese medicine, jamu prescriptions are not well documented.

This study identified and investigated plants with dual active functions that could serve as anti-diabetes and anti-obesity agents. These plants were selected for use in this study because they are commonly used by local herbal industries in Indonesia. Other plants that are often used locally for treating diabetes complications were also selected. Certain plants in this study were expected to have unique mechanisms that could increase glucose uptake, while simultaneously suppressing lipid production.

To the best of our knowledge, this work is the first to report the *in vitro* screening of Indonesian medicinal plants for the dual activity of insulin-induced glucose uptake enhancement and lipid-lowering activity in 3T3-L1 adipocyte model cells (Lahrita et al., 2015). The findings of the study were expected to corroborate the traditional application of Indonesian jamu medicines, in treating diabetes and its complications, thus contributing to a more complete understanding of their efficacy.

Description

Dried samples of Indonesian plants were collected from the Central Java region and identified by a herbalist.

The processes surrounding adipocyte differentiation have been well studied using 3T3-L1 cells, cell lines that are committed to the adipocyte lineage. The 3T3-L1 preadipocytes differentiate into mature adipocyte cells and accumulating triglyceride lipid droplets. Needless to say, 3T3-L1 adipocytes have been used as a well-established cell culture system that can be employed to study adipogenesis, fatty acid metabolism, and insulin-regulated trafficking.

Insulin-induced glucose uptake-enhancing assay

On day 8 after differentiation, 50 µg/mL of each of the plant extracts was added to mature adipocytes. The reference compound used in this study was rosiglitazone, which acts as an insulin sensitizer, while the control cells were treated with 0.5% of a standard solvent. Insulin stimulation and glucose uptake were conducted on day 12, followed by an enzymatic fluorescence assay. On day 12, the cells were washed twice with KRPH buffer and incubated in a serum-free D-MEM medium. After being washed twice in a buffer solution and incubated with or without to perform insulin stimulation in the same buffer. The cells were then incubated with 2-DG solution. To remove excess 2-DG and halt its uptake, the cells were washed with cold KRPH buffer three times.

After washing again to stop the reaction, cells were lysed adding 50 μL of Sodium hydroxide (NaOH) to each sample. The cell lysate was frozen and then heated to 85°C for 45 minutes. The lysate was neutralized by adding hydrochloric acid (HCl) and 350 μL of a second buffer was subsequently added. The measurement of 2-DG uptake was done using an enzymatic fluorescence assay and normalization of protein using the Bradford protein assay.

Lipid accumulation assay

For lipid accumulation studies, plant extracts were added to 3T3-L1 cells after differentiation induction.

To detect the accumulated lipid droplets, a standard Oil Red O staining assay was performed when the 3T3-L1 adipocytes reached maturity.

Cytotoxicity assay

Cell viability was tested using the cell counting kit solution (CCK-8) from Dojindo Molecular Laboratories Inc. (Kumamoto, Japan).

All experiments were undertaken at least in triplicate under identical conditions.

Results and Discussion

Screening of plant extracts for insulin-induced glucose uptake-enhancing activity

Fifty-nine plant extracts were screened for their ability to enhance glucose uptake upon the induction of insulin against fully differentiated 3T3-L1 cells. Out of 59 plants, 13 plant extracts that demonstrated insulin-induced glucose uptake-enhancing activity at the concentration of 50 $\mu\text{g}/\text{mL}$ were assessed, including green chiretta *Andrographis paniculata* (Burm.f.) Nees (whole plant), areca palm *Areca catechu* L. (fruits), sappanwood *Caesalpinia sappan* L. (wood), *Eleutherine bulbosa* syn. *americana* (Aubl.) Merr. ex K. Heyne (bulb part), pasak bumi *Eurycoma longifolia* Jack (root), moringa *Moringa oleifera* Lam. (leaves), betel *Piper betle* L. (leaves), red betel *P. crocatum* Ruiz & Pav. (leaves), black pepper *P. nigrum* L. (fruits), clove *Syzygium aromaticum* (L.) Merr. & L.M. Perry (fruits), beleric *Terminalia bellirica* (Gaertn.) Roxb. (fruits), *Tinospora crispa* (L.) Hook. f. & Thomson (bark), and aniseed *Pimpinella anisum* L. (seeds).

Lipid suppression from glucose uptake-enhancing plants

Overproduction of lipids in adipose tissue is considered a sign of dyslipidaemia, which could lead to obesity and exacerbate type 2 diabetes mellitus conditions. For this reason, studies on the regulation of adipogenesis inform the medical community of the best

method for treating obesity-associated diabetes. The 13 plant extracts that exerted insulin-induced glucose uptake-enhancing activity were subjected to a lipid accumulation assay to examine their biological effects on the lipid production of adipocytes. Various concentrations of the extracts were added on day 4 after the induction of differentiation, to investigate their effects on intracellular lipid formation (lipo-genesis).

Lipid accumulation assay demonstrated that treatment in 3T3-L1 adipocytes with four of the plant extracts substantially reduced the accumulation of intracellular lipids in a concentration-dependent manner. They were *A. paniculata* (Burm.f.), Nees (whole plant), *E. longifolia* Jack (root), *P. betle* L. (leaves), and *P. nigrum* L. (fruits).

Cytotoxicity

It is important to examine cytotoxicity when observing potential lipid-suppressing activity in order to distinguish between selectivity of action and cell death. Out of the four tested plants in which lipid reduction had previously been demonstrated, there were two species that exerted their lipid-lowering activity without causing cytotoxicity, namely pasak bumi root and black pepper fruits.

Collectively, the glucose uptake, lipid accumulation, and cytotoxicity results demonstrate the potential of pasak bumi root and black pepper fruits as anti-diabetic agents for the regulation of glucose and lipid metabolism (Fig. 1).

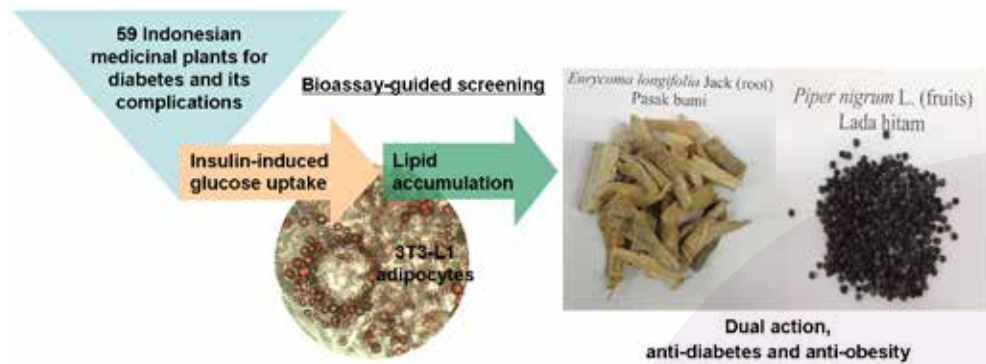


Figure 1: Bioassay scheme of screening Indonesian traditional medicines for dual actions for the potential treatment of type 2 diabetes.

Partnerships

If there is a field where people can think globally and act locally, it is traditional medicine, and the path to knowledge and innovation has always been travelled best in company. More things can be achieved in this field when people work together than when they work alone, because in the end, research on exploring traditional medicine tends to be an interdisciplinary process that requires collaboration among colleagues within an institute or with other institutes beyond national borders. For this reason, Hokkaido University (Japan) has research interest in Indonesian traditional medicine. Through intellectual knowledge exchange, academic institutions can synergize research strengths in exploring traditional medicine for the benefit of public health.

Impact

This study has uncovered the potential of both pasak bumi root and black pepper fruits to increase insulin sensitivity and suppress lipid production without causing significant cell death in the 3T3-L1 adipose cells.

Replicability

With more than 20,000 plant species, Indonesia is rich in biodiversity for treatment of diabetes and other disorders. Following the Amazon rain forests, Indonesia has the second greatest biodiversity in the world, reflected in the high number of indigenous medicinal plants. To promote biodiversity for the benefits of public health, Indonesia needs to assist pharmacists and scientists to develop proper intellectual property rights (IPR), indigenous knowledge, benefit sharing, efficacy and safety for the further development of Indonesian medicinal plants.

So far, the country lacks this kind of legislation. Jamu has been handed down from one generation to the next based on traditional knowledge and community experience. When new plant-derived therapeutics based on indigenous knowledge are explored, it is important that the benefit be returned to native populations and the local governments from which the research material was obtained.

Lessons Learned

This work provides a scientific basis for the use of pasak bumi root and black pepper fruits in obesity-associated diabetes mellitus. Further studies of both plants should be conducted to investigate their cellular mechanisms as well as the isolation of their bioactive constituents.

Future Plans

It is expected that the bioactive components of the plants could become lead compounds in the field of new drug discovery as well as functional foods. To expand the project, the findings of this study could be used to promote the traditional application of jamu in treating obesity-related diabetes diseases. We plan to publicize this encouraging result with other research institutes in Asia through workshops and international seminars.

Publication

- Lahrita, L., Kato, E. & Kawabata, J. (2015). Uncovering potential of Indonesian medicinal plants on glucose uptake enhancement and lipid suppression in 3T3-L1 adipocytes. *J. Ethnopharmacol.* 168, 229-236.

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The author thanks the Elsevier Publisher for the opportunity to disseminate this seminal work to a wider scientific community in the field of traditional medicine for the benefit of public health.

**From the Traditional Recipe
to the Modern Remedy: The
antitumoural activity of the
small burr parsley
(*Caucalis platycarpos*)**

Croatia

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Summary

While studying old manuscripts of folk pharmacopoeias for the treatment of diseases, written mostly by Croatian priests in past centuries, we found a recipe for the preparation of a remedy used in cancer treatment. It included instructions for its use. The basis of the remedy was a plant which we identified as small burr parsley *Caucalis platycarpus* L. The results of initial experiments, performed at the M.D Anderson Hospital and Tumor Institute, Houston, Texas, USA, demonstrated an antimetastatic effect as a result of the stimulation of the host's immunological system. During the last ten years many additional experiments have been performed. While some results were published, most experiments were performed for the purpose of obtaining the documentation needed to meet the registration procedure and EU Pharmacopoeia requirements. Under

the trade name Primus[®], the product passed the standard legal procedure in Croatia, a license was obtained and the drug was put on the market. A company, Fitofarmacija d.o.o., based in Zagreb, was founded with the intention of developing and producing drugs based on the plant. The firm grows *C. platycarpus* a rare plant, and develops and produces Primus[®], a herbal medicine. Primus[®] is recommended as an adjuvant therapy for patients whose immune system has been weakened by chemotherapy and radiotherapy for tumoural diseases. It is also recommended for persons liable to recurrent infections. Its active compounds strengthen the non-specific resistance of the body by increasing the number and activity of the cells of the immune system. Recently the remedy has been registered and classified as a food supplement.

Background and Justification

Our experience of developing the plant remedy based upon the old recipe books is unique in Croatia, there being no similar experience based upon such sources so far, although the potential for such investigations is strong. Before the beginning of the project dealing with the antiproliferative properties of the small burr parsley we had been exploring many old traditional pharmacopoeias (called "ljeکارuše" in the Croatian language) written in Croatia between the 14th and 19th centuries. During this screening process we noticed and singled out some which we considered worth testing (Fig. 1).

Among them was small burr parsley, *Caucalis platycarpus* L., an annual plant that grows in the Mediterranean region and central Europe (Fig. 2).

23. *Spiz.* Iko isanu (čeljadetu) od udarca ili kakve namete (nepozna) nametne se oivina, koja je spize, veliku muku podnese i ritko ritko prikuca. Spiz more napasti svaki dio tela, a najčešće dote na lice, na usta ili nos. Ženam na sasu jednu ili drugu, ritko na obe. Spiz se nove, jer spize - isida živo meso. Nema melemu koji se ne privija. Bobemite pita pomoći u svakoga, u nikoga je ne nakazi.

U ovoj bolesti - kažu - da je jedini lik trava, podlanica".

Podlanica je slična dlanu a raste u šenici. Travi trakovu po zemlji, ka' prste.

Ove podlanice ućupaju jednu rskovet i rgnjeće, ratim sok njihov isarom u ranu, ono ^{ot} podlanice što ostane, privija na ranu. Triba ponovit nekoliko puta, i - kažu - da pomogne. Ne samo da muka prite, nego i rana raste, ali brango, time ostane, ne do smati se poznaju.

Figure 1: Old recipe recommending small burr parsley as remedy against cancer.



Figure 2: *Caulis platycarpus* L, according to Thome, 1885.

A range of traditional pharmacopoeias from 14th to late 19th centuries are preserved in Croatia. Some of them have been published as facsimile editions while many more are preserved as manuscripts within various institutions or private collections. The unique aspect of this corpus is the fact that preserved sources were written in the Latin, Glagolitic and Cyrillic alphabets. The oldest pharmacopoeias, written in Glagolitic script, are preserved within the Croatian Academy of Sciences and Arts and date from the 14th and 15th centuries. The Croatian Academy of Sciences and Arts pays a lot of attention to their preservation as well as to the editing and publishing of manuscripts of traditional pharmacopoeias, particularly those which were written in scripts are no longer in use. Part of this corpus presents a rich source of data which inspires the need for further investigations and scientific testing of the healing properties described. Here we present a case which illustrates such an approach. The small burr parsley tested in our investigation was identified in the old recipe collection from the manuscript written by a Franciscan priest, Silvester Kutleša, and preserved in the archives of the Croatian Academy of Sciences and Arts. In the recipe the plant was proposed for use in the treatment of certain tumours. Tracing this information, we performed a number of experiments which demonstrated the experience described in the pharmacopoeia in question.

The first issue involved was to prove that a water extract from small burr parsley has an antiproliferative effect. The next was to explore the mechanism of its activity. The third element was to make a detailed analysis of its active constituents. As the plant is relatively rare and poorly described in the literature, some of its morphological, anatomical and growth characteristics had to be studied with an aim to its correct cultivation. For the purpose of the registration of the plant remedy all the demands of the European pharmacopoeia (a monograph of the plant, methods for its qualitative and quantitative analysis, elements for microscopy of powdered material, stability, toxicology, etc.) had to be answered.

Description

Small burr parsley contains several chemical groups known as substances with immune-stimulating properties. About 30 phenolic compounds (flavonoids and phenolic acids) were structurally identified and characterized. Their activity is based upon enhancing a non-specific immune response by increasing the number and activity of certain immune system cells. Various experiments were performed on different animal tumour models which resulted in the demonstration of this anti-tumoural activity. A set of experiments proposed by the European pharmacopoeia were performed to meet the demands for the registration of the remedy. Finally, the remedy, named Primus[®], was registered and

distributed throughout the Croatian pharmaceutical market. The remedy is used as a tea and recommended for persons who are liable to recurrent infections, and as an adjuvant therapy in patients whose immune system is weakened by chemotherapy and radiotherapy for tumoural diseases.

The steps taken during the investigation and implementation process are outlined as follows:

The small burr parsley extract was tested for anti-tumoural activity using the method of artificial lung metastases. The studies used fibrosarcoma induced in mice with 3-methylcholantrene, a spontaneously induced fibrosarcoma. The extract strongly decreased the number of metastases of both tumours (Fig. 3).

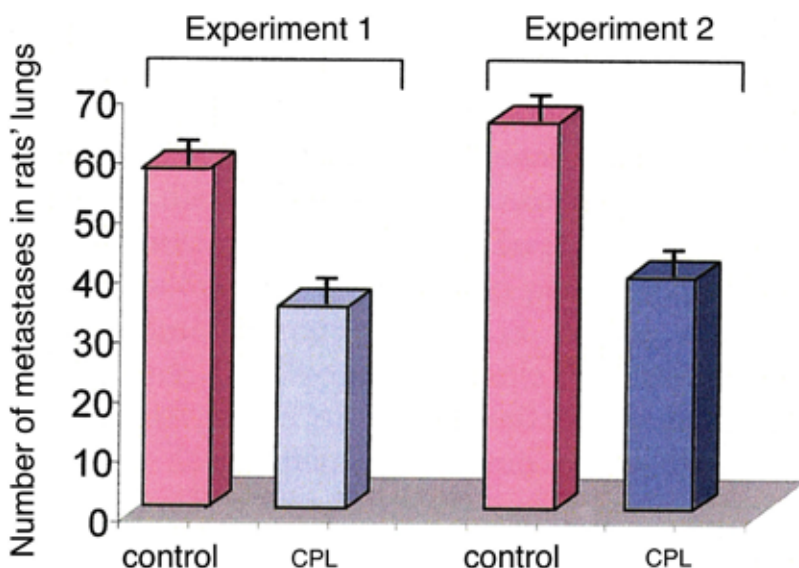


Figure 3: Experiment 1: Fibrosarcoma tumour cells were injected. The second and the fifth day after that, 10 mg of small burr parsley extract *Caucalis platycarpus* L. (CPL) was injected. Control group: experiment no CPL treatment. Experiment 2: The same conditions as in experiment 1 only with double the dose (20 mg) of CPL.

The substance was even more effective if injected before the intravenous injection of tumour cells. These data suggest that the anti-metastatic effect is probably the result of the stimulation of the host's immune system. In addition, no anti-tumoural activity of small burr parsley was observed in mice that had been immunosuppressed by irradiation.

When small burr parsley extract was injected before irradiating the mice, the facilitation of the metastases induced by the immunosuppression was completely abolished. Since one of the two tumours was non-immunogenic, it seems that the mechanism of action of the small burr parsley involved natural killer (NK) cells and/or macrophages in that similar effects were observed. The same *modus operandi* for small burr parsley chemoprotective

effects is proposed. The extract increases spleen weight and cellularity as well as NK activity. It is also a stimulator of the haematopoietic system in general. Antioxidant activities of small burr parsley were also monitored (Fig. 4).

In addition to these experiments, antitumoural activity of small burr parsley was demonstrated on the models which included rats with artificial liver metastases of colorectal cancer, when a comparison was made with the anti-cancer drug levamisole (Fig. 5), and with tumor metastases in the lungs (Fig. 6).

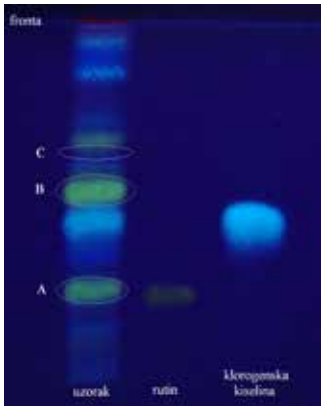


Figure 4: High performance thin layer chromatography (HPTLC) of small burr parsley showing the presence of antioxidants: flavonoids and phenolic acids (left side track).

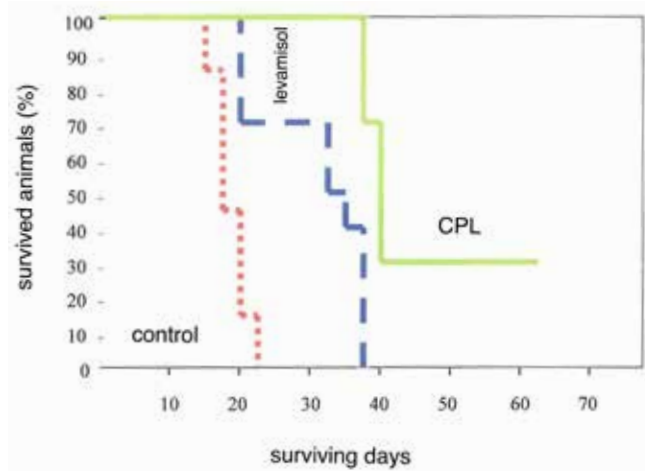


Figure 5: The effect of *Caucalis platycarpus* L. (CPL) and levamisole on the survival of rats with colorectal cancer metastases of the liver.



Figure 6: The effect of small burr parsley on the number of tumour metastases in a rat's lung. Left: Lung section of rat from the control group showing metastases. Right: Lung section of rat from the group treated with small burr parsley with reduced number of metastases.

On the basis of these results, herbal remedy was developed. In order to meet the requirements of commercialization, the plant had to be domesticated for horticultural production (Fig. 7). The product, Primus®, is now available on the market in Croatia (Fig. 8).

The target market includes patients whose immune system has been compromised by chemotherapy and radiotherapy for tumourous diseases, as well as persons liable to recurrent infections..



Figure 7: Cultivating small burr parsley for commercial production.



Fig. 8: The final product, Primus®, now available on the market in Croatia.

Technical data

Toxicological studies on mice showed that the doses proposed for human use are safe. There are now 15 years of experience with the product and no side effects have been noted.

Results

The results therefore correspond to the observations and description in the old traditional pharmacopoeia. Three PhD theses were completed during the research. The results were presented at various scientific meetings and published in the proceedings book (*N. Kujundžić, L. Milas, H. Ito. Antimetastatic effects of the plant extract P-VE-A. Proceedings of 8th Medical Days of Sombor, Sombor, 16-18 June 1985 p. 155*). Many of the results have also been published in scientific journals (see below).

Recently the remedy has been registered and classified as a food supplement.

Partnerships

Those who helped research and developed this successful experience included:

- Nikola Kujundzic, PhD, with expertise in pharmacy (herbal medicine, history of pharmacy), the chemistry of metal complexes of pharmaceutical importance and analytical chemistry. He is retired as Professor of Chemistry and former head of the Department of Analytical Chemistry at the Faculty of Pharmacy and Biochemistry, University of Zagreb, Croatia.
- Luka Milas, MD, PhD, is an expert in tumoural immunotherapy and radiotherapy. He was head of the Department of Radiotherapy, M.D. Anderson Hospital and Tumor Institute, Houston, TX, USA.
- Ivo Basic, PhD, is an expert in tumour immunology. He was Professor of Animal Physiology and head of the Department of Animal Physiology at the Faculty of Sciences at the University of Zagreb.
- Stella Fatovic-Ferencic, MD, PhD, medical historian, head of the Department of the History of Medicine of the Croatian Academy of Sciences and Arts.

The company Fitofarmacija d.o.o. was established exclusively for the production, development and marketing of this plant remedy. It is still operative. The small-scale and expensive production has always made the drug's development difficult.

Impact

Prizes for this study include The Prize of the City of Zagreb in 2008; The Croatian Chamber of Economy has granted Primus® tea product with the title "Croatian Creation" as a mark of high standard of quality (Fig. 9). The product also won a diploma and bronze medal at the Eco World Fest 2009 held in Opatija, Croatia (Fig. 10).



Figure 9: Croatian Chamber of Economy Prize for Primus® tea, 2009.



Figure 10: Diploma presented to Fitofarmacija d.o.o. for Primus® tea, Eco World Fest 2009.

Sustainability

The potential for growing the plant on a wider scale has been demonstrated. Its larger scale cultivation is now planned which may provide a basis for the development of the local community.

The knowledge and experience gained from exploring old pharmaceutical manuscripts (pharmacopoeias) proved the main trigger for further exploration. The experience of traditional medicine, particularly old recipes noted in various manuscripts has strong potential to aid the search for new remedies always subject to testing using modern scientific methods.

Patents

The product is not protected by patent, since it is beyond the financial potential of the company and only the logo is protected (legally named *seal* – it is the visual identity, marked with®).

Lessons Learned

The 1991-95 war affecting Croatia and the very modest financial funds available were the main obstacles in conducting effective research. On the other hand, the prejudices of Western medicine towards traditional medicine were among difficulties the research faced from time to time. In addition, legislation and registration remain limiting factors for the marketing of herbal remedies.

Apart from scientific papers, targeting a technical audience, information about the project was presented to the public through the media, especially regarding the prizes the project was awarded.

Future Plans

Although preclinical studies were sufficient for marketing the product, the clinical proof of the concept is planned, as well as the patenting of the product. Furthermore, the large scale planting of the small burr parsley is planned. Greater and more reliable availability of the source material will mean that export can be considered.

Publications

- Z. Plazonic, A. Mornar, Ž. Maleš N. Kujundzic (2013). Phenolic Content and Antioxidant Activities of Small Burr Parsley (*Caucalis platycarpus* L.). *Molecules*, 18: 8666-8681.
- Z. Plazonic, Ž. Males, A. Mornar, B. Nigovic, N. Kujundzic (2011). Characterization and Quantification of Flavonoid Aglycones and Phenolic Acids in the Hydrolyzed Methanolic Extract of *Caucalis platycarpus* Using HPLC-DAD-MS/MS, *Chemistry of natural compounds*, 47(1):27-32.
- N. Orsolic, M. Bevanda, N. Kujundzic, A. Plazonic, D. Stajcar, M. Kujundzic (2010). Prevention of Peritoneal Carcinomatosis in Mice by Combining Hyperthermal Intraperitoneal Chemotherapy with the Water Extract from Small Burr Parsley (*Caucalis platycarpus* L.), *Planta Medica*, 76: 773-779.
- Z. Plazonic, F. Bucar, Ž. Maleš, A. Mornar, B. Nigovic, N. Kujundzic (2009). Identification and Quantification of Flavonoides and Phenolic Acids in Small Burr Parsley (*Caucalis platycarpus* L.) Using High-performance Liquid Chromatography with Diode Array Detection and Electrospray Ionization Mass Spectrometry, *Molecules*, 14: 7; 2466-2490.
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Antiplasmodial Activities of Fractions and Natural Compounds from *Icacina senegalensis* (Icacinaceae)

Sénégal

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Duration: September 2007 - June 2011

Total cost: USD 7,000 (Grant partially covered by Agence Universitaire de la Francophonie)

Summary

Our project sought to contribute to the fight against malaria in Senegal. The main envisaged application remains both the treatment and prevention of malaria. We conducted our study on an antiplasmodial plant, *Icacina senegalensis*. The properties of this plant were discovered during a previous ethnopharmacological survey conducted in Senegal.

Our experiment in laboratory with *Icacina senegalensis* showed an inhibitory concentration, IC_{50} of $<5 \mu\text{g} / \text{mL}$ on the malaria parasite *Plasmodium falciparum* (pLDH method) and a selectivity index higher than 10 for mouse hepatocytes (Hepa 1-6) and human dermal fibroblasts (NHDF). This result shows interesting antiplasmodial activity without cytotoxicity. Moreover, our finished product could be an effective and well-tolerated treatment against malaria. Orientin, isoorientin, cis-clovamide and trans-clovamide were already identified from this plant during our study. It is interesting to

emphasize the structural differences between these molecules and quinine and artemisinin. Furthermore, the compounds of interest can be produced by total or partial synthesis from the plant material and the antiplasmodial activity optimized by pharmacomodulation.

The raw material has been used by Senegalese traditional healers to cure malaria and no adverse effects have been reported from its use. The absence of toxicity observed by the traditional healers was later confirmed by haemolysis and *in vitro* cytotoxicity tests which showed good tolerance at a concentration 100-fold higher than those we used.

Our experience shows that the “ethno-bio-analytical” approach can provide access to more interesting results in the exploration of traditional medicine. This multidisciplinary approach combining ethnopharmacological surveys and bio-analytical assays deserves to be promoted for a better health in sub-Saharan Africa.

Background and Justification

Despite intensive efforts to control malaria, this disease continues to be one of the greatest public health problems in Africa. There were 247 million malaria cases among 3.3 billion people at risk in 2006, causing nearly one million deaths, mostly children under five years of age. One hundred and six (106) countries were still endemic for malaria in 2009 and of them 45 were within the WHO African region.

In Senegal, interesting progress against malaria has been achieved. The number of deaths due to malaria was estimated as 1678 in 2006 dropping to 577 in late 2009 according to data from the National Malaria Control Programme. However the burden of the disease

still remains a major public health threat. Almost all the most efficient antimalarial drugs are from natural sources (quinine and artemisinin derivatives). The global scope of malaria and the spread of drug-resistant *Plasmodium falciparum* makes the need for improved therapy urgent.

This experience was conducted in Senegal where malaria is still a public health problem and chemoresistance of *P. falciparum* is increasing. In this country, most of the antimalarials used officially are imported from Europe and Asia.

- Main issues involved:
- Assessment and promotion of traditional medicine
- Research of alternative treatment against malaria
- Bio-guided fractionation of natural extracts
- Multidisciplinary collaborative research
- Partnership between scientists from academic fields and local traditional healers

Description

We carried out chemical and bio-analytical methods to evaluate the biological properties of the plant in general and its antiplasmodial activity in particular.

Plants extracts were tested on strains of *P. falciparum* 3D7 Africa (chloroquine-sensitive) and 7G8 Brazil (chloroquine-resistant). Solid-liquid and liquid-liquid extractions techniques were carried out using organic solvents including methanol, methylene chloride and pentane. Samples tested were the defatted IM extract called IM^d, the fraction obtained by liquid-liquid extraction using methylene chloride (IMD) and IMM which constituted the residual polar fraction.

We evaluated the haemolytic activity of extracts and fractions using a haemolytic agent, 5% sodium dodecyl sulphate (SDS) as a positive control. The negative control contained erythrocytes diluted (v/v) with the sterile malaria culture media (MCM). Haemoglobin content in the supernatants was determined by absorbance measurements at 538 nm in a microtitre plate spectrophotometer.

The cytotoxicities of both the extracts and the fractions on mouse liver cells (Hepa 1-6) and normal human dermal fibroblast (NHDF) were also assessed *in vitro* using the MTT colorimetric assay (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide). The assessment was performed in order to determine the selectivity index (SI). The SI was determined as the ratio between the IC₅₀ of cytotoxicity activity (concentration level

at which 50% of the parasite growth is inhibited) to that of anti-plasmodial activity. SI values were assessed with the NHDF cells (IC_{50} cytotoxicity) and on *P. falciparum* 3D7 (IC_{50} antiplasmodial activity). The IC_{50} was calculated using a nonlinear regression method. The results from the three independent experiments were reported as means with their standard deviations (SD).

The purification of the fractions was done using reverse semi-preparative chromatography using a C18 column type of 1 cm internal diameter. Biological techniques used comprised an assessment of the viability of the parasites in the presence of natural extracts at different concentrations. Thus, we used the enzymatic immunoassay technique based on the detection of *Plasmodium* Lactate Dehydrogenase (PLDH). PLDH is a metabolic enzyme produced during the parasite's development and is a good indicator of its viability.

Although time consuming, a bio-analytical and transdisciplinary approach was implemented in order to select and fractionate the natural active extracts on different biological models and *in vitro* cultures of *P. falciparum* respectively. The analytical approach adopted in this work enabled the isolation and the purification of active fractions from *Plasmodium* strains.

To optimize the biological activity and modulate the toxicity of such molecules, it appears useful to determine the chemical structure of the molecules previously purified. In our study, we highlighted the contribution of both modern chromatography and detection techniques such as Ultra High Performance Liquid Chromatography (UHPLC) with different detection systems.

Moreover, scientific studies such as ours will improve the methods of preparation of traditional medicines, providing scientific information on their potential toxicities and establish modern analytical methods for characterization. Finally, upon completion, this work will contribute to the enhancement of development of the Senegalese Pharmacopoeia.

Steps taken in the innovative and implementation process:

- Ethnopharmacological survey involving traditional healers and the local population
- Selection and identification of plants of interest
- Verification whether the selected plant is on the IUCN red list
- Production of bio-active extracts and fractions
- Haemolytic activity on red blood cells
- Antiplasmodial and cytotoxicity tests (*Plasmodium falciparum*, Hepa 1-6 cells, NHDF cells)
- Bio-guided chemical characterization of active extracts
- Separation, purification, isolation and identification of bioactive compounds.

Results

Icacina senegalensis (Icacinaceae) leaves (Fig. 1A) used against malaria and symptoms suggestive of possible malaria, were harvested in October 2007 in the plant's natural habitat in the province of Medina Sabakh (Senegal) (Figure 2).

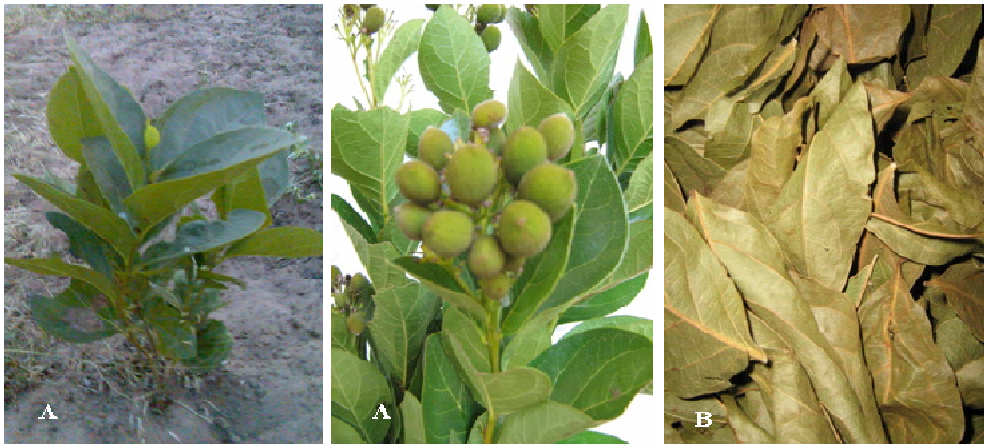


Figure 1: *Icacina senegalensis* at different development stages (A) and dried leaves (B).



Figure 2: Map of Senegal showing the area where the plants were collected.

I. senegalensis revealed no haemolytic effect on red blood cells *in vitro*. Malaria Culture Media (MCM) was used as a negative control. These first results, which were not quantity-dependent, tended to confirm the traditional use, orally, by way of the decoctions and macerations of these natural extracts by the local and traditional tribes and without any side effects.

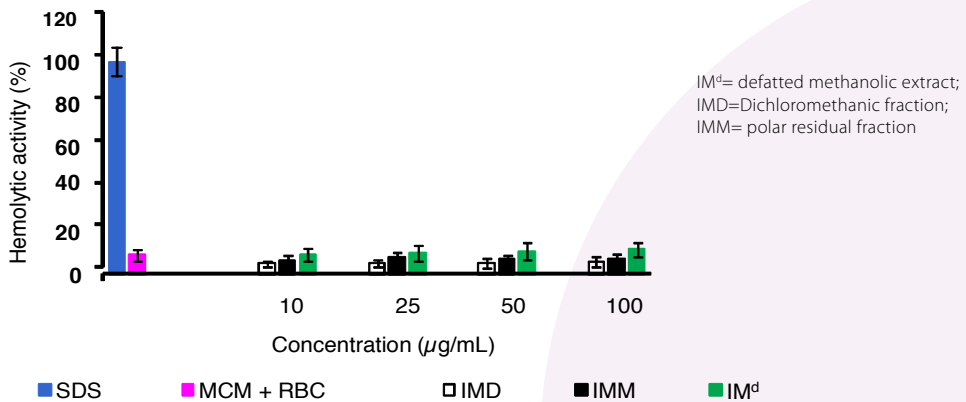


Figure 3: Haemolytic activity of extract and fractions.

Samples	IC ₅₀ ± SD (µg/mL) on <i>P. falciparum</i>		*IC ₅₀ (Hepa 1-6) (µg/mL)	IC ₅₀ (NHDF) (µg/mL)	*SI
	3D7	7G8			
IM ^d	4.7 ± 0,2	8 ± 1	133 ± 20	> 500	> 106
IMD	0.9 ± 0,2	4.1 ± 0.1	122 ± 4	> 500	> 500
IMM	14.2 ± 0.7	32 ± 2	447 ± 16	> 500	> 35
Chloroquine (nM)	44 ± 1	658 ± 14	> 800	> 800	> 18

*IC₅₀= Inhibitory Concentration 50%; SI= selectivity index

Table 1: Antiplasmodial activity, cytotoxicity and selectivity indexes of samples IM^d, IMD and IMM (n=3).

According to the WHO recommendations and previous works [32-34], anti-plasmodial activities of plant extracts were classified as follows: highly active extracts with IC₅₀ < 5 µg/mL, promising activity at 5-15 µg/mL, moderate activity at 15-50 µg/mL and inactivity at > 50 µg/mL.

With the aim of finding active subfractions and consequently identifying the substance(s) active against *P. falciparum*, many bio-analytical strategies were adopted, followed by a semi-preparative chromatographic method that was also used to produce five fractions from the active IM^d fraction. The chromatographic profile is presented in Fig. 4.

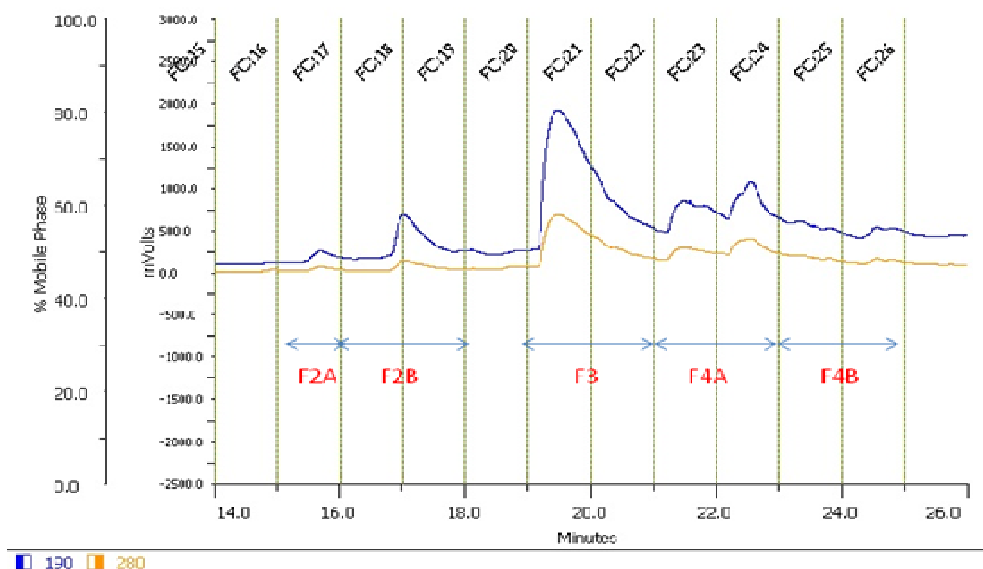


Figure 4: Chromatographic profile of defatted methanolic extract (IM^d).

Fractions F2A, F2B, F4A et F4B collected from IM^d were tested on *P. falciparum* strains 3D7 and 7G8. The results obtained are presented in table 2:

Fractions	IC ₅₀ 3D7	IC ₅₀ 7G8
F2A	0.73 ± 0.02 µg/mL	3.59 ± 0.24 µg/mL
F2B	4.52 ± 0.03 µg/mL	0.75 ± 0.03 µg/mL
F4A	3.78 ± 0.10 µg/mL	4.61 ± 0.41 µg/mL
F4B	3.53 ± 0.28 µg/mL	2.06 ± 0.41 µg/mL

Table 2: Antiplasmodial activity (IC₅₀) of fractions F2A, F2B, F4A and F4B (n=3) of defatted methanolic extract (IM^d).

Partnerships

The project was conducted at University Cheikh Anta Diop in partnership with the Faculty of Pharmacy of University of Strasbourg in France. The weaknesses between partners were strengthened by an efficient communication approach using email exchanges, meetings and periodic reports on the project.

Impact

The project was conducted to a PhD degree level, obtained from Strasbourg University (France) However, the originality of the project and results obtained were emphasized during many national and international congresses, specialist meetings, etc.

The plant studied is a native species and could be cultivated on a large scale. The cultivation would provide the raw material and economic resources for local populations.

- This experience is an original approach from traditional use to scientific evaluation of the antiplasmodial activity of *Icacina senegalensis*.
- The route of administration is the traditional oral one and should allow a simple mode of administration and excellent bioavailability of the active ingredient(s).
- For the “user”, no adaptation will be required to use the product. However, it remains desirable that the cost of the finished product be as low as possible because of financial constraints and poverty in user countries. This status should allow them access to the new treatment. Furthermore, the solidarity fund grants or assistance to development will also promote free access to this product for disadvantaged populations.

- The extraction of active principles requires large amounts of expensive and toxic organic solvents. However the aqueous decoction could be an alternative “ecological” way of obtaining products of interest.
- A final advantage is the delay in the occurrence of chemoresistance of *Plasmodium* with this natural antimalarial.
- The part of the plant (endemic in Senegal) that contains active ingredient(s) is the leaf which allows intensive cultivation under industrial and sustainable development in the country that would be the main producer and supplier of the raw material. The opportunity to cultivate the plant on a large scale in Senegal would generate employment.

Replicability

Until now there have been no policy implications. But the protection of traditional knowledge must be ensured by major political decisions.

Our experience has not brought about changes in legislation for the moment but it is urgent to define mechanisms and legislation to protect traditional medical knowledge which constitutes a national legacy. We hope that further discussion will be engaged in by the authorities on account of our findings.

No patents were applied for although a patent project existed but was never finalized.

- The innovative experience is important for other regions particularly in Africa where malaria remains a major public health threat.
- This plant grows in western and central Africa and therefore the raw material would be accessible in many countries.
- Our findings were exploited by Nigerian colleagues (University of Calabar, Nigeria) to run *in vivo* antiplasmodial activity on *Icacina senegalensis* extracts in 2014.

Lessons Learned

Obstacles faced and steps taken to overcome them:

- Availability of the plant material with the plant being harvested during and just after the rainy seasons;
- Financial resources to conduct all experiments necessary at the time required;

- Duration of the project to obtain the first results;
- Inconsistent governmental technical help during the research.

Preparing public perception for acceptance of the innovation:

- It is planned to share the results with the public after the finalization of the innovation (*in vivo* studies, analytical monograph for quality control, etc.);
- A communication strategy will be adopted in relation to this with the public health authorities.

Future Plans

Plans for further improvement and expansion of the project:

- *In vivo* studies with natural extracts and hemi-synthetic molecules obtained from those isolated;
- Metabolomic approach to characterizing the natural extracts' chromatographic profiles;
- Extract profiling according to the main seasons to ensure the quality of the plant in any given season (rainy season and dry season);
- Validation of an analytical monography for this plant to establish a modern quality control tool;
- A multidisciplinary team research on traditional medicine is being set up with a phytochemist, analytical chemist, biologist, toxicologist, biostatistician, etc.;
- Purification and identification of other active secondary metabolites of the plant;
- Research on other plants traditionally used to cure neglected diseases which constitute public health problems in Africa in general and in Senegal in particular.

Plans for collaborations and sharing of the results with other organizations/countries:

- Open access publication;
- Participation in national and international congresses;
- Organisation of national, regional and sub-regional workshops;
- Creation of open access database for sharing experiences and information exchanges between researchers and local population.

Publications

- Sarr S.O., Perrotey S., Fall I., Ennahar S., Diop Y.Mb., Candolfi E., Marchioni E. 2011. *Icacina senegalensis* A. Juss. (Icacinaceae), traditionally used for the treatment of malaria inhibits *in vitro* *Plasmodium falciparum* growth without host cell toxicity. *Malaria Journal* 2011, 10:85 doi:10.1186/1475-2875-10-85.
- Sarr S.O., Zhao M., Perrotey S., Diop A., Fall I., Fall A.D., Ndiaye B., Diop Y.M., Candolfi E., Marchioni E. Activité antiplasmodiale d'*Icacina senegalensis* et isolement, purification, identification du cis et du trans-clovamide, de l'orientine et de l'isoorientine. Oral communication. VIIIèmes Journées des Départements de Pharmacie de l'UCAD, Dakar (Sénégal), 11-12 December 2013, p.69.
- Sarr S.O., Perrotey S., Nagarathinam C Ennahar S., Fall I., Candolfi E., Marchioni E. Intérêt de l'approche ethno-bio-analytique dans la recherche de propriétés antiplasmodiales d'une plante de la Pharmacopée Traditionnelle Sénégalaise. Réunion conjointe de la Société Française de Parasitologie, de la Société Française de Mycologie Médicale et du Groupement des Protistologues de Langue Française, Strasbourg (France), 18-20 May 2011.
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Network Target: A novel approach to deciphering traditional Chinese medicine

China



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Summary

Having been practiced for thousands of years, traditional Chinese medicine (TCM) has accumulated a wealth of clinical experience and plenty of herbal formulae (*Fang-Ji*) that typically consist of various medicinal herbs to balance the disordered human body. It is challenging to access such clinically-effective but mechanistically-unclear medicine due to the complexity of both the chemical compositions of, and the biological systems targeted by, TCM. To tackle the puzzle, we proposed a new approach of the “network target, multi-component drug” that seeks to update the current “one target, one drug” paradigm. The core idea of the “network target” is to construct a biological network that can be used to capture complex diseases as well as TCM syndromes (*Zheng*) at the system level. Such a network is then used as the therapeutic target to which multi-component remedies such as herbal formulae are applied. Based on the network target concept, we have created a set of methods to infer the network associations between herbs, compounds, biomolecules,

phenotypes, diseases and TCM syndromes. By conducting clinical and experimental verifications, we demonstrated the good performance of the methods in constructing the biological networks specific for Cold/Hot Syndrome for the first time, identifying Cold / Hot Syndrome-associated metabolism-immune biomarkers and tongue coating microbiota in a cohort of gastritis patients, connecting Cold/Hot Syndrome and related diseases in the network, and revealing new indications, active compounds, synergistic combinations and network regulation mechanisms for classic herbal formulae including *Liu-Wei-Di-Huang* and *Qing-Luo-Yin*. The results suggest that the network target-based methodology promises to be an innovative way to explore and understand TCM. With the arrival of “big data” and the era of precision medicine, the network target approach ushers in a new research field, TCM network pharmacology, which permits exciting advances in traditional medicine and will contribute to narrowing the gap between Eastern and Western medical practices.

Background and Justification

Holistic thinking has long been central to TCM. TCM has rich clinical experience in using herbal formulae that typically consist of various medicinal herbs to restore imbalances in patients suffering from different TCM syndromes. This holistic medicine meets the philosophy of curing diseases in a systematic and personalized manner. However, is difficult to be understood by current reductionist research strategies that treat both biological entities and herbal ingredients separately. By reason of lacking appropriate methods, the biological basis underlying TCM syndromes and herbal medicine is still

unclear. Meanwhile, the “one gene, one target, one drug” approach currently use widely in Western medicine is being questioned due to its reductionist thinking, trial-and-error methodology and high failure rates.

We are now in the era of big data. To update the current “one target, one drug” approach and find a way to unveil traditional Chinese medicine, we proposed a new approach of “network target, multi-component drug” to shift the research paradigm of TCM (Fig.1). The human body can be viewed as a living system with interconnected networks of molecular entities, and its regulation requires multi-component interventions. The core idea of the “network target” is to use a network to capture the complex biological systems of diseases, and then treat the disease-specific biological network as a therapeutic target. With the rapid growth of various knowledge databases, omics technologies, bioinformatics and systems biology, the network target approach is able to take advantage of big biological data, integrate computational and experimental methods, and develop a holistic way to study TCM.

The novel network target approach with systems thinking and powerful computing capacity can help make sense of traditional practices and explore the modern indications of herbal medicine, showing promise in unveiling how the herbal medicines work and providing valuable insights into current medical study. Network target methods can be widely applied in constructing biological networks for TCM syndromes and related diseases, elucidating mechanisms of actions and combinatorial rules of TCM herbal formulae, and identifying targets and actions of herbal ingredients. This novel approach also leads to a new cutting-edge research field, TCM network pharmacology (TCMNP).

Description

The network target approach attempts to map TCM syndromes and herbal formulae to the complex biological systems of the human body in a network manner. To meet this goal, we have created dozens of network analysis tools and integrated into a platform of TCM network pharmacology (Table 1). The platform is capable of predicting genes relating to diseases and target profiles of herbal compounds, and making discoveries from the network connection of disease genes and herb targets. For example, with the idea that “like attracts like”, a drugCIPHER algorithm performs well in *de novo* prediction of target profiles for any herbal compounds by inferring the global network association to all available US Federal Drug Authority (FDA)-approved drugs including drug chemical similarity, drug-target interaction, and protein-protein interaction. A sibling method, CIPHER, also shows good performance in gene prediction for diseases or even *de novo* gene prediction for TCM phenotypes. Other methods have been developed to identify

biomarkers or active herbal ingredients, reveal drug-gene-disease associations, and screen synergistic herbal compounds by evaluating feedback, redundancy and modularity properties from target interactions. These methods make the network target approach an innovative and powerful way to explore the complexity of both TCM syndrome and herbal formulae, to reveal biomarkers, active components, and mechanisms of action of TCM. Overall, two facets of the network target approach are, making full use of current big data to accelerate TCM research; and the outputs can improve the efficiency of biomarker and drug discovery

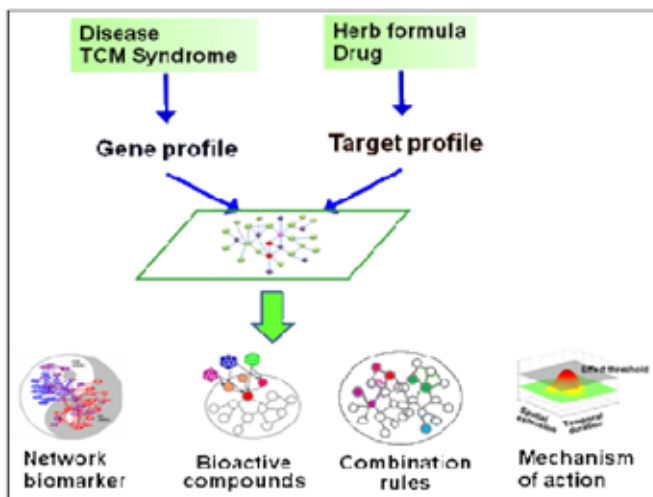


Figure. 1: Schematic diagram of the network target approach and its applications on traditional Chinese medicine.

Concepts and methods	Description	Year	Reference
Network target concepts	Proposed the relationship between TCM Syndrome and biological networks	1999	Li. The First Academic Annual Meeting of the China Association for Science and Technology. 1999
	Proposed the network-based TCM research framework	2007	Li. J Chin Integrat Med 2007, 5: 489-493.
	Proposed the "Network biomarker" concept	2007	Li. JTCM 2009,50:773-776.
	Proposed the "Herb network-Biological network-Phenotype network"	2009	Li. Curr Bioinform 2009, 4:188-196
	Proposed the "Network target" concept	2011	Li. China J Chin Mater Med 2011, 36:2017-2020; Li et al. BMC Syst Biol 2011, 5(S1): S10
	Proposed the TCM network pharmacology	2011	Li. China J Chin Mater Med 2011, 36:2017-2020; Li & Zhang. CJNM 2013, 11:110-120
	Proposed the "Network target model"	2015	Li. Science 2015;350(6262):S72-S74
LMMA	Disease-specific biological network construction	2006	Li et al. Bioinformatics 2006,22:2143-2150
CIPHER	Network-based prediction for disease genes	2008	Wu et al. Mol Syst Biol 2008,4:189
drugCIPHER	Network-based prediction for drug targets	2010	Zhao & Li. PLoS ONE 2010,5:e11764
DMIM	Herb network construction and co-module analysis	2010	Li et al. BMC Bioinformatics 2010,11(S11): S6
NADA	Network-based assessment for drug action	2010	Li et al. Chin Sci Bull 2010,55: 2974-2980
Drug combination model	A formal model to analyze drug combinations	2010	Yan et al. BMC Syst Biol 2010, 4: 50
CSPN	Disease-specific pathway network construction	2010	Huang & Li. BMC Bioinformatics 2010,11(S1): S32
ClustEx	Disease-specific responsive gene module identification	2010	Gu et al. BMC Syst Biol 2010,4:47
NIMS	Network-based identification of synergistic drug combinations	2011	Li et al. BMC Syst Biol 2011, 5(S1): S10
CIPHER-HIT	Modularity-based disease gene and subtype prediction	2011	Yao et al. BMC Syst Biol 2011, 5:79
comCIPHER	Drug-gene-disease co-module analysis	2012	Zhao & Li. Bioinformatics 2012, 28: 955-961
DGPsubnet	Drug-gene-disease coherent subnetwork analysis	2014	Wang et al. CPT Pharmacometrics Syst Pharmacol 2014;3:e146
GIFT	Global inference of drug substructure-target domain interaction	2015	Zu et al. Bioinformatics 2015;31:2523-9
SidePro	Network-based inferring proteins related to drug side effects	2015	Li et al. Quantitative Biology 2015

Table 1: Network target concepts and method established in author's laboratory.

Results

As summarized in Table 2, the network target approach can make sense of TCM syndromes and explore the modern indications of herbal medicine. For example, the traditional use of herbal formulae is to treat TCM syndromes, e.g. the Cold/Hot Syndrome, a pair of Yin-Yang imbalance conditions. Correspondingly, most medicinal herbs can be categorized into Cold, Cool, Warm and Hot properties for tailored treatment of individual syndromes. Taking Cold and Hot as examples, as illustrated in Fig. 2, a biological network for Cold/Hot Syndrome is first constructed. With this network in hand and subsequently conducting measurements including microarray and deep sequencing, we identified the Cold/Hot related metabolism-immune imbalance patterns, candidate network biomarkers, as well as two groups of tongue-coating microbes in a cohort of chronic gastritis patients. We further found that hub genes in Hot network may get involved in the process from inflammation to cancer. A case from one of our cooperative works is, IL1 β /IRAK1 signaling can promote gankyrin expression in inflammation-enhanced hepatocarcinogenesis (Su et al., 2015). These results indicate that the TCM Cold/Hot concept may have an imbalanced network as its molecular basis, providing a new way to subtype complex diseases.

For the treatment, we found that some herbs with Cold or Hot properties can differentially restore the Hot or Cold network imbalance. For instance, a Hot-Cooling formula, *Qing-Luo-Yin*, can synergistically suppress the cytokine and VEGF pathways to clear away Hot in the treatment of inflammation and angiogenic disorders. A Yin-nourishing formula, *Liu-Wei-Di-Huang*, can restore the metabolism-immune pathways by groups of bioactive ingredients. We also predicted modern indications of this formula by its target network. Several anti-angiogenesis, anti-cancer, anti-tumorigenesis compounds or synergistic combinations were subsequently identified. The results suggest that the network target approach could help reveal the “component–target/biomarker–indication” relations of herb formulae and accelerate TCM-based precision medicine.

	Subject investigated	Network target contents	Year	Reference
TCM Syndrome	Cold Syndrome / Hot Syndrome	Network construction, topological temperature measure	2007	Li et al. IET Syst Biol 2007, 1: 51-60.
	Gastritis patients with Cold / Hot Syndrome	Imbalance of metabolism-immune, network biomarker	2013	Li et al. Sci Rep 2013;3:1543
	Tongue coating of Cold / Hot Syndrome patients	Cold / Hot microbe network	2012	Jiang et al. Sci Rep 2012;2:936
Herb medicine	Qing-Luo-Yin (Hot-Cooling formula) Wen-Luo-Yin (Cold-Warming formula)	Network relationship between Herbal formula and Cold / Hot Syndrome	2007	Li et al. IET Syst Biol 2007, 1: 51-60.
	3865 herbal formulae including Liu-Wei-Di-Huang	Herb network construction and herb-target-diseases co-module	2010	Li et al. BMC Bioinformatics 2010,11(S11): S6
	61 herbs or compounds including Qing-Luo-Yin ingredients	Synergistic combination screening	2011	Li et al. BMC Syst Biol 2011, 5(S1): S10
	Qing-Luo-Yin	Jun-Chen-Zuo-Shi network regulation	2013	Zhang et al. ECAM 2013:456747
	Man-Jing-Zi (<i>Vitex rotundifolia</i>), a Qing-Luo-Yin compatible herb	New activity of anti-tumor angiogenesis and target network	2013	Zhang et al. ECAM 2013: 278405
	Liu-Wei-Di-Huang formula	Network regulation mechanism, active compounds, modern indications	2014	Liang et al. Mol BioSyst 2014,10:1014-22
	Ge-Gen-Qin-Lian decoction	Network effects on type 2 diabetes	2014	Li et al. ECAM 2014:495840
	Tetramethylpyrazine from <i>Ligusticum wallichii</i> Franchet (Chuan-Xiong)	Activity of ameliorating methotrexate-induced oxidative organ injury	2015	Zhang et al. J Ethnopharmacol 2015
	Wu-Tou decoction	Network effects on rheumatoid arthritis	2015	Zhang et al. Sci Rep 2015; 5: 9463
	871 TCM doctor's clinical prescriptions including Qing-Luo-Yin	Network effects on rheumatoid arthritis	2015	Li et al. ECAM 2015:451319

Table 2: Applications of the network target approach from the author's laboratory.

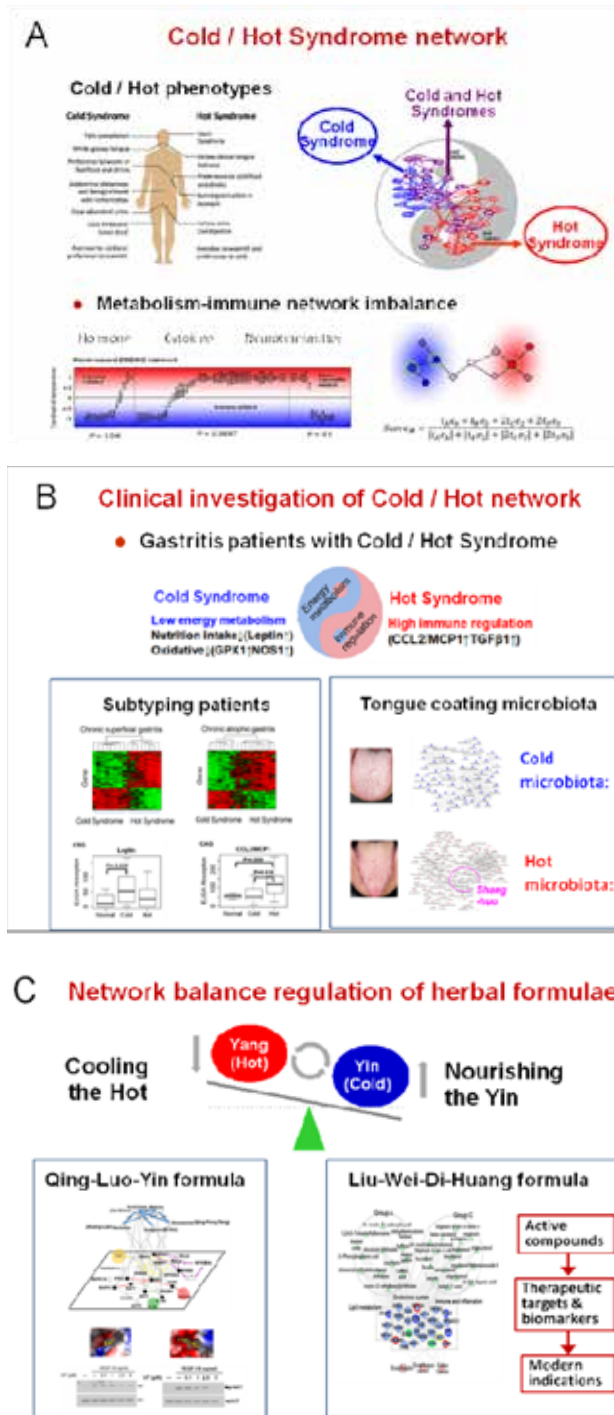


Figure 2: Network-based case studies on TCM Syndromes and herbal formulae.

Partnerships

We collaborated with hospitals to validate the network biomarkers of TCM syndromes as candidates in subtyping diseases such as inflammation disorders and cancer and with drug companies by patent transformation to discover TCM-derived natural products and elucidate their mechanisms of action.

Impact

The author, Shao Li won the National Outstanding Young Scientist Award, China, in 2012 for his contributions to the field of “new technology and new methodology in traditional Chinese medicine”. His works have been positively evaluated by over 2000 papers by Chinese and international researchers, including publications in journals such as *Nature*, *Cell* and *Science Translational Medicine*.

He has also been considered as a pioneer in TCM systems biology and network pharmacology by some publications (e.g. *Indian Journal of Traditional Knowledge*).

He is Chairman of the Specialty Committee of network pharmacology, World Federation of Chinese Medicine Societies (2015), vice chair of the Specialty Committee of network pharmacology, Chinese Pharmacological Society (2014), leading guest editor of the first special issue on “Network Pharmacology in Traditional Chinese Medicine” in *Evidence-Based Complementary and Alternative Medicine* (2012-2013), and the leading scientist of the first book entitled “Network Pharmacology: A New Way to the Modernization of Traditional Chinese Medicine” published by China Science and Technology Press (2014).

He has delivered keynote speeches and invited talks at more than 20 international conferences. His works made the headlines in *The Wall Street Journal* with the title of “New data on ancient remedies” on 4 November 2014. This news was selected as one of “The World top 10 news of traditional Chinese Medicine 2014” by the World Federation of Chinese Medicine Societies.

Replicability

The author and his colleagues have had seven patents awarded, while another five patent applications are currently under review. In addition, copyright has been granted on four pieces of computer software developed by the group.

Lessons Learned

The main obstacle faced is the complexity both in TCM diagnosis (syndrome) and treatment (herbal medicine). TCM syndrome focuses on the observation of the human body at the macro-level. Herbal medicine always consists of many herbs with a large number of ingredients. Currently, the understanding of the biological basis of TCM diagnosis and treatment is far from complete. To tackle this complexity, the network target approach uses the biological network, a representation of the complex biological systems of patients, as a common basis that enables information exchange between traditional and modern medicines. Then, the network association analysis can be created to make full use of current big data to reveal the biological basis underlying TCM syndromes and herbal formulae, which can also narrow the gap between traditional and modern knowledge and practices.

The network target-based TCM network pharmacology is essentially an interdisciplinary frontier in both TCM and modern medical research fields. The development of this innovation is dependent upon a cooperative and multidisciplinary team. Now the approach is being accepted by a growing number of people from different disciplines, especially TCM practitioners, pharmacologists, clinical doctors, and researchers from related fields.

Future Plans

For the network target methods, we will continue to improve the prediction accuracy of computational algorithms by integrating multilayer omics data, more comprehensive databases, and network-level experiments to make the discovery of TCM more efficient.

For the application, we will conduct more clinical investigations to find new biomarkers that combine TCM phenotypes and Cold / Hot network molecules to subtype patients, and make the pattern of “component–target/biomarker–indication” much clearer to improve the modern use of herbal formulae.

For the collaboration of the novel approach, we will work closely together with TCM and Western medicine doctors, as well as biomedical, pharmacological and computational scientists to make the discovery of TCM more productive and more prosperous.

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Zedupex, an Anti-herpes Herbal Medicine for Management of Human Herpes

Kenya

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Duration: January 2001 to April 2006

Total cost: USD 520,000

Summary

The KEMRI/JICA Infectious Diseases Research Project (2001–2006) was a collaborative study between the Kenya Medical Research Institute (KEMRI) and the Japan International Corporation Agency (JICA), the main purpose of which was to improve and enhance research and production capacity, human resources and human/information networks at KEMRI in collaboration with other institutions in Kenya and the region. The aim of the project was to strengthen effective control of targeted diseases (HIV/AIDS, viral hepatitis and opportunistic infections) through the identification of new management therapies and tools. A sub-project carried out by the Plant Drug Research Group (PDRG) was to produce effective, safe and chemically well-characterized herbal-based products that could be developed for use against opportunistic viral infections caused by the herpes simplex virus. Herpes is caused by two

sub-types of the human herpes simplex virus (HSV-1 and HSV-2) which share a 50% gene sequence homology. The viruses can initiate and establish recurrent orofacial lesions and are clinically indistinguishable from initial episodes of genital herpes.

Studies on HSV seroprevalence reveal rates of up to 80% in Africa. Increased prevalence rates have been attributed to immunosuppression, mainly due to HIV/AIDS infection, when HSV is a major opportunistic pathogen. Even though the chemotherapy of HSV infections has improved tremendously over the years, the management of these infections in Africa has been hampered by the high costs of the drugs. Identification of local therapies for management of the infection is therefore paramount given that over 60% of the population depends on herbal medicine for primary health care.

Background and Justification

Herpes is a major health concern around the world and in Africa in particular. In sub-Saharan Africa, seroprevalence of herpes simplex virus type 2 (HSV-2) is among the highest in the world, and records of 50–80% have been registered in population-based studies (Looker *et al.*, 2015). In Kenya, according to the 2009 Kenya AIDS indicator survey report that provided the first report on herpes prevalence in the country, over 6 million adults were HSV-2-infected (Mugo *et al.*, 2009). In the region, HSV is the leading cause of genital ulcer disease (GUD), an infection that enhances the infectiousness of HIV-positive subjects and the susceptibility of HIV-negative subjects to HIV infection. The widespread nature of herpes infection in sub-Saharan Africa is attributable to malnutrition on one hand and immunosuppression due to HIV/AIDS on the other, compounding factors which contribute to the manifestation of the opportunistic nature of the HSV.

Despite the strengths of the epidemiological evidence of herpes presence in sub-Saharan Africa, treatment is not readily available even though great achievements have been realized in the development of new therapeutic agents for its management. In instances where these drugs are available, they are too expensive and therefore unaffordable by most people in the region. For this reason, there is a need for the development of affordable therapeutic agents to address the problem in Africa. Another reason is the fact that most widely used drugs for prophylaxis and treatment of HSV infection are acyclovir-based but unfortunately, they have high toxicities and long-term therapy associated with their use leads to the development of clinically resistant viral strains.

The KEMRI/JICA Infectious Diseases Research Project (2001-2006) was a collaborative study between Kenya Medical Research Institute (KEMRI) and the Japan International Corporation Agency (JICA), aimed at strengthening medical research. The main goals of the project were to identify ways to strengthen effective control of problematic diseases, such as HSV, in the region through identification of new management therapies and tools. The Plant Drug Research Group (PDRG) at KEMRI thus aimed at producing effective, safe and chemically well-characterized herbal-based products that could be developed for use against opportunistic viral infections caused by herpes causing viruses.

Description

Herpes is a virus infection of the skin and mucosa caused by the virus, human herpes simplex virus (HSV), sub-type 1 (HSV-1) or sub-type 2 (HSV-2) and manifests as cutaneous skin lesions of the genitals (genital herpes) and/or oral facial infections (oral labial herpes) (Fig. 1). The two sub-types of the virus are among the most common causes of viral infections of humans worldwide, resulting in a spectrum of illnesses ranging from asymptomatic to life-threatening disease. These two viruses share a 50% gene sequence homology and can initiate and establish recurrent orofacial lesions and similarly cause clinically indistinguishable first episodes of genital herpes. Herpes can be transmitted through sexual contact, kissing and skin-to-skin contacts.

Oral labial herpes



Genital herpes



Figure 1: Symptoms of oral labial herpes (left), and genital herpes in the male (centre) and female (right).

The association of herpes infection with human immunodeficiency virus type HIV/AIDS acquisition and manifestation continues to provide a worrying challenge. Increasing evidence demonstrates a substantial link between the epidemics of sexually transmitted HIV-1 and genital herpes in developing countries, which is a matter of great public health concern. Over the years, data from Africa, Asia and the Americas have highlighted the parallel and intersecting epidemics of HIV-1 and HSV-2, with a growing understanding about the impact of genital HSV infection on increased risk of HIV-1 acquisition. Another compounding factor of HSV infection is its role as a major cause of genital ulcer disease (GUD) in both developed and developing countries. A report of the International Herpes Management Forum (IHMF) indicates that HSV infection has overtaken bacterial sexually transmitted disease (STD) infection as the most common cause of GUD disease worldwide. GUD enhances the infectiousness of HIV-positive subjects and the susceptibility of HIV-negative subjects to HIV infection.

Zedupex is prepared from a medicinal plant which grows in its natural habitat in the Kenyan forest. A voucher specimen (No. 0077: Mungai, Rukunga and Tolo) is deposited at the East African Herbarium, Nairobi, Kenya. The bark of the plant is dried and ground into fine powder from which a hot water extract is prepared and lyophilized. The lyophilized powder is then evaluated for the presence of an active compound as a standardization measure before formulation (Fig. 2).

Phytochemical screening of the raw material of Zedupex has shown the presence of several classes of compounds with potential pharmaceutical activity, including alkaloids, phenolics, flavonoids, anthraquinones, terpenoids, steroids and saponins.

Product formulation



Peeled bark of the medicinal plant being processed for grinding.



Ground bark ready for phytochemical screening/weighing and packing or extraction/ lyophilisation/cream formulation.



An aqueous extract of the plant being lyophilized in 200ml aliquots.



Formulated Zedupex cream prepared from the lyophilized extract.

Figure 2: Procedure for preparing standardized formulation of Zedupex from the bark of a medicinal plant native to Kenya.

Product standardization (Quality assurance and control)

The presence of a lead compound that has been isolated from the extract of the medicinal plant from which Zedupex products are prepared is always determined. The compound is the biological marker for activity and its presence in any freshly collected and processed material is determined as a quality control (QC) measure. Quality assurance (QA) is verified at each major stage in the production process for each batch. At the end of the production line, the finalized product, Zedupex powder or cream, is evaluated for anti-HSV activity before the batch is released (Fig. 3).

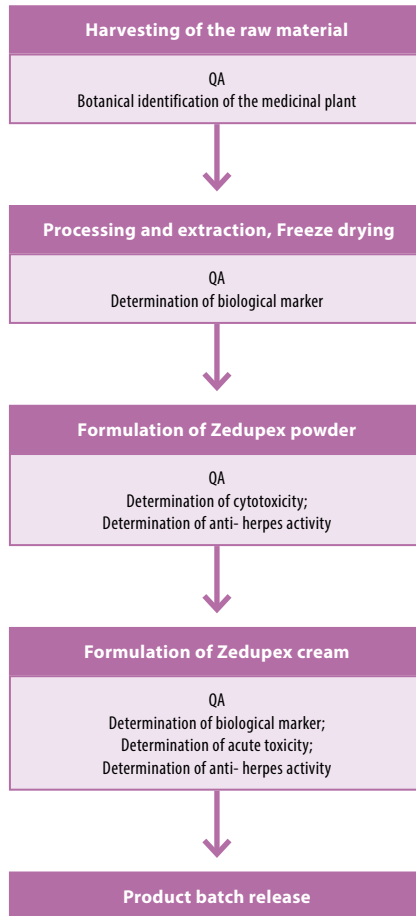


Figure 3: Quality assurance (QA) at major production points of Zedupex.

The product is presented in two forms: powder and cream. Zedupex powder is a preparation of a finely ground dried raw material of the medicinal plant. For herpes management, one single oral administration for an adult of an average weight of 70kg consists of boiling water and adding 1 teaspoonful of the powder (1.0 g approx.) to a cup of hot water (70 mls approx.) and stirring, letting it settle for 10 minutes, sieving and drinking. A child, of age above 10 years, can take half this dosage. The extract can be taken three times a day for a week or until the herpes lesions clear completely.

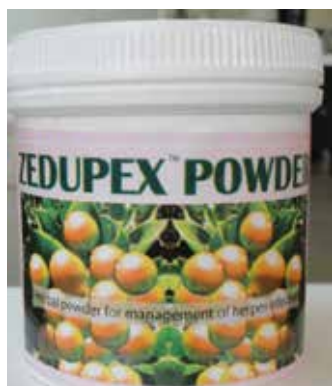


Figure 4: Commercially available packs of Zedupex powder (left) and cream (right).

Zedupex cream is a formulation of an extracted and freeze dried portion of the medicinal plant. The cream has no chemical additives besides a preservative and its brown colour and aromatic smell originate from its raw materials. The cream contains a 10% freeze dried portion (10mg w/w) in an aqueous cream base. The cream is for topical use only to be applied evenly on the affected area three times daily. The treatment should be continued for at least one week, or until the herpes lesions clear completely.

The efficacy of Zedupex was confirmed in a series of laboratory tests.

Anti-herpes activity of Zedupex on mammalian cell lines on wild type and acyclovir resistant strains of herpes simplex virus

Table 1 shows the effective concentrations of Zedupex to cause 50% viral death (EC_{50}) in vitro, as well as the cell cytotoxic concentration of the product which causes 50% cell death (CC_{50}) in the absence of viral infection. It is important to note that the CC_{50} (480 μ g/ml) is well above the EC_{50} (15.1 μ g/ml) meaning that the formulation has a high selectivity index and is therefore very safe and that it works well against both sensitive and resistant strains of HSV.

Table 1: Effective concentrations of Zedupex causing 50% viral death (EC_{50}) in vitro against both sensitive and resistant strains of HSV; and the cell cytotoxic concentration of the product causing 50% cell death (CC_{50}) in the absence of viral infection. (The results are means of three independent experiments).

Formulation/ Drug	$EC_{50}^a \pm S.D$ (μ g/ml)				CC_{50}^b (μ g/ml)
	HSV-1	HSV-2	HSV-1 AP ^r	HSV-1 TK ^r	
Zedupex	15.1±0.57	6.9±1.27	8.1±1.56	11.1±5.66	480.00
Acyclovir (Zovirax)	0.91±0.46	0.87±0.44	>5.0	>5.0	>100

^a Effective concentration for 50% plaque reduction;

^b Cytotoxic concentration causing 50% cell lysis.

HSV-1 Herpes simplex virus type 1;

HSV-2 Herpes simplex virus type 2;

HSV-1 AP^r Acyclovir resistant strain of HSV type 1;

HSV-1 TK^r Another type of acyclovir resistant strain of HSV type 1.

Figure 5 shows that 50µg/ml Zedupex completely stops the replication of the virus in both sensitive (HSV-1 and HSV-2) strains and resistant (HSV-1 and AP^r HSV-1) strains, whereas the commonly-used acyclovir has little effect on the resistant strains as evidenced by the persistence of the white viral plaques.

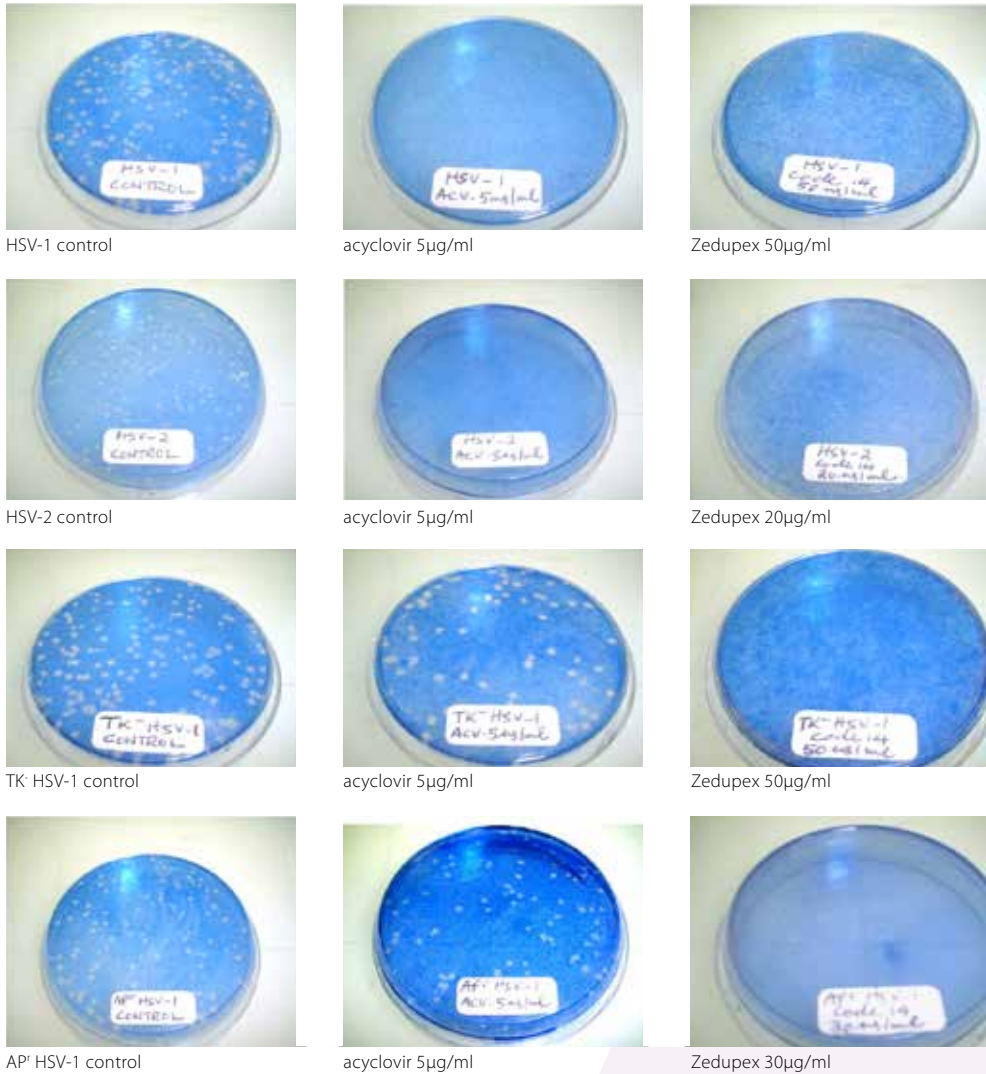


Figure 5: Effects of acyclovir and Zedupex against sensitive and resistant strains of HSV. White spots are viral plaques (areas infected with the virus, most evident in no-treatment controls, left column).

Following these successful *in vitro* trials, we moved on to the animal experiments with mice and guinea pigs once our protocols had been approved by KEMRI's Animal Care and Use Committee (ACUC). Again, these experiments were successful in treating HSV and showed no ill effects.

The plant powder was then formulated as either a cream and pre-clinically evaluated for herpes infection and intended for use in herpes management in humans. As herbal products, their ethnomedical use in crude form for human disease management over the years established their safety and this has now been confirmed experimentally. An evaluation of efficacy in controlled pre-clinical settings provides indicators of their potential as antiviral agents and an evidence base for clinical evaluation. Proper clinical evaluations have yet to be done. However, given the magnitude of herpes infection in Kenya, the formulated products have been made available to registered traditional health practitioners (THPs) for prescription in their clinics. This use is being monitored through observational studies by qualified clinical doctors. Reports so far indicate a positive response in management of herpes infection. Data on observational studies is still limited and will be made available once ethical clearance has been received.

Partnerships

During this project, KEMRI researchers collaborated with partners from the National Commission for Science, Technology and Innovation (NACOSTI), Nairobi, and JICA.

In addition, collaboration with Kenyan THPs registered by the Ministry of Culture and Social Services, Gender and Sport was critical.

Impact

The study has demonstrated the important role herbal medicine could play in health management if quality control, efficacy and safety of herbal preparations are established and products formulated in suitable dosage forms for ease of use. The formulated herbal medicines could be relatively cheaper than conventional therapies since the raw materials could be sourced locally and their preparation would not entail complex pharmaceutical processing. Importantly, the documented scientific information on them would enhance the evidence base for their integration into mainstream healthcare systems in Africa and beyond.

In October 2012, the team won a prize for the best innovative researched natural product at the Nairobi International Trade Fair, Kenya, awarded by His Excellency the President of the Republic of Kenya.

Replicability

As explained above, herpes is a burden not only in Africa, but across much of the rest of the world. Once fully researched with positive results, Zedupex would contribute significantly in lessening the suffering experienced following infection. As much as it cannot be claimed to be a cure for herpes, indications have shown that the frequency of clinical presentation of infection following a latent HSV phase is remarkably reduced with Zedupex use. As with any other health product, the benefits of Zedupex could therefore be experienced in any region of the world.

KEMRI has thus applied for a patent for: "A novel anti-viral plant extract". In the meantime, Zedupex has been listed by the Pharmacy and Poisons Board of Kenya.

Presentation at workshops, seminars and scientific conferences, both locally and internationally, will provide evidence of the scientific merit of the product as an antiviral agent and propel its integration into healthcare management systems.

Lessons Learned

A major obstacle faced in the early stages of research was the sourcing of the raw material. Before the current site was identified, the raw material was obtained from a range of geographical localities which gave rise to difficulties in standardization. This was because the different localities provided different qualities of raw material. Presently, the project is planning to develop a herbal garden of the medicinal plant for large-scale production to guarantee supplies of the raw material.

Future Plans

There are plans to mount proper clinical studies of the Zedupex product. A study protocol has been developed for Phase 1 and 2 clinical trials at the Centre for Clinical Research (CCR) of KEMRI. However, lack of funds has delayed the onset of the study. Project funding is being sourced from the Government of Kenya and possible collaborators.

Once clinical trials are conducted and concluded, a pharmaceutical company will be approached for partnership in product production and sales. Discussions and agreements will be guided by KEMRI's Intellectual Property Rights (IPR) policy in tandem with regulations of the partnering entity.

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Exploring Medicinal Plants for Better Health: Antiplasmodial properties of *Clerodendrum myricoides* and *Dodonaea angustifolia*

Ethiopia

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Duration: 2011-2014

Total cost: USD 6,000 (School of Graduate Studies, Addis Ababa University, Addis Ababa, Ethiopia)

Summary

Malaria is still a public health problem in Ethiopia, affecting mainly children under five years of age and the elderly. Anti-malarial drugs are mainly available in major cities and some rural towns and health posts. However, the rural community still uses herbal medicine to control malaria-related illnesses. Therefore, the validation of medicinal plants is a very important engagement of researchers in the biomedical fields. The leaves of the sand olive (*Dodonaea angustifolia*) and *Clerodendrum (Rotheca) myricoides* are used to treat malaria in traditional practices in some parts of Ethiopia. The antiplasmodial activity of both plants was checked using the

rodent parasite *Plasmodium berghei* in Swiss albino mice using a standard Peter's four day suppressive method. Extracts made using different organic solvents (methanol, ethyl acetate and others) from the dried leaves of *D. angustifolia* and *C. myricoides* showed strong activity in suppressing the plasmodial parasite load in the mice. In conclusion, the present study demonstrated significant suppression of *P. berghei* in the infected mice that now needs to be further substantiated using human plasmodium parasites. Establishing the efficacy and safety of the plant extracts is also important to develop herbal preparations against malaria.

Background and Justification

The majority of the rural Ethiopian population relies on traditional herbal remedies for its primary health care needs. However, the scientific merit and health benefits of many of the country's medicinal plants is not yet fully studied.

Malaria, caused by *Plasmodium* parasites, is still a public health problem in Ethiopia, affecting mainly children under five years of age and the elderly. Anti-malarial drugs are available mostly in the major cities and rural towns.

Over-use of antimalarial drugs however, is leading to growing problems with drug resistance. Therefore, development of new drugs is being given due attention. The discovery of artemisinin from *Artemisia annua*, based on traditional Chinese medicine, was a breakthrough in the development of antimalarial drugs. Attempts are now being made to discover antimalarial constituents in other potentially antimalarial medicinal plants such as *Dodonaea angustifolia* and *Clerodendrum (Rotheca) myricoides*.



Figure 1: *Dodonaea angustifolia* (l.) and *Clerodendrum (Rotheca) myricoides* (r.), Ethiopian plants with potential antiplasmodial activities (source Wikipedia).

Dodonaea, commonly called hop-bushes, is a genus with 70 identified species. The sand olive (*Dodonaea angustifolia*) (*Syn: Dodonaea viscosa*) (family Sapindaceae) is a shrub that grows up to 3 metres tall and is indigenous in most Ethiopian regions. Ethnopharmacology reporting in different parts of the world indicates a variety of therapeutic uses. Stem or leaf infusions are used to treat sore throats and root infusions to treat colds. Extracts of leaves of this plant exhibited antibacterial and antioxidant properties (Teffo *et al.*, 2009; Riaz *et al.*, 2012). Furthermore, the crude extract of the leaves (Tekalign *et al.*, 2010) and of the seeds (Mengiste *et al.*, 2012) showed antimalarial activity

The genus *Clerodendrum* L. (family Lamiaceae) is widely distributed in tropical and subtropical regions. Locally, in Amharic, the Ethiopian language, it is called Misrch and is an open shrub reaching 2 to 3 metres tall and 2 metres in diameter with dark green glossy leaves about 10 cm long. It is used for various ailments. For example the bark of the plant is used for abdominal pains, malaria and against snake bites. The roots and leaves of *Clerodendrum myricoides* are also used to treat gonorrhoea, rabies, measles, eye diseases, malaria, swelling in the body, wound dressing, haemorrhoids and asthma.

Anti-malarial drugs are not affordable for the rural poor community and a preference for herbal medicine by many people is driving the search for effective antimalarial plants. In addition, *Plasmodium* species especially *P. falciparum*, are becoming resistant to the majority of today's available anti-malarial drugs and the infection is one of the most serious causes of morbidity and mortality globally, particularly in sub-Saharan Africa. Therefore, there remains a need to develop new and highly efficient antimalarial drugs that are efficacious and affordable. Many plants are claimed to have antimalarial effects but more often than not the claims prove to be incorrect. Our long years of testing medicinal plants with claimed antiplasmodial activity have showed that *D.angustifolia* and *C. myricoides* have good potential for further development.

Thus, the main objective this case study was to come up with the validation (or otherwise) of the claimed antimalarial effects of the two plants by testing them in animal models using a *Plasmodium* parasite that can easily infect mice and use them as test systems for further detailed investigation in human *Plasmodium* parasites.

Description

The research was undertaken in our research laboratory following established laboratory practice. The protocols were set based on our experience and adapting experimental methods already in use.

Both *D. angustifolia* and *C. myricoides* were collected from a hilly area in the eastern part of Addis Ababa in September 2011 and 2012. Identification and authentication of the plant specimens was done at the National Herbarium of Addis Ababa University by a botanist and voucher specimens were deposited as voucher number GG04/2011 and GG04/2012 in the Herbarium.

Plant collection and the preparation of laboratory animals for the experiments were done after ethical approval from the College of Natural & Computational Sciences Ethics Board.

The antiplasmodial activity of the plants was checked in Swiss albino mice using a modified version of the standard Peter's four day suppressive method (Peters *et al.*, 1975).

The leaves of the plants were separately dried and powdered before supplying an oral dose of a known amount to the mice.

For *in vivo* antiplasmodial assay, the plant extracts (fractions, subfractions) and the standard drug chloroquine (CQ) and the mouse infective CQ sensitive strain of *Plasmodium berghei* was used. The parasite was maintained by serial passage of blood obtained from infected mice to non-infected ones on a weekly basis in the Animal House of the College of Natural Sciences, Addis Ababa University.

Each mouse used in the experiment was infected intraperitoneally with 0.2ml of infected blood containing about 1×10^6 - 10^7 *P. berghei*-parasitized erythrocytes. For each experiment about 1 ml of *P. berghei*-infected blood sample was obtained by a gentle cardiac puncture of the donor mice with rising parasitaemia of about 25-35% in such a way that 1 ml blood contains 5×10^6 - 10^7 *P. berghei*-parasitized erythrocytes per ml. This was prepared by determining the percentage of parasitaemia and diluting 1 ml of blood in 4 ml of physiological saline solution (0.9% NaCl).

After the fourth day, blood was taken from the tail end of each mouse and parasitized red blood cells were counted to determine the parasitaemia (parasite load). The percentage parasitaemia and percentage suppression in control and treated mice were calculated using the formulae below.

Percentage parasitaemia in each field is calculated as:

$$\frac{\text{Total number of PRBC} \times 100}{\text{Total number of RBC}}$$

Where, PRBC= Parasitized Red Blood Cells
RBC= Red Blood Cells.

Percentage suppression is calculated as:

$$\frac{\text{Parasitaemia in control (\%)} - \text{Parasitaemia in treated group (\%)} \times 100}{\text{Parasitaemia in control (\%)}}$$

Results

***Dodonaea angustifolia*:** The methanol crude extract of the dried leaves of *D. angustifolia* significantly inhibited parasitaemia in the mice. Further fractionations resulted in subfractions that showed significant inhibition of the percentage parasitaemia and percentage suppression of the parasite load. The aqueous phase (AP) was found to be inactive. The non-polar hexane fraction (HF) of the crude extract was inactive since it only reduces the parasitaemia by 19%. The detailed results are given in Table 1.

Table 1: *In vivo* antiplasmodial effect of ethyl acetate, hexane fraction and aqueous phase extracts of *D. angustifolia* leaves against *P. berghei* in mice.

<i>D. angustifolia</i> 80% methanol extract	Doses mg/kg	Antiplasmodial activity		MST±SEM
		% Parasitaemia ±SEM	% Suppression	
Aqueous phase of extract (AP)	NC	27.48±0.68 ^a	0.00 ^a	7.20±0.37
	150	20.32±0.57 ^b	26.05 ^b	8.20±0.45
	300	19.60±0.81 ^b	28.68 ^b	8.60±0.51
Ethyl acetate (EA) soluble phase	NC	33.00±1.87 ^a	0.00 ^a	7.20±0.37
	150	7.00±0.71 ^b	78.80 ^b	8.10±0.45
	300	8.60±0.51 ^b	74.00 ^b	8.40±0.40
Hexane fraction (HF)	NC	58.90±0.84 ^a	0.00±0.00 ^a	8.20±0.58
	150	50.80±0.97 ^a	14.00±0.81 ^c	8.40±0.51
	300	49.00±0.71 ^a	19.00±0.51 ^c	8.60±0.40
Chloroquine	25	0.00±00 ^b	100.0 ^d	ND

NC = Negative control.

SEM = Standard Error of the Mean. a, b, c indicate values that do not differ significantly ($P < 0.05$)

Values are presented as mean plus or minus standard error of mean ($M \pm SEM$), $n=5$. ND = Not done, NC = Negative control, MST = Mean survival time, %Para = Percentage Parasitaemia, %Supp = Percentage Suppression; a, b, c indicate values in the same column that do not differ significantly ($P < 0.05$).

Most test groups have a statistically significant increase in mean survival time (MST) compared to their corresponding negative control, the mice with no administration of plant extract and chloroquine. All the test mice died between 7- 12 days, except the group of mice treated with CQ that survived longer.

***Clerodendrum myricoides*:** Methanol fraction (MF) and ethyl acetate fraction (EF) obtained from successive fractionation of the ethanol crude extract of *C. myricoides* showed highest activity with suppression of 77.24% and 65.21% parasitaemia at an oral dose of 300 mg/kg/day respectively. Further bioactivity guided fractionation of the ethanol extract provided some fractions which exhibited good antiplasmodial activity. On the other hand, the hexane fraction did not suppress the parasitaemia significantly (Table 2).

The ethyl acetate fraction obtained from ethanol extract of the dried leaves of *C. myricoides*, did not show much difference in its percentage suppression at different doses and it therefore proved better to treat the mice with lower dose as to avoid potential toxicity at higher doses.

Table 2: *In vivo* effects of methanol, ethyl acetate and hexane fractions of ethanol extract of *C. myricoides* leaves against *P. berghei* in mice.

Fractions of <i>C. myricoides</i> ethanol extract	Dose (mg/kg/day)	Antiplasmodial activity	
		% Parasitaemia \pm SEM	% Suppression
Methanol fraction (MF)	NC	35.47 \pm 0.46 ^a	0.00
	150	9.61 \pm 0.38 ^b	72.90
	300	8.07 \pm 0.39 ^b	77.24
Ethyl acetate, fraction (EF)	NC	35.47 \pm 0.46 ^a	0.00
	150	14.23 \pm 0.34 ^b	59.88
	300	12.34 \pm 0.45 ^b	65.21
Hexane, fraction (HF)	NC	28.48 \pm 0.41 ^a	0.00
	150	24.60 \pm 0.84 ^a	15.77
	300	23.51 \pm 0.64 ^a	17.45
Chloroquine (CQ)	25	0.00 ^b	100.00

NC = Negative control.

SEM = Standard Error of the Mean. a, b indicate values that do not differ significantly ($P < 0.05$)

Partnership

The partnership is within our own college, the Addis Ababa University (AAU), College of Natural and Computational sciences, Departments of Biology and Chemistry.

Sustainability

Currently the importance of using medicinal plants in the form of herbal preparations, as well as in industrially-formulated drugs, is gaining momentum in many parts of the world. This is also true for Ethiopia. At the same time, more and more focus is also being given to the medicinal value of endemic Ethiopian medicinal plant species.

There is an encouraging legal framework to get permission for those medicinal plant products that can be safely used as herbal medicines in Ethiopia. At the national level, the recognition of traditional medicine in Ethiopia was accorded in 1942 (Proc. 27) where the legality of the practice is acknowledged as long as it does not have a negative impact on health. Articles in the Ethiopian Penal Code (512/1957) and the Civil Code (8/1967) provide guidelines for the practice of traditional medicine.

The Ethiopian government has policies and strategies with regard to medicinal plants that support the development and utilization of plant resources in a sustainable manner. The institutions that currently have the main mandates in this field are primarily the Institute of Biodiversity and Conservation (IBC) and the Ethiopian Health and Nutrition Research Institute (EHNRI).

The marketing opportunity of medicinal plant seedlings and herbal products is a new area that is developing. Local people are now much more aware of the importance of medicinal plants, and the need for conservation and protection measures to be taken for safeguarding these natural resources.

In light of the current case study, some recommendations can be made for sustaining such an innovative experience:

- Institutionalization and capacity-building of local people; for the transfer of indigenous knowledge, the conservation of medicinal plants as well as the proper utilization of the resources could help with regard to the utilization of plant biodiversity.
- Local indigenous groups could come together to protect their plant biodiversity and sustainably utilize the benefit.
- Local government and other non-government agencies can also assist in this endeavour.

- A community register of the uses of medicinal plants would be very useful, along with proper documentation for ensuring property rights and, eventually, obtaining patents.

Replicability

Many countries including Germany, Japan, Korea and the USA are major consumer countries of raw medicinal plant materials for their large pharmaceutical industries. In this regard our research results also have a potential market outside the country. In addition, in some African countries including Botswana, Kenya and Mozambique, the healthcare systems use traditional plants in a more formal trade in plant-derived allopathic drugs. Thus, it may be possible for Ethiopia to collaborate in similar endeavours and strengthen the contact and benefit from the results of medicinal plants such as *D. angustifolia* and *C. myricoides*.

Lesson Learned

Some of the major constraints were harvesting the plant material in time due to the growing season, acquisition of supplies including chemicals, and sufficient funds to run the research. Some chemicals are imported and we had to wait until they were delivered. The steps taken to overcome some of the obstacles included trying to obtain some materials from other sources. Communities use medicinal plants. However, this use is not always justified scientifically. What should be done is first of all to create a discussion forum at the village level about the need to scientifically prove the medicinal plants they use on a day-to-day basis. We must come to terms with this and then, when the innovative experience is fully justified, they can become the beneficiaries of their own knowledge, because the knowledge is theirs and not of the researcher.

Future Plans

The project is in its first phase of proving the efficacy of the plants against *Plasmodium* parasites in an animal model. In the next phase the following aspects need to be considered.

- Checking the extracts of both plants; *D. agnustifolia* and *C. myricoides* in human Plasmodium parasites (*P. falciparum*, *P. vivax*) in vitro models.
- Checking the toxicity of the active extracts/fractions on animal models.
- If these are found to be safe then proceeding to the preparation of herbal formulations.

- Further work on the chemistry of the active extracts followed by testing of their efficacy *in vivo* on mice models as well as *in vitro*.
- The final phase, if the plant extracts are found to be efficacious and safe, would be the start of using the plant products as leads to develop antimalarial drugs.

It is very important to identify the target groups and beneficiaries to ensure the success of any project. For this project, a stakeholder analysis was carried out during the inception phase and this helped in designing activities effectively. Research institutes, higher learning institutes, community leaders, religious leaders, members of local government bodies and the private sector will be involved in the project. They play a crucial role in shaping local people's opinions and the decision-making process and provide important technical and financial support.

Sustainable use of medicinal plants is becoming a serious concern in Ethiopia because of resource degradation in the lowlands and highlands alike. Ecosystem conservation will ensure *in situ* conservation of medicinal plants and enable sustainable harvesting methods for collecting medicinal plants from wild habitats.

If the project is sustained and comes to a positive outcome it will contribute to the healthcare system and the economic development of the country.

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Exploring Cameroonian Medicinal Plants for a New Generation of Anti-malarial Compounds

Cameroon

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Duration: 1988 to 2015

Total cost: USD 145,000 (IFS: USD 20,000; TWAS: USD 18,000; OAPI: USD 7,000; IPICS: USD 100,000)

Summary

Malarial drug resistance is the major challenge, sapping control efforts as it affects virtually all drugs available on the market today. At the Biotechnology Unit, University of Buea, the Malarial Drug Discovery and Development Research Group aims to explore the rich Cameroonian flora as a potential source of new antimalarial compounds. This initiative has received support from the Cameroon Government, local partners and international bodies, including the International Programme in Chemical Sciences (IPICS), the International Foundation for Science (IFS) and The World Academy of Sciences (TWAS). Over the past 25 years, we have succeeded in thoroughly documenting more than 200 plant species commonly employed for the treatment of malaria, and establishing antiplasmodial activity in more than 50 of them. Among

the plant species tested, *Alastonia bonei*, *Bersama* spp., *Dacryodes edulis*, *Guibourtia tessmani*, *Hypericum lanceolatum*, *Kigelia africana*, *Perperomia* spp. and *Tectona grandis*, showed the most promising activity against drug-resistant malaria. More than 70 pure molecules, from Cameroonian medicinal plants, were isolated and tested, showing a wide range of activities. A medium-scale screening platform has been established for malaria including both *in vitro* and *in vivo* models. We plan to develop this platform further to include automated systems with improved screening output, as well as proceeding to more advanced drug development stages including pharmacokinetics and pharmacodynamic profiling of the most promising products identified thus far.

Background and Justification

Malaria has steadily inflicted a heavy socioeconomic burden on the people of Africa and other tropical regions for centuries, despite enormous efforts to control it by different stakeholders both at the local and international levels. For several decades, drug resistance has remained the greatest challenge to malaria control, and is one of the formidable obstacles that foiled earlier attempts at seeing malaria eradicated in the 1970s. The late 1980s witnessed an unprecedented spread of chloroquine-resistant *Plasmodium* strains across Africa. So far, resistance has been well established in three of the five *Plasmodium* species responsible for human malaria (*P. falciparum*, *P. vivax*, and *P. malariae*), and this concerns virtually all drugs in current use. This enduring challenge underline the urgent need to broaden the repertoire of anti-malarial drugs, especially for the countries where malaria is endemic.

For hundreds of years, plants have constituted the basis of traditional medicine systems

and a plant-derived products have been a major source for drug development (Titanji *et al.*, 2008). Quinine and artemisinin are examples of plant-derived drugs that have been used successfully against resistant strains of malarial parasites. In some rural areas of Cameroon, traditional anti-malarial medicines are even preferred to pharmaceutical compound drugs, suggesting that the herbal preparations used by traditional healers might contain useful active ingredients. Most central African countries converge in the Congo basin, which represents the second largest contiguous rainforest in the world after the Amazon. The Congo basin forests hold 20% of the world tropical moist forests and 80% of the tropical moist forests in Africa. In Cameroon, traditional medicine practitioners and communities use more than 500 medicinal plants species in their various remedies including antimalarials (Zofou *et al.*, 2014).

The idea of designing novel antimalarial drugs especially from Cameroonian medicinal plants was prompted within our research team in the late 1980s, with the main focus on identifying and evaluating locally-used plant species acclaimed for their efficacy and safety. In order to either standardize these as phytomedicines or exploit them as sources of new drug leads. Thus, for many years, our team has been working closely with traditional healers in Cameroon with the main aim of improving the exploitation of natural resources in handling malaria and other endemic diseases.

The main research objectives of the Biotechnology Unit in the field of malarial drug research include:

- the identification and characterization (*in vitro*) of antimalarial products from selected medicinal plants used to treat malaria in Cameroonian folk medicine;
- the preclinical study of potential drug leads identified through *in vivo* screening for antimalarial activity and toxicity and pharmacokinetics; and
- drug target prediction and validation using bioinformatics tools.

Description

Drug research and development (R&D) is, in general, a long and laborious process requiring substantial funding, good infrastructure, qualified personnel and an enabling political and institutional environment. The starting product, from a range of sources (plants, marine flora and microorganisms) may be chosen and screened at random or the source-materials may be selected based on previous knowledge (ethno-botanical or ethno-pharmacological surveys) of their use in communities. A wide range of technologies is available for the extraction of active components and essential oils from medicinal and aromatic plants. The choice depends on the nature of the source material,

the nature of experimental techniques and models employed as well as cost feasibility and the suitability of the process to the particular context. Crude extracts with promising activity typically undergo bioassay-guided fractionation towards detection and isolation of potentially active pure ingredients.

The systematic isolation and testing of molecules from plant parts against the various parasites is not a feasible option because of the prohibitive cost of such a vast enterprise. The use of ethno-botanic information to select plants for screening has proved to be a fruitful and cheaper approach for drug discovery. Once identified, active principles can be optimized by chemical synthesis to obtain more active products at affordable costs. The entire pool of molecules present in different parts of the plant under consideration can also be isolated and tested against different parasite types in the search for potentially active compounds (Fig. 1).

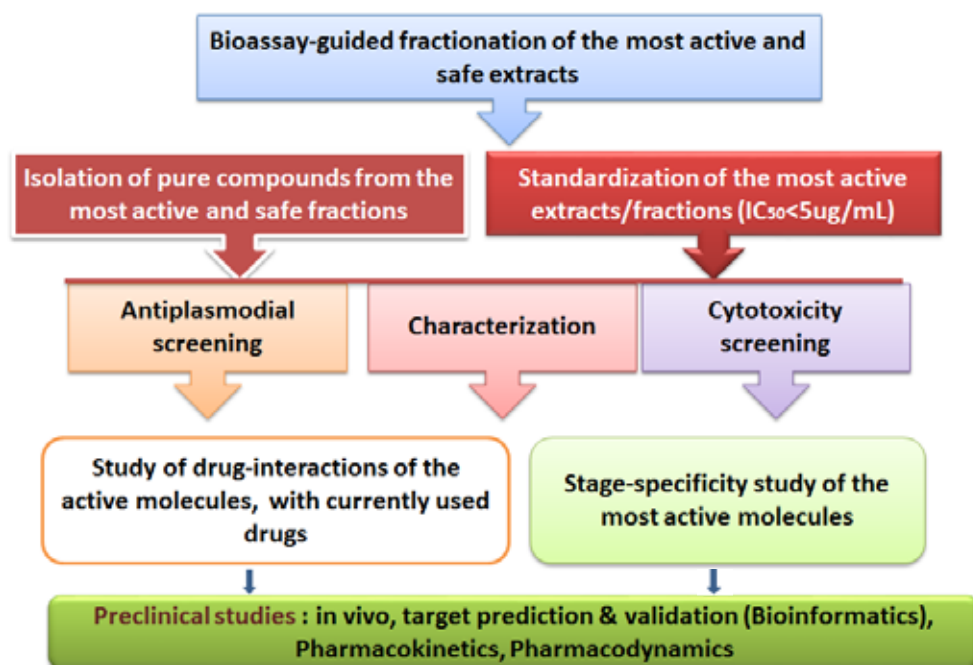


Figure 1: General protocol for malaria drug discovery and development from medicinal plants (Zofou *et al.*, 2011a).

Both chloroquine-sensitive (3D7, D6) and multi-drug-resistant (W2, W2mef, K1 and Dd2) *Plasmodium* strains were kindly donated by BEI-Resources/MR4 (MR4, Manassas, VA, USA), and maintained in continuous culture, with back up stored in liquid nitrogen. In earlier studies, the Vietnamese strains were used (Tantchou *et al.*, 1986). Field isolates made up of active parasites collected locally from malaria patients were also used in testing the plants products identified. The *in vitro* screening employs the semi-automated techniques of Desjardins, using both light microscopy (normal light and fluorescence) and the parasite lactate dehydrogenase assay (pLDH). Fig. 2 illustrates a plate result of pLDH-based screening.

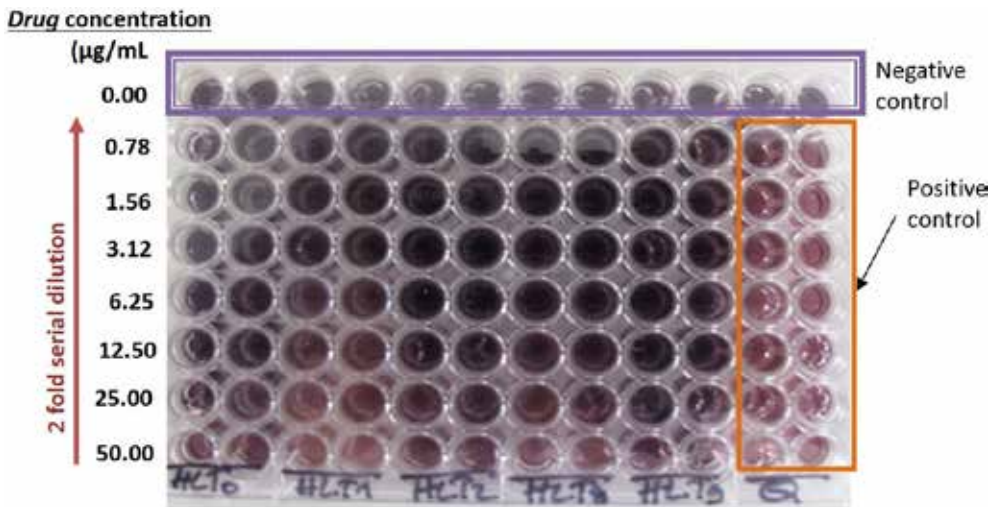


Figure 2: Photograph of the pLDH plate obtained with some compounds of *H. lanceolatum* (Zofou *et al.* 2011a). The experiment was conducted in duplicate and included, from left to right the following compounds: HLT0, HLT1, HLT2, HLT3, HLT8, HLT9 and QN. The denser the coloration, the higher the parasitaemia in the concerned well.

From the medicinal plants identified as well as reports of species from other parts of Africa, we have screened more than 200 plant species, some of which exhibit very interesting properties in terms of antimalarial activity and safety. From these, 70 compounds (including three newly-discovered ones) were isolated from four plant species, including the edible plant species *Dacryodes edulis*, and their antiplasmodial profile established. Twelve of these 70 compounds that scored the highest ratings were selected and screened following the bioassay-guided fractionation approach. The antimalarial activity was tested alongside with toxicity of the different products against one of the most fragile mammalian cell lines, the LLC-MK2 monkey kidney cell line, to define their relative safety. For the most active pure molecules isolated, we also evaluated their interaction patterns with drugs already in use such as artemether and quinine. Likewise, the activities of the most active and non-toxic candidates were tested against different life cycles in order to have preliminary information on their mechanism of action.

Results

These studies confirmed the antimalarial properties of seven plant species used in Cameroon to treat malaria and other fevers, and five new antimalarial molecules were identified from these, with *Dacryodes edulis* (Zofou *et al.*, 2013), *Bersama* spp. (Nondo *et al.*, 2015), *Perperomia* spp. (Ngemenya *et al.*, 2014), *Tectona grandis* (Kopa *et al.*, 2014), *Hypericum lanceolatum* (Zofou *et al.*, 2011a) and *Kigelia africana* (Zofou *et al.*, 2011b, 2012) being the most active. These species underwent further purification through bio-assay-guided fractionation to yield several compounds of which 15 were found to be highly active against both chloroquine-sensitive and drug-resistant malaria parasites. Table 1 summarizes the *in vitro* activity of the compounds isolated from *K. africana* while Fig. 3 illustrates their drug-interaction profiles with reference malaria treatments.

Product code	Quantity (mg)	Extraction yield (%)	IC ₅₀ on W2	IC ₅₀ on W2mef	IC ₅₀ on CAM10	IC ₅₀ on SHF4
Atranorin	30	0.10	4.41 ± 0.35	1.78 ± 0.18	2.81 ± 1.07	2.78 ± 0.29
KAE3	17	0.06	1.60 ± 0.00	1.86 ± 0.15	2.17 ± 0.55	8.02 ± 0.55
KAE7	103	0.15	1.54 ± 0.00	1.02 ± 0.17	2.34 ± 1.15	2.70 ± 0.29
KAE10	62	0.09	53.84 ± 19.39	12.89 ± 0.87	7.13 ± 3.35	6.71 ± 0.12
CQ	-	-	0.29 ± 0.02	0.26 ± 0.02	0.25 ± 0.04	0.19 ± 0.02
QN	-	-	0.23 ± 0.02	0.27 ± 0.04	-	0.14 ± 0.05
ART	-	-	0.03 ± 0.01	0.04 ± 0.00	-	0.03 ± 0.01

Table 1: Extraction yield and antiplasmodial activity of pure compounds from *Kigelia africana* (Zofou *et al.*, 2011b). W2, W2Mef, CAM10 and SHF4 are different strains of *Plasmodium*. CQ = chloroquine, QN = quinine, ART = artemether, three standard antimalarial drugs.

More interestingly some of these compounds showed synergy with artemether and antagonistic effects with quinine, suggesting their potential as partner molecules in new antimalarial combination therapies (ACT) or quinine-based drug combinations against multi-drug-resistant malaria. Further studies including *in vivo* testing of the combinations and the study of their toxicity are required to move forward in the exploration of this plant species as source of new antimalarials.

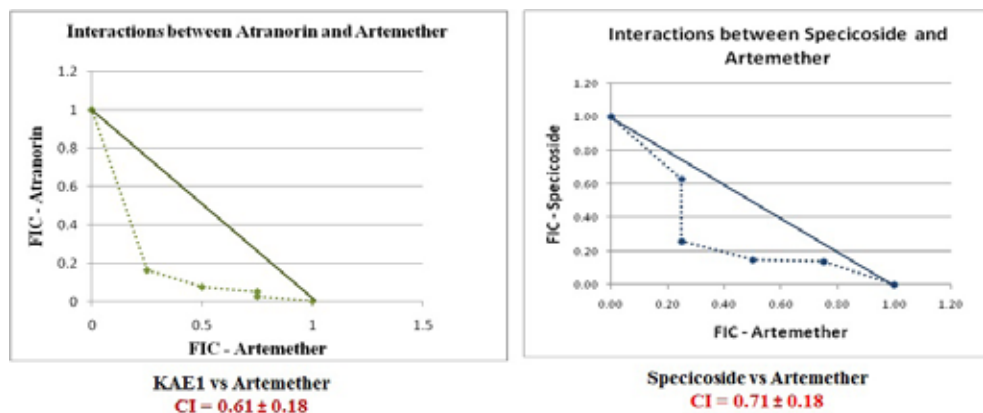


Figure 3: Synergistic effects between selected compounds from *Kigelia africana* and artemether (from Zofou *et al.*, 2012).

Partnerships

Over the years, we have succeeded in establishing a mutually-fruitful collaboration between various teams. Our local network in Cameroon is illustrated as shown in Fig. 4 and includes traditional medicine practitioners through the National Order of Tradipracticioners in Cameroon; the Natural Product Laboratory of the University of Dschang; the Phytochemistry Laboratory of the Institute of Medical Research and Studies of Medicinal Plants (IMPM) for phytochemistry work; the Gallenic Pharmacy and Drug formulation team of the IMPM; the Department of Botany and Plant Physiology and the National Herbarium for plant identification; the Department of Sociology of the University of Buea; the Botanic Garden, Limbe; and the National Herbarium, Yaounde’.

A research collaboration has recently been established with the Muhimbili University of Health and Allied Sciences (Dar es Salaam, Tanzania) aimed at fostering the efforts of both institutions as far as drug discovery from African flora is concerned. In this regard, a PhD student has already been trained at the Biotechnology Unit, University of Buea, on malaria culture and screening techniques. From this highly promising partnership, a scientific paper has been published on the documentation and primary investigation of over 100 hundred plants species from Tanzania (Nando *et al.*, 2015).



Figure 4: Collaboration and networking in drug discovery research at the Biotechnology Unit, University of Buea, Cameroon.

Impact

The main impact of our work (see Titanji *et al.*, 2008 and Zofou *et al.*, 2014) so far has been to stimulate research in and provide a framework for the scientific study of medicinal plants. Work is now ongoing in various laboratories in the country not only on malaria, but also on filarial diseases, bacterial infections, and even non-communicable conditions like diabetes and obesity.

We have also trained a cohort of researchers in the field of medicinal plant studies including three PhD and more than a dozen MSc degree holders.

Finally, some the active antimalarial compounds discovered in our work are in pre-clinical studies with a view to developing phytomedicines for the treatment of malaria. Meanwhile, local communities continue to use the plants which we have validated as active and non-toxic for the management of fevers and malaria.

Replicability

The Government of Cameroon, through its Institute of Medical Research and Study of Medicinal Plants has instituted a project for the production of improved phytomedicines for malaria and other diseases. Our group provides screening services and technical advice to this project. The team was recently honoured with the selection of Denis Zofou as the laureate for the 2014 TWAS-ROSSA Young Scientists Prize in Applied Sciences.

It was not our aim to seek patents from our work; rather we sought to scientifically validate herbal medicines for the most common maladies in our setting as a contribution to the fight against diseases of poverty.

Lessons Learned

The main lessons learned are that many of the claims of traditional medicine practitioners can be verified by using simple bio-assays, and the success rate is far higher than if random systematic screening of plants is carried out. Another lesson is that confidence can be built and maintained if the traditional practitioners and community members are certain that they are not being exploited for financial gain.

Future Plans

We plan to collaborate with chemists to scale up the production of the most active compounds discovered by our group and to carry out preclinical and subsequently clinical trials of these compounds for malaria treatment. This is a major undertaking which will need the collaboration of the private sector.

Meanwhile, several research projects funded by the IFS, TWAS and the African Union to develop malaria phytomedicines will continue to serve as a training ground for MSc and PhD students.

We also plan to expand our programme to look for plant-derived remedies for the neglected tropical diseases other than malaria.

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Protective Effect from Ulcerative Colitis to Colitis- associated Cancer in Experimental Models with Huangqin Decoction

China

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Duration: September 2013 to May 2015

Summary

We demonstrated the therapeutic potential of Huangquin Decocotion-1 (HQD-1) for acute/chronic ulcerative colitis. Furthermore, HQD-1 was effective for colitis-associated cancer prevention. The mechanism of HQD-1 may involve the inhibition of inflammation

and oxidative stress in mice. All these data confirm the effects of the clinical application of HQD-1 for ulcerative colitis patients. HQD-1 can also be used as a preventive agent against colitis-associated cancer in chronic ulcerative colitis patients.

Background and Justification

Colorectal cancer (CRC) ranks as the neoplasm with the third highest incidence and death rate in humans. Evidence is accumulating that inflammatory bowel disease (IBD) is strongly associated with CRC, with ulcerative colitis and Crohn's disease being the two main forms of IBD in humans. Indeed, the natural pathology of IBD sufferers, and particularly ulcerative colitis patients, may be marked by the development of CRC. During the course of IBD, inflammatory cytokines contribute to the formation of a tumour-supportive microenvironment. In addition, the production of large amounts of reactive oxygen species (ROS) in inflamed tissue by inflammatory cells, neutrophils and macrophages results in oxidative stress and impairment of antioxidant defences. Increased levels of ROS lead to protein damage, lipid peroxidation and DNA damage, all leading to genetic and epigenetic alterations, which promote the occurrence of carcinoma. However, progression from IBD to CRC is a long process, so it may prove feasible to prevent CRC through IBD treatment.

Aminosalicylates have been extensively used to treat patients with IBD with several studies demonstrating their potential in the prevention of colitis-associated cancer. However, some groups were not able to reproduce the findings when these drugs were used. Corticosteroids play an important role in the treatment of IBD, and some studies have found beneficial effects in the prevention of CRC with corticosteroids, although the adverse effects of their long-term use are considerable. Treatment with herbal products represents a novel approach to IBD. However, although the development of new drugs for IBD and IBD-associated CRC is an urgent necessity, there is insufficient data on the preventive role of herbal products in CRC development in IBD patients.

Description

Colitis sufferers are at high risk of developing CRC. Huangqin Decoction (HQD), one of the traditional Chinese formulae chronicled in *Shang Han Lun* (the Treatise on Cold Damage Disorders), was commonly used to treat a range of gastrointestinal symptoms. In our study, we used current biomedical approaches to investigate the protective/preventive efficacy and possible mechanism of HQD in mice models with dextran sulphate sodium (DSS)-induced acute/chronic ulcerative colitis and azoxymethane (AOM)/DSS-induced colitis-associated cancer. Four herbs of HQD were extracted using four processes named HQD-1, HQD-2, HQD-3 and HQD-4. Acute/chronic ulcerative colitis was induced in mice by adding DSS to drinking water, while AOM and DSS in combination were used to induce colitis-associated cancer. The mice were administered at a dose of 9.1 g/kg HQD via oral gavage per day. Body weight, stool characteristics and haematochezia were observed daily. Measurements of survival rate, length of the colorectum, tumour numbers and tumour size were conducted. The colorectal tissues were used to detect levels of inflammatory cytokines using quantitative reverse transcription PCR (qRT-PCR). The expression of 8-oxoguanine, nitrotyrosine and proliferating cell nuclear antigen (PCNA) were examined using immunohistochemistry. The activity of superoxide dismutase (SOD), which prevents damage from ROS, the levels of other chemical markers, and the apoptosis (cell death) rate of colorectal tissues were measured using respective kits.

HQD-1 may significantly inhibit acute DSS-induced ulcerative colitis. HQD-3 and HQD-4 exhibited a mild ameliorative effect. However, HQD-2 had almost no protective effect and resulted in a higher mortality rate than DSS. The high mortality rate of HQD-2 may have resulted from the higher content of baicalein, wogonin and oroxylin A or their combination. Furthermore, HQD-1 possessed the ability to protect against DSS-induced chronic ulcerative colitis in mice, to inhibit the production of inflammatory cytokines and to improve anti-oxidative activity. The preventive effect of HQD-1 on AOM/DSS-induced CAC in mice was notable. HQD-1 also evidently inhibited the expression of inflammatory cytokines and oxidative damage in mice. The four herbs in the HQD extract mix (HQD-1) could perhaps be used to cure acute/chronic colitis and, furthermore, prevent colitis-associated cancer. The protective/preventive mechanism of HQD-1 may involve the inhibition of inflammation and oxidative stress.

Partnerships

Partnerships were undertaken with Qiang Yu of the Department of Pharmacology, Shanghai Institute of Materia Medica, Chinese Academy of Sciences, and with Chung S. Yang of the Department of Chemical Biology at the Center for Cancer Prevention Research and Rutgers Cancer Institute of New Jersey, Rutgers University in the USA.

Replicability

There is no patent and Huangqin Decoction (HQD) is one of the traditional Chinese formulas chronicled in Shang Han Lun.

Lessons Learned

The unit of measurement in Shang Han Lun is different from that of modern units. Therefore, the confirmation of the dosage is difficult. After consulting specialist books, we solved this issue.

The results showed the content of baicalein, wogonin and oroxylin A were higher in HQD-2 than in HQD-1.

The high mortality rate of HQD-2 might have resulted from the higher content of these three compounds or their combination. Whether the three compounds or their combination result in a higher death rate will be confirmed in future in vivo experiments.



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