Volume 30 • Issue 4 • **2018**

Australian Journal of Herbal and Naturopathic Medicine

A publication of the NHAA

Australian Journal of Herbal and Naturopathic Medicine

naturopaths & herbalists association of australia

The *Australian Journal of Herbal and Naturopathic Medicine* is a quarterly publication of the NHAA. The Journal publishes material on all aspects of Western herbal medicine and is a peer-reviewed journal with an Editorial Board.

ISSN 2209-119X

The *Australian Journal of Herbal and Naturopathic Medicine* is an independent academic entity and as such does not necessarily reflect the views of the NHAA.

The NHAA is a peak professional association representing appropriately qualified naturopathic and Western herbal medicine practitioners. A foundation member of the World Naturopathic Federation and founded in 1920, it is a member-based association, the oldest professional association of complementary therapists in Australia and the only national professional association specifically concerned with the practice and education of Western herbal medicine (WHM) in Australia. Our mission is to support naturopaths and Western herbal medicine practitioners to deliver excellence in healthcare in Australia and our vision is naturopathy and Western herbal medicine for the health of Australia.

Aim and scope

The *Australian Journal of Herbal and Naturopathic Medicine* (*AJHNM*) is Australia's leading herbal publication. A thoroughly modern, peer-reviewed and clinically relevant journal, the *AJHNM* can trace its origins back to publications issued by the Association as long ago as the 1930s. Issued quarterly, the *AJHNM* publishes material on all aspects of herbal medicine including philosophy, phytochemistry, pharmacology and the clinical application of medicinal plants.

Editorial policy

- Subject material must relate to herbal medicine.
- Accepted articles become the property of the NHAA.
- Contributions are subject to peer review and editing.
- Contributions to the *Australian Journal of Herbal and Naturopathic Medicine* must not be submitted elsewhere.

Advertising

For advertising enquiries, please contact the NHAA office on telephone (02) 9797 2244, fax (02) 8765 0091, email nhaa@nhaa.org.au or visit www.nhaa.org.au / Publications and Products / AJHNM

Publisher Cambridge Media

Postal Address
10 Walters Drive
Osborne Park, WA 6017

Follow AJHNM on facebook
www.facebook.com/pages/Australian-Journal-of-Herbal-and-Naturopathic-Medicine/1416725668550367

Twitter
www.twitter.com/TheNHAA

NHAA postal address:
PO Box 696, Ashfield NSW 1800

Telephone +61 (0) 2 9797 2244

Fax +61 (0) 2 8765 0091

Email nhaa@nhaa.org.au

Website www.nhaa.org.au

Editor Susan Arentz

Email editorajhnm@nhaa.org.au

Design of electronic platform and layout for hard copy Gordon McDade

Copy editing Rachel Hoare

CONTENTS

Australian Journal of Herbal and Naturopathic Medicine **2018** 30(4)

Futures and histories

Susan Arentz
Editor, *Australian Journal of Herbal and Naturopathic Medicine*
PO Box 696, Ashfield, NSW 2131
editorajhnm@nhaa.org.au

December 2018, the eve of NHAA's 100-year anniversary, and perfect for reflection and looking forward. This is the final issue of the year and introduces the NHAA centenary conference in March 2019, *Traditional Wisdom, Future Practice*. This particular conference will be a one-off event, not only because it represents 100 years of the NHAA, but also because it will host the General Assembly of the World Naturopathic Federation, and enable us to present Australian naturopathy and herbalism, and mingle with international peers, exchange and share knowledge.

A record number of abstracts have been submitted for peer review and presentation at the conference. This shows how we willingly engage with critique, and signifies a strengthening of our professions in recognising the value of peer review and transparent information. It signifies our transition towards a stable occupation and secure professional identity. I would like to congratulate all who submitted abstracts — it takes courage to put forward ideas and data, to invite feedback that may not be entirely positive, and it takes skill to redevelop and evolve our ideas; a process from which we all grow and benefit.

So where are we heading as health professionals? A round table of experts, including naturopaths and herbalists, recently explored the challenges facing complementary medicine professions in Australia[1]. They aimed to identify future direction and develop leadership, action and debate. Group consensus was achieved on two main themes — these were to ensure clients remain at the centre of decisions and that evidence informing clients' needs and safety is prioritised. These findings acknowledge clients as our biggest employers; people from the public, coming to our private practices. So what are our clients' needs, and what is it about us that they most value and expect? The evidence provides a clear and consistent answer. Our clients seek to be involved in their own tailored health care, and they want optimal health, wellbeing and performance and help to prevent and treat chronic disease[2], with minimal risks in a way that fits in and complements other health care practices. They are attracted to our holistic philosophy and our natural treatments. We know this because there is high-quality, peer-reviewed, robust evidence that repeats this message[3,4]. These reasons for people's use are key to our continued viability as health care professionals as they outline what our main employers expect, and elude to our capacity to meet unmet health needs and to our traditional and historical foundations in interdisciplinary, evidence-based practice.

Philosophically informed health care is an expressed and interesting need of our clients; I have had the privilege to delve deeper into this recently. Naturopathy and Western herbal medicine are types of holistic Western medicine. Holistic, opposite to reductionist, means including all of a person's characteristics in a big-picture case synthesis. It requires expertise and knowledge of inter-, extra- and intracellular detail, associated organ compensation, micro and macro biology, emotional and social impacts and relationships between people and their environment. Amongst many things, it means providing simultaneous relief of symptoms and preventing disease, and providing treatment that is personal, effective, safe, affordable, accessible and meaningful.

Traditional naturopathy as a holistic practice is uniquely differentiated by six principles. This is an important point because these codified principles distinguish naturopathy from other natural medicine practices such as homeopathy and traditional Chinese medicine[5]. They communicate identity and were the sole identifying motivating factor of naturopaths' inclusion by the World Health Organization, in the development of the Alma Ata declaration at Asanta[6]. The Alma Ata declaration emphasises the rights of all people to primary health care, prioritises primary health needs, and directly informs international and local governance, policy, political decisions and direction. An invited contribution to development of the Alma Ata recognises naturopathy as a distinct form of traditional primary health care for all people, including marginalised, at-risk and disenfranchised people.

These issues are close to many of our hearts. I look forward to hearing and sharing more about these developments at the NHAA conference in March. And I look forward to catching up with you, reflecting on our traditional wisdom and crafting our future practice. Our profession is evolving and it is essential that we contribute to and steer our way towards the future we want.

This issue presents one of our champions, Ruth Trickey. In an interview by Michelle Boyd, Ruth explains how she got into herbal medicine and reflects upon her amazing contribution. Brad Leech and Jessica Bays provide a conference report on the International Congress on Integrative Medicine and Health (ICIMH) that took place in Baltimore, America, in early 2018, with messages aligned to the round table findings mentioned earlier and the way forward. It also includes two herbal medicine articles: the first a summary of the pre-clinical and clinical evidence for *Nigella sativum* and the second on the comparative effects of Mistletoe and chemotherapy on colon cancer cells. It also a case study illustrating naturopathic treatment for a woman with interstitial cystitis.

Please join me in welcoming Dr Wendy McLean as our new MedJourn, MedPlant and CPE question writer. Wendy was selected from several high-quality applicants for her critical appraisal experience. I'd like to thank all who applied; it was encouraging to receive so many high-quality applications. Wendy has contributed the MedPlant component to this issue and will take over the MedJourn from Jodie in the next issue. Thanks again, Jodie, for your support with Wendy's transition.

I wish you all the best for the festive season, it has been a big year and this journal is really finding its feet with submissions coming from many areas of our professions. And the calibre is notably improved. Well done you! Now is time for celebration, reflection, rest and replenishment. I look forward to publishing more of your work in this peer-reviewed journal in 2019.

References

1. Canaway R, Leac M, Hunter J. Setting an agenda for strengthening the evidence-base for traditional and complementary medicines: Perspectives from an expert forum in Australia. Adv Integr Med 2018.
2. Steel A *et al.* Complementary medicine use in the Australian population: Results of a nationally-representative cross-sectional survey. Sci Rep 2018;8(1):17325.
3. Reid R *et al.* Complementary medicine use by the Australian population: a critical mixed studies systematic review of utilisation, perceptions and factors associated with use. BMC Complement Altern Med 2016;16(1):176.
4. Bishop FL, Yardley L, Lewith GT. A systematic review of beliefs involved in the use of complementary and alternative medicine. J Health Psychol 2007;12(6):851–867.
5. Wardle, J., *Naturoapthic Curriculum Development Personal communication.* S. Arentz, Editor. 2018.
6. Bhutta ZA *et al.* Alma Ata and primary healthcare: back to the future. British Medical Journal Publishing Group, 2018.

Australian Journal of Herbal and Naturopathic Medicine **2018** 30(4)

Private health insurance (PHI) Reforms: Profession committee report

On behalf of the Professions Committee of the NHAA Board:
David Casteleijn (Professions Committee, Chair), Tobey-Ann Pinder (Vice-President), and Natalie Cook (President)

The Honourable Greg Hunt, MP (the federal Minister for Health)'s recent decision has effectively banned private health insurers (PHIs) from covering naturopathy and Western herbal medicine, despite a high level of evidence of their efficacy and valued use in chronic conditions. The Naturopaths and Herbalists Association of Australia (NHAA) is opposed to this decision. In our opinion, these new rules do not help patients, save virtually no money, create new risks to the public, deny care that is effective, and potentially encourage care that is ineffective and/or unsafe.

Most of the issues with the new rules stem from their prohibitive nature, so, whilst the purpose was to remove public subsidies for these services, the new rules actually *prohibit* their provision as part of a private health insurance plan under any circumstances. What has *not* been made clear in the new rules is why removal of public subsidies from these therapies *requires* that their supply be prohibited in complying policies.

Removal of naturopathy and Western herbal medicine from private health insurance refundable status is of particular concern to the NHAA, primarily due to the anticipated impact on public safety. In the absence of statutory regulation (such as under AHPRA), naturopathy and Western herbal medicine currently operate in a self-regulated environment. A key safety lever in the current self-regulatory model is the need for practitioners to join a professional association to access PHI provider numbers in order to allow patients to claim private insurer rebates on the services rendered. In the face of inaction on the part of various governments to regulate naturopathy and Western herbal medicine, the PHIs have assumed the pseudo-regulatory role in this area by requiring practitioners be members of a professional association, to maintain current first aid training, and meet continued professional development requirements, which has acted as a steadying influence on the profession. This allows professional associations such as the NHAA to enforce standards of public safety. For instance: 'registered' practitioner listings, codes of conduct, education standards and educational accreditation, monitoring of compliance with first aid certification, overseeing member professional development and continuing education activities, and ensuring practising members hold appropriate levels of professional indemnity and public liability insurance.

Removal from private health insurance refundable status in relation to naturopaths (and Western herbal medicine practitioners) removes the 'pseudo-regulatory' function currently operating in the public interest and is likely to facilitate underqualified and non-compliant persons to practise naturopathy or Western herbal medicine with no professional checks or balances. There are already high-profile cases of misconduct by people claiming to be naturopaths, while not meeting even the most basic requirements set by some of the other representative bodies. Had there been statutory registration, cases like this would have been clearly described in the press as 'fake naturopaths' in similar terms to those used for other fake medical practitioners. The NHAA is concerned the regulatory vacuum resulting from removal of PHIs for naturopaths (and Western herbal medicine practitioners) will enable people with little or no qualifications in the discipline to misrepresent themselves and anticipate more harm to the community and to the standing of the profession.

The Minister purports to use the "Review of the Australian Government Rebate on Private Health Insurance for Natural Therapies"[1] as the justification for preventing PHIs from providing rebates for naturopathy and Western herbal medicine. The limitations of that review (for example, restricted to systematic reviews from 2008 onwards, missing an important review from 2007), as well as the large number of randomised controlled trials undertaken in the years since that review, make that reliance questionable. Not only was the report limited to published systematic reviews, but evidence has grown considerably since then: for example, in 2013 there were six randomised controlled trials (RCT) comprising a total 692 patients for "whole practice" naturopathy; however, there are now 31 RCTs comprising a total 9798 patients. Even then the review found that there was evidence to suggest that whole-system naturopathic practice is effective in improving patient health for a range of chronic health conditions, including anxiety, multiple sclerosis, cardiovascular disease and musculoskeletal conditions. Of the studies which were included, their positive outcomes were dismissed as not relevant to the Australian context due to the unregulated nature of the workforce.

For Western herbal medicine, the review noted that while there is a large body of research on the effects

of individual herbal agents and remedies, the study of the real-life practice and outcomes of Western herbal medicine as a health service was separate to this research and as there were no studies of the real-life practice and outcomes of individualised Western herbal medicine as a health practice they could not reach any conclusions as to its effectiveness or potential harms.

Had naturopathy already been registered as a profession then it would not have been subject to the review in the first place. Naturopathy and Western herbal medicine have previously been assessed against the requirements for registration and found to meet the requirements[2]. It is the NHAA's position that the lack of statutory regulation of naturopaths and Western herbal medicine practitioners under the National Scheme (through AHPRA, as the agency that supports the National Boards to implement the National Scheme) is the key difference, to naturopaths and Western herbal medicine practitioners' adverse treatment under the policy change (that removes the government rebate to PHIs in relation to a broad range of natural therapies). Registration under the National Scheme would address this issue and the NHAA has been, and will continue to, work to facilitate this.

In the interim, the NHAA will be working to have naturopathy and Western herbal medicine removed from the "banned list". This is an administrative process, but it requires understanding of the issues by the Health Minister so that it becomes easier to make the change rather than to leave it as it is. This requires a grassroots campaign with naturopaths and their patients contacting their local member and the health minister to bring this to their attention and give them a reason to implement the change required. The message needs to be succinct and to the point. The NHAA will be keeping members updated with advice on practical action they can take to assist with the amendment process of the new PHI rules in the best interest of members and the public alike.

References

1. Baggoley C. Review of the Australian Government Rebate on Natural Therapies for Private Health Insurance. Canberra: Australian Government Department of Health, 2015.
2. Lin V *et al.* The practice and regulatory requirements of Naturopathy and Western Herbal Medicine. Melbourne: Department of Human Services, 2005.

Interview: Ruth Trickey

Interviewer: Michelle Boyd Date: 18 November 2018
On behalf of Naturopaths and Herbalists Association of Australia (NHAA); transcribed for publication in the *Australian Journal of Herbal and Naturopathic Medicine* (AJHNM).

Introduction and thank you

Why naturopathy (herbal medicine)?


Ruth Trickey

Although I liked working in the health care arena, most of my work was in hospitals and I was very dissatisfied with the way we didn't deliver, what I later came to know of, as the holistic approach to health care. So, you could say I started in complementary medicine more for what I *didn't* like about medicine rather than what I *did* like about naturopathy [she says with a smile].

When I first started, I believed my practice would consist of homeopathy, acupuncture and osteopathy, mostly for philosophical reasons. Along the way I realised I was a much better herbalist than a homeopath. In fact, I thought if I waited for homeopathy to work on my patients they might die in the meantime! [She giggles.]

Osteopathy was regulated so I couldn't continue to practise as an osteopath, and I brought on a chiropractor and masseurs to look after my osteopathy patients. Acupuncture also went by the wayside when I became too busy to see my acupuncture patients twice a week, necessitating that we add acupuncturists to the team. Overall, this resulted in the expansion of the practice to a multidisciplinary clinic, which, in addition, included psychologists and other natural therapists.

Over the years herbal medicine became my true love and I studied Western and Chinese herbal medicine and Kampo.

What prompted the writing of *Women, Hormones & the Menstrual Cycle?*

The book was written because I was lecturing in gynaecology and obstetrics at the Southern School of Natural Therapies. Most of my students asked me if I could write a book as there was just nothing available at the time about the naturopathic treatment of these conditions. The real problem was I was only given six lectures and it was patently clear to everyone that I couldn't do justice to these topics in 12 hours!

The book has been through three editions, four if you include the e-version. This represents a large slab of my life that has been devoted to researching and writing. In order to break the monotony, I spent three months in Austin, Texas, and two months in Columbia, South America, while rewriting the third edition. This made what would otherwise be an onerous task, much more manageable and enjoyable.

Places of clinical experience

I started my first clinic in 1978 from home. In 1980, I was one of the founding members of Carlton Healing Centre and I also started a small clinic in Clifton Hill, called Clifton Hill Natural Health Centre. This clinic was expanded over the years to accommodate the increasing numbers of practitioners who needed to work there, to satisfy the demand for our professional services.

In 2000, I moved my clinic to Fairfield to an even larger premises and we became known as Melbourne Holistic Health Group. It was here that I really started to get involved in working collaboratively with other medical professionals to attempt to gain the best outcomes for patients. I was also invited to work in a multidisciplinary medical clinic for the treatment of obesity; this proved to be stimulating and challenging work.

What was your experience of working with gynaecologists?

There were many occasions when I was asked to present to groups of medical practitioners about the use of herbal medicine in gynaecology. In the early days, gynaecologists were interested in the use of *Vitex agnus-castus* as there was evidence for this herb's efficacy in premenstrual syndrome (PMS). At these talks I was continually amazed by how interested they were about the wider scope of herbal medicine.

Over time, relationships developed, many had read my book, and they became more and more informed about herbal medicine. For example, when I went to talk to a self-help group at the Royal Women's Hospital, the gynaecologist who was speaking alongside me gave a full evidence-based talk on why herbal medicine was a suitable addition to these women's health care. From the inception of my practice in the early '80s, referrals were common, particularly for complaints for which medicine had little to offer. This expanded over the years to include more and more specialists and for more and more different types of complaints.

I also regularly presented with gynaecologists to self-help groups, principally on polycystic ovary syndrome (PCOS), endometriosis, infertility and menopause.

Three strengths of naturopathy

The provision of holistic health care and all this entails

This has always been an important aspect of my treatment. Years ago, a gynaecologist remarked that we (naturopaths) continue to see patients for years instead of curing them and sending them on their way. My response, given that the only way he was going to understand holistic medicine, was that we worked similarly to the old-fashioned country doctor, who was responsible for every aspect of health for all family members, from birth to death. The gynaecologist thought about this and then apologised saying that this aspect was sadly missing from modern medicine, which now largely focused on conditions rather than patients.

Safer interventions

Lifestyle correction and natural remedies are overall safer than their medical counterparts.

Unique treatment options

There are some herbal medicines that provide treatments for conditions that medicine has no answer for. For example, Vitex in certain types of premature ovarian failure.

Three areas (of the profession) in need of development

Delaying appropriate intervention

More attention needs to be given to the potential harm that can be done when practitioners undertake the treatment of patients with conditions that may not be suitably responsive to natural therapies alone.

Mentoring

The profession needs to have a more cohesive approach to mentoring. I have done this throughout my career without ever charging for this service, since I believe experienced practitioners have an obligation to pass on their knowledge to students and the less experienced. This benefits not only individual practitioners but improves the overall development of the profession.

Diagnosis, particularly differential diagnosis

One of the most common questions I would ask of practitioners learning from me was "What else could this be?" And it was, by far, the weakest aspect in their knowledge. Not only did they not know what it could be, but they had not even thought to consider a differential diagnosis.

Future hopes and dreams

Future hopes

That the world at large wakes up and realises what a mess we are in and that we have strong leaders who take global warming and the associated problems seriously. At an individual level, that we all strive to protect and heal the planet in the same way that we do for our patients.

Dreams

To grow veggies and herbs, and to contribute to my community, especially in relation to the housing crisis.

———

Ruth then walked me around her tranquil garden and gathered a bunch of basil, zucchinis and cucumber for me to take home 😊

Australian Journal of Herbal and Naturopathic Medicine **2018** 30(4)

Nigella sativa (black seeds): Panacea or hyperbole? A critical review of experimental and clinical observations

Shahid Akbar MD, PhD

Former Professor of Pharmacology, Medical University of the Americas, Nevis, and Department of Pharmacology, Director, College of Pharmacy Research Center, Qassim University, Buraidah, Saudi Arabia

Contact information

Dr Shahid Akbar, 2651 Eagle Rock Circle, Stockton, CA 95209, USA

Email shahidakbar@sbcglobal.net drakbarmdphd@gmil.com

Abstract

Introduction: Black seeds, also called black cumin, and known as *Habb-es-Sauda* and *Habbat-ul-Barakah* (Seeds of Blessing) in Arabic, are reverently used by Muslims all over the world, because their usefulness and health benefits are mentioned in a Hadith of the Prophet Mohammad (PBUH). All ancient and modern cultures, be it Greek, Roman, Egyptian, Mediterranean, Hindu or others have used black seeds in one or the other form since millennia.

Purpose: This review is an attempt to critically evaluate the experimental and clinical evidence in support of the health benefit claims about black seeds or lack thereof. The major focus of this review was on studies that involved whole seeds, powdered seeds, their aqueous decoction/extract or black seeds oil, administered orally. Reports that did not specify the nature of the extract used in the studies, or studies involving isolated constituents were generally avoided.

Results: In most clinical trials, a daily dose of 2 g of black seeds was found optimum to achieve health benefits, including on blood glucose, lipid profile, inflammation, gastrointestinal symptoms and the central nervous system (CNS). Most of the effects on the CNS were reported by the volatile oil. Other positive results reported in clinical trials have not been reproduced or expanded to validate their outcomes.

Discussion: A number of positive results were achieved in many clinical trials on black seeds, but a paucity of clinical studies reproducing these reported clinical benefits necessitates clinical trials in more diverse populations, of longer duration, and involving a significantly higher number of patients to establish the reproducibility.

Keywords: Nigella sativa, black seed, black cumin, *Habb-es-Sauda*, *Habbat-ul-Barakah*

Introduction

Despite breath-taking advances in modern medicine we are still grappling to find cures for the most common diseases of our times, like diabetes and hypertension, and the increasing incidences of various types of cancer that affect populations in every part of the globe. Since natural or plant-based drugs have been used by humans to address their health care needs for millennia and are still depended upon by large sections of societies throughout the world, they become our focus of attention when modern medicine fails to provide sufficient, safe, inexpensive or long-term answers. Black seeds, also called black cumin, are known as *Habb-es-Sauda*, and *Habbat-ul-Barakah* (Seeds of Blessing) in Arabic and are reverently used by Muslims all over the world, both medicinally and as a spice, because the Prophet Muhammad (Peace Be Upon Him) is quoted to have said "Use black seed regularly, since it is a cure for every disease except death"[1]. Black cumin are the seeds of an annual flowering plant, *Nigella sativa*, native to the Mediterranean, and south and south-west Asian countries, belonging to the family Ranunculaceae. Use of black cumin seeds dates back to the ancient Egyptians, Greeks, and Romans. Hippocrates and Dioscorides mentioned them as *Melanthion*, and Pliny called them *Gith*[2]. Black cumin seeds were found in the tomb of the Egyptian Pharaoh, Tutankhamen (also spelled as Tutankhamun), who ruled Egypt c. 1332–1323 BC[3], and were also recovered in north-central Turkey from a pilgrim flask of the Old Hittite period of Boyali Höyük, dating from around 1650 BC[4]. "Muslim physicians of Greco-Arab medicine in India describe them as heating, attenuant, suppurative, detergent and diuretic, and believe that they increase menstrual flow and the secretion of milk, and stimulate uterine activity"[2]. They are also claimed to have been used to treat nervous system diseases such as memory impairment, epilepsy, neurotoxicity, and pain[5]. Hindu physicians of India use them with other aromatics and Plumbago root in dyspepsia, loss of appetite, diarrhoea and intermittent fevers, and a decoction of the seeds is given after delivery to stimulate uterine contraction and to stimulate milk secretion[6]. In India, Arab countries, Europe and Iran, the seeds and oil are also traditionally used in the treatment of asthma, hypertension, diabetes, inflammation, tumour, cough, bronchitis, headache, eczema, fever, dizziness, gastrointestinal disturbances, impotence, painful menstruation, flu, and as a carminative, diuretic and anti-parasitic agent[7]. The seeds are one of the most frequently

used plant medicine to treat diabetes and hypertension in the south-eastern Moroccan folk medicine[8], and more than 70% of Jordanian diabetic patients use them as adjunct to conventional therapy and 80% of them with the knowledge of their physicians[9]. This review is intended to evaluate the published experimental and clinical studies, listed on PubMed, to determine if the claims made about health benefits of black seeds stand scientific scrutiny.

Methodology

Published articles in English listed on PubMed until 2018 were included in the review. The focus of this review were studies, both animal and human clinical trials, that used orally, either whole seeds, powdered seeds, their aqueous decoction/extract or the oil, to reconcile with the common forms of traditional human use. Studies on organic solvent extracts, routes of administration other than oral, or isolated individual constituents, such as thymoquinone (TQ), the major active constituent of the oil, have been sparingly used where relevant or in cases a comparison was needed. Similarly, *in vitro* studies have rarely been mentioned, except only where their inclusion was relevant to complement other findings. The nationalities of the patients involved in clinical studies have been identified for the simple reason that the racial differences, cultural and psychological influences, and dietary habits may affect the observed outcomes, and observations in one set of individuals may, at times, not be replicated in other individuals. Also, the chemical contents of the seeds and volatile oil (VO) from various countries and within a country may vary remarkably and produce variation in results.

Results

Chemical constituents

The seeds are reported to contain 36%–38% fixed oil, proteins, alkaloids, saponin, and 0.4%–2.5% essential oil[10]. Alkaloids, anthraquinones, flavonoids, phenolic compounds, proteins, carbohydrates, saponins, lipids, sterols and tannins have also been reported present in aqueous and methanol extracts[11,12]. The alkaloidal fraction include indazole-type alkaloids, such as nigellidine and nigelanoid[13,14], and dolabellane-type diterpene alkaloids, such as nigellamines[15,16], and various triterpene glycosides[17], such as α-hederin[18], and steroidal glucoside[19], have also been isolated from the seeds. The seeds contain both the fixed and volatile oil, and thymoquinone is present in both oils[7]. The chemical composition of VO of seeds from various countries and within a particular country has been reported to vary remarkably. Volatile oil of seeds from India contained 47 compounds, of which TQ, dithymoquinone, thymohydroquinone and thymol were the major phenolic compounds, varying dependent upon extraction method[20]; TQ and *p*-cymene were the major constituents of the 38 volatile compounds identified in seed oil from Turkey[21]; but TQ (62.17%), carvacrol (16.84%), 2-methyl-5-Prop-2-enyldihydroquinone (8.29%), dihydro-thymoquinone (6.99%), monoterpenes (3.11%) and terpini-4-en-1-ol (2.07%) were identified as major constituents of VO of seeds from Morocco[22]. However, major variations were reported from Iran; while trans-anethole (38.3%), *p*-cymene (14.8%), limonene (4.3%) and carvone (4.0%) were identified as major compounds in VO out of the 32 compounds by Nickavar *et al.*[23], TQ (42.4%), *p*-cymene (14.1%), carvacrol (10.3%) and longifolene (6.1%) were reported as the major components by Mahmoudvand and associates[24]; whereas, *p*-cymene (58.2%), α-thujene (11.2%), carvacrol (3.63%) and longifolene (3.32%) were the main components out of 14 identified in the steam distilled VO[25], all from Iran.

Experimental studies

The results of experimental studies are tabulated in Table 1.

Anticancer activity

The seeds have been reported to exert anti-proliferative, pro-apoptotic, cytotoxic, antimutagenic, antimetastatic, and NK cells cytotoxic activity enhancing effects against various primary cancer cells and cancer cell lines[26]. Black seeds supplemented diet inhibited DNA damage in azoxymethane-induced colon cancer in rats[27], and oral treatment of rats with seeds significantly suppressed ferric nitrilotriacetate-induced renal carcinogenesis[28], and with honey protected 100% against methylnitrosourea (MNU)-induced oxidative stress and renal carcinogenesis in rats[29]. Topical application of seed extract delayed the onset of dimthylbenz [a] anthracene (DMBA)-induced skin papillomas, and intraperitoneal administration significantly reduced the incidence of 20-methylcholanthrene (MCA)-induced soft tissue sarcoma in mice[30]. The VO also inhibited 1,2-dimethylhydrazine (DMH)-induced colon carcinogenesis of rats in the post-initiation stage, with suppression of cell proliferation in the colonic mucosa[31], and the seed oil treatment decreased the expression of Brca1, Brca2, Id-1 and P53 mutations in mammary tissues of female rats with DMBA-induced breast cancer and reduced the activities of tumour markers[32]. While the MCF-7 breast cancer cells exposed to alcohol seed extract were completely inactivated, aqueous extract showed weak activity[33]. Injection of the VO into the tumour also significantly inhibited solid tumour development[22]. TQ inhibited DNA synthesis, proliferation, and viability of cancerous prostate epithelial cells by down-regulating androgen receptors and E2F-1, a regulator of cell proliferation and viability, without affecting the noncancerous cells[34], inhibited benzo(a)pyrene (BP)-induced forestomach carcinogenesis[35], prevented MCA-induced fibrosarcoma tumours[36], and blocked tumour angiogenesis in a xenograft human prostate cancer in mice[37]. TQ is reported to exert anticancer effects through activation of tumour suppressor gene, phase II gene/enzymes, peroxisome proliferator-activated receptors (PPARs), inactivation of angiogenesis and anti-inflammatory gene, and induction of apoptosis[38].

Table 1: Brief descriptions of experimental studies on black seeds (*Nigella sativa*)

Activity	Type of study	Form and route of use	Model/Animals	Results	Reference(s)
Anticancer	*In vitro*	Alcohol seed extract	MCF-7 breast cancer cells	Anti-proliferative, and cytotoxic effect	Farah and Begum, 2003
	In vivo	Seed oil (oral)	DMBA-induced breast cancer in female rats	Decreased expression of the Brca1, Brca2	Linjawi *et al.*, 2015
	In vivo	Dietary seed supplementation	Azoxymethane-induced colon cancer in rats	Prevented DNA damage	Al-Johar *et al.*, 2008
	In vivo	Intra-tumour injection of VO	Solid grafted tumour in mice	Significantly inhibited tumour development, liver metastasis and improved survival	Ait Mbarek *et al.*, 2007
	In vivo	Dietary seed supplementation	Nitrilotriacetate-induced renal carcinogenesis in rats	Suppressed oxidative stress, and hyperproli-ferative response	Khan and Sultana, 2005
	In vivo	VO (oral)	DMH-induced colon carcinogenesis in rats	Inhibited colon carcinogenesis and colonic mucosa cell proliferation	Salim and Fukushima, 2003
	In vivo	Powdered seeds 0.2 g/day (oral)	MNU-induced carcinogenesis in rats	Protected against oxidative stress and carcinogenesis by 80%	Mabrouk *et al.*, 2002
	In vivo	Topical application of seed extract	DMBA-induced skin papillomas in mice	Delayed onset of skin papillomas	Salomi *et al.*, 1991
	In vivo	Seed extract (i.p.)	MCA-induced soft tissue sarcoma in mice	Reduced the incidence of soft tissue sarcoma	Salomi *et al.*, 1991
	In vivo	TQ (oral)	BP-induced forestomach carcinogenesis in mice	Significant inhibition of tumourigenesis	Badary *et al.*, 1999
	In vivo	TQ (oral)	MCA-induced fibrosarcoma in mice	Significantly prevented fibrosarcoma development	Badary *et al.*, 2001
	In vivo	TQ (oral)	Human prostate cancer xenograft in mice	Blocked tumour angiogenesis	Yi *et al.*, 2008
Antidiabetic	*In vitro*	Aqueous seed extract	Isolated rat jejunum	Inhibited sodium-dependent glucose transport	Meddah *et al.*, 2009
	In vivo	Aqueous seed extract (oral)	Normal rats	Improves glucose tolerance	Meddah *et al.*, 2009
	In vivo	Aqueous seed extract (oral)	Diabetic rabbits	Lowered blood glucose, and prevented lipid peroxidation-induced liver damage	Meral *et al.*, 2001
	In vivo	Fixed oil (oral)	Normal rats	Lowered blood glucose, TC, and TGs	Zaoui *et al.*., 2002
	In vivo	Fixed and VO (oral and i.p.)	Diabetic rats	Reduced oxidative stress	Kanter *et al.*, 2004; Sultan *et al.*., 2014
	In vivo	VO (oral and i.p.)	Diabetic rats	Lowered blood glucose, increased phagocytic activity and βcell regeneration	Fararh *et al.*, 2002, 2004; Kanter *et al.*, 2003, 2009
Antihyperlipidaemic	*In vivo*	Dietary seed supplementation	Normal and hypercholesterolaemic rats	Lowered TC, LDL-C, TGs, and improved antioxidants activity	Kocyigit *et al.*, 2009; Muneera *et al.*, 2015; Al-Rasheed *et al.*, 2014
	In vivo	Diet supplemented with seed powder, VO	Hypercholesterolaemic rabbits	Lowered TC, LDL-C, increased HDL-C, and inhibited aortic plaque formation	Al-Naqeep *et al.*, 2011
	In vivo	Fixed oil (oral)	Normal rats	Lowered blood glucose, TC, TGs, WBCs, and platelets	Zaoui *et al.*, 2002a,b
Analgesic/Anti-inflammatory	*In vivo*	Aqueous seed suspension (oral)	Paw oedema in rats; hot plate in mice	Antinociceptive, and anti-inflammatory effects	Al-Ghamdi, 2001
	In vivo	VO (oral), TQ (oral, i.p., i.c.v.)	Hot-plate, tail-pinch, acetic acid-writhing and formalin tests	Antinociceptive effect	Abdel-Fattah *et al.*, 2000
	In vivo	TQ (i.p.)	Airway inflammation, adjuvant-arthritis,	Anti-inflammatory effects	Abbas *et al.*, 2005; El Mezayen *et al.*, 2006; Tekeoglu *et al.*, 2007
	In vitro	Fixed oil and TQ	Peritoneal leukocytes	Inhibition of COX and 5-LOX	Houghton *et al.*, 1995

Table 1: Brief descriptions of experimental studies on black seeds (*Nigella sativa*) *continued*

Activity	Type of study	Form and route of use	Model/Animals	Results	Reference(s)
Antimicrobial	*In vitro*	Aqueous extract		More effective against multiple antibiotics-resistant Gram -ive isolates	Morsi, 2000
	In vitro	VO		Strongly active against *Aspergillus fumigatus*, *T. mentagrophytes*, *M. canis* and *M. gypseum*	Islam *et al.*, 1989; Mahmoudvand *et al.*, 2014
	In vivo	VO (i.p.)	Murine cytomegalovirus (MCMV) infected BALB/c mice	Inhibited MCMV titres in spleen and liver, increased serum IFN-gamma	Salem *et al.*, 2000
Cardiovascular	*In vivo*	Dietary seed supplementation	Normal rats	Cardiac hypertrophy, improved cardiac contractility and performance	Al-Hariri *et al.*, 2009; El-Bahai *et al.*, 2009; Yar *et al.*, 2008
	In vivo	Oral seed powder for 12 weeks	Normal rats	Increased resistance to oxidative stress and I/R damage	Seif, 2013
	In vivo	Aqueous seed extract (i.v.)	Normal rats	Reduced BP and HR	Hebi *et al.*, 2016
	In vivo	Aqueous seed extract (i.p.)	Normal rats	Diuresis, natriuresis and kaliuresis	Asif *et al.*, 2015
CNS	*In vivo*	Aqueous extract	Normal rats	Anxiolytic effect	Bano *et al.*, 2014
	In vivo	VO (oral)	Normal rats	Antidepressant effect, improved learning and memory, prevented morphine tolerance and dependence	Perveen *et al.*, 2013; Sahak *et al.*, 2013; Abdel-Zaher *et al.*, 2010
	In vivo	VO (oral)	Normal and diabetic rats	Prevented oxidative brain damage and diabetic neuropathy	Ezz *et al.*, 2011; Ilhan *et al.*, 2005; Hosseinzadeh *et al.*, 2007; kanter *et al.*, 2006; Kanter, 2008
	In vivo	VO (oral)	Normal rats	Prevented haloperidol-induced movement disorders and dyskinesia	Malik *et al.*, 2016
GIT	*In vivo*	VO (oral)	Normal rats	Reduced experimental gastric ulcers, gastric oxidative stress, and protected against TNBS-colitis	Bukhari *et al.*, 2011; El-Dakhakhny *et al.*, 2000; Kanter *et al.*, 2005, 2006; El-Abhar *et al.*, 2003; Isik *et al.*, 2011
Liver	*In vivo*	Dietary seed supplementation	Normal rats	Protected against hepatotoxic agents	Farrag *et al.*, 2007; Hassan *et al.*, 2012; Jaswal *et al.*, 2016
	In vivo	Water suspension and extract of seeds	Normal rats	Protected against CCl4-induced hepatotoxicity	Al-Ghamdi, 2003; Jaswal and Shukla, 2015
	In vivo	VO (oral)	Normal rats	Protected against CCl_4-, *d*-galactosamine, hypervitaminosis A hepatotoxicity, and I/R injury	Al-Seeni *et al.*, 2016; Meral and Kanter, 2003; Türkdoğan *et al.*, 2003; Ibrahim *et al.*, 2008; el-Dakhakhny *et al.*, 2000; Al-Suhaimi, 2012; Yildiz *et al.*, 2008
Kidneys	*In vivo*	Powdered seeds	Normal rats	Protected against I/R injury	Mousavi, 2015; Mousavi and Mohajeri, 2014
	In vivo	VO (oral)	Normal and diabetic rats	Ameliorated gentamicin, sodium nitrite, bromobenzene acetaminophen, and cyclosporine nephrotoxicity, and protected against I/R injury and diabetic nephropathy	Ali, 2004; Saleem *et al.*, 2012; Yaman and Balikci, 2010; Al-Gayyar *et al.*, 2016; Hamed *et al.*, 2013; Ahmed and El-Mottaleb, 2013; Uz *et al.*, 2008; Bayrak *et al.*, 2008; Havakhah *et al.*, 2014; Yildiz *et al.*, 2010; Yusuksawad and Chaiyabutr, 2011
Reproduction	*In vivo*	Dietary seed supplementation	Normal rats	Increased testosterone level	Mohajeri *et al.*, 2015
	In vivo	Low dose seeds (oral)	Ovariectomised rats	Oestrogenic like effects on reproductive organs	Parhizkar *et al.*, 2016
	In vivo	Aqueous extract	Normal rats	Increased milk production	Agrawala *et al.*, 1971; Hosseinzadeh *et al.*, 2013
	In vivo	VO (oral)	Normal rats	Improved semen quality, and sperm motility	Mosbah *et al.*, 2016; Cho Ping *et al.*, 2014

VO: Volatile oil; BP: Benzo(a)pyrene; CCl_4: Carbon tetrachloride; COX: Cyclooxygenase; DMBA: Dimethylbenz[a]anthracene; DMH: Dimethylhydrazine; I/R: Ischaemia/Reperfusion 5-LOX: Lipoxygenase; MCA: Methylcholanthrene; MNU: Methylnitrosourea; TC: Total cholesterol; TGs: Triglycerides

Antihyperglycaemic effects

The aqueous seed extract (AE) was shown to inhibit *in vitro* sodium-dependent glucose transport across isolated rat jejunum, and *in vivo* improved glucose tolerance in normal rats after six weeks of treatment (2g/kg/d), comparable to metformin[39], reduced blood glucose and malonaldehyde (MDA) concentrations, increased glutathione (GSH) level and prevented lipid peroxidation-induced liver damage in diabetic rabbits[40]. Oral administration of fixed oil to normal rats for 12 weeks significantly decreased glucose levels, serum cholesterol (TC), and triglycerides (TGs), and leukocyte and platelet counts[41]. The fixed and VO significantly improved diabetes-induced oxidative stress in rats[42,43], and the VO significantly reduced blood glucose, increased antioxidant enzymes activity[42], significantly increased phagocytic activity of peritoneal macrophages of diabetic hamsters, at least partly, from a stimulatory effect on β-cells function[44,45], caused a partial regeneration/ proliferation of pancreatic β-cells in diabetic rats[42,46], and preserved β-cells integrity[47]; an extrapancreatic component is suggested for the hypoglycemic effect because the fall in blood glucose is not parallel to the stimulated insulin release[48].

Antihyperlipidaemic effects

The positive effects on lipid profile were evident when diet supplemented with seeds significantly decreased TC, LDL-cholesterol (LDL-C) and TGs in normal[49], and hypercholesterolaemic rats[50], and powdered seeds highly significantly reduced serum LDL-C and TGs, and improved antioxidant enzymes activities in rats with fructose-induced metabolic syndrome[51]. Hypercholesterolaemic rabbits supplemented in diet with seed powder and VO had significantly reduced TC and LDL-C and enhanced HDL-cholesterol (HDL-C) levels and significantly inhibited aortic plaque formation[52]. One-week administration of the seed oil afforded significant and strong protection (94.5%) against methionine-induced hyperhomocysteinemia and the associated changes in triglycerides, lipid peroxidation, and cholesterol[53]. Twelve weeks oral treatment of rats with fixed oil significantly decreased serum TC, TGs and glucose levels, slowed body weight gain, significantly increased haematocrit and hemoglobin levels, but significantly reduced leukocyte and platelet counts[41,54].

Analgesic and anti-inflammatory effects

The AE exhibited both analgesic and anti-inflammatory activities[55], whereas ethanol extract is reported to produce anti-inflammatory effect of longer duration[56] but a lesser analgesic effect than diclofenac[57]. Oral administration of the VO produced dose-dependent analgesic activity, that was significantly blocked by naloxone, indicating the possibility of involvement of opioid mechanism[58], but only i.p. dose exhibited significant anti-inflammatory effect[59]. Both fixed oil and TQ inhibited cyclooxygenase (COX) and 5-lipoxygenase (5-LOX) pathways in rat stimulated peritoneal leukocytes, significantly reducing synthesis of thromboxane B2 and leukotriene B4, respectively. However, the inhibition of eicosanoid generation rendered by the fixed oil was greater than was expected from its TQ contents, an indication that constituent(s) other than TQ may contribute to the anti-inflammatory effect[60]. Pretreatment with TQ (i.p.) attenuated ovalbumin-induced airway inflammation in mice[61,62], suppressed adjuvant-induced arthritis in rats[63], and potently inhibited the formation of leukotrienes in blood cells[48,64].

Antimicrobial activity

The AE was more effective against multiple antibiotics-resistant Gram negative isolates than the Gram positive ones[65], and *Candida albicans*[66]. Aqueous infusion of ground seeds from *Hadramout* (Yemen) was reportedly more effective in inhibiting *in vitro* growth of *Staphylococcus aureus* (*S. aureus*) than the ground seeds from Ethiopia, but without any effect on *Escherichia coli* (*E. coli*) or Enterobacter[67]. Ethanol seed extract was reported active against various strains of methicillin-resistant *S. aureus* (MRSA)[68], and against *Salmonella typhi* (*S. typhi*), *S. aureus*, *Bacillus subtilis*, *Bacillus cereus*, *Klebsiella pneumoniae*, *E. coli*, Xanthomonas, *Salmonella heidelberg*, Clostridium, *Aspergillus niger*, *Aspergillus flavus*, *Alternaria alternata* and Penicillium[69]. VO was significantly active against *S. aureus*, *Micrococcus lysodeikticus* and *Sarcina lutea*, and moderately active against *E. coli*, *S. typhi*, *Pseudomonas aeruginosa*, *B. subtilis* and *C. albicans*, with thymohydroquinone identified as the active principle[70], and strongly active against *Aspergillus fumigatus*[71], on dermatophytes, *Trichophyton mentagrophytes*, *Microsporum canis* and *Microsporum gypseum*[24]. Nearly half of the 19 isolates of multidrug resistant *S. aureus*, from Nigerian diabetic patient's wounds were susceptible to different concentrations *N. sativa* oil[72]. Alpha-zam, an indigenous Nigerian formulation of *N. sativa*, selectively inhibits hepatitis C virus (HCV) replication[73]. Oral administration of the oil was protective against hematological and biochemical changes in schistosomiasis-infected mice and markedly improved the antioxidant capacity[74], whereas administration of VO (i.p.) to BALB/c mice infected with a susceptible strain of murine cytomegalovirus (MCMV), strikingly inhibited the MCMV titres in spleen and liver, and increased serum level of IFN-gamma[75].

Cardiovascular effects

Dietary supplementation of normal rats with seeds produced a homogeneous cardiac hypertrophy and enhanced cardiac contractility and improved cardiac performance[76-78], preserved and augmented exercise-induced physiological cardiac hypertrophy[79], and significantly increased vascular endothelial growth factor (VEGF) in heart similar to aerobic exercise, that could potentially induce coronary angiogenesis[80]. However, both aqueous and macerated extracts significantly reduced heart rate (HR) and contractility of isolated

guinea pig heart, due to calcium channel blocking effect that was greater than nifedipine[81,82], and hearts isolated from rats administered seed powder orally for 12 weeks showed resistance to oxidative stress and damage due to ischaemia/reperfusion (I/R) injury, further reinforcing the positive effect on heart[83]. Seeds incorporated in rats' diet for four weeks induced significant hyper-fibrinogenemia, while doubling the dose for two weeks significantly, but transiently prolonged prothrombin time and reduced thrombin time[84]. Intravenous administration of aqueous seed extract lowered mean arterial pressure (MAP) with a significant decrease in HR in normal rats, that was partly dependent on endothelium[85]. Oral administration of oil prevented increase in systolic blood pressure (SBP) of L- NAME-induced hypertensive rats, along with reduction in cardiac lipid peroxidation product, NADPH oxidase, angiotensin-converting enzyme activity and plasma nitric oxide[86]. The arterial BP and HR lowering effects of VO (i.v.) in anaesthetised rats were antagonised by cyproheptadine, hexamethonium, atropine and pretreatment with reserpine[87]. However, the vasorelaxant effect of oil was reportedly not mediated by nitric oxide and is independent of endothelium[88]. Intraperitoneal administration of AE also produced significant diuresis coupled with natriuresis and kaliuresis in rats[12], that could also contribute to the hypotensive effect.

CNS effects

Aqueous extract of defatted seeds exhibited potent CNS depressant and analgesic effects[89], the AE also showed anxiolytic activity[90], and the VO produced antidepressant effect with increase in brain 5-HT levels and decreased 5-HT turnover in rats[91]. Pretreatment with VO for 20 days improved learning and memory of normal rats[92], and repeated administration of VO attenuated the development of tolerance and dependence to morphine[93], probably through some mechanism other than opioid receptors. The VO effectively prevented brain oxidative injury due to experimental seizures, better than valproate[94,95], ameliorated brain ischaemia and spinal cord injury-induced oxidative damage[96,97], and diabetic neuropathy[98], but failed to significantly affect pilocarpine-induced spontaneous recurrent seizures in rats[99]. Pretreatment with hydro-alcohol extract improved learning and memory in rats[100] and prevented hippocampal neural damage in pentylenetetrazole-induced repeated seizures in rats[101], protected against scopolamine-induced spatial memory impairment[102], and against hypothyroidism-associated learning and memory weakening during neonatal and juvenile growth in rats[103]. Treatment of aged female rats with VO for two months improved the structure and the thickness of olfactory epithelium, that tends to reduce in thickness due to ageing[104]. Co-administration of VO to rats significantly protected against haloperidol-induced EPS-like effects, including movement disorders and oral dyskinesia[105]. Chronic toluene exposure caused neurodegeneration in the frontal cortex and brain stem of rats was completely prevented by concurrent oral administration of seeds[106] and thymoquinone[107].

Gastroprotective effects

Pretreatment with seeds and VO significantly reduced experimental gastric ulcers and gastric mucosal histamine, and significantly increased gastric glutathione level, mucin content and free acidity[97,108-110]; they also protected against I/R-induced gastric oxidative stress and lesions[111], and the oil was reported to decrease pro-inflammatory cytokines, lactate dehydrogenase, TG, and TC in trinitrobenzene sulfonic acid (TNBS)-induced colitis in rats[112].

Hepatoprotective effects

Liver is the main organ for the detoxification of harmful substances that enter the body, but it is also the target of various hepatotoxic agents, such as prescription drugs and environmental toxins. Black seeds supplemented diet protected against lead acetate-induced[113], and isoniazid-induced hepatotoxicity[114,115], and fixed oil and VO protected against ethanol-induced oxidative stress and liver damage[116,117]. Oral administration of water suspension and extract of the seeds was also protective against carbon tetrachloride (CCl4)-induced hepatotoxicity[118,119], so were both the fixed oil[120], and VO.[120-122]. VO completely protected against *d*-galactosamine hepatotoxicity[123], alleviated CCl4-induced suppression of CYP2B, CYP3A2, CYP2C11 and CYP1A2[124], protected liver against the effects of hypervitaminosis A[125], against I/R injury[126], and decreased lipid peroxidation, liver enzymes and increased antioxidant enzyme levels[127,128]. Overall, the seeds and oils were significantly protective against various hepatotoxic agents[129]. TQ was the only constituent of VO that could efficiently protect against chemically-induced hepatic damage[130].

Nephroprotective effects

Powdered seeds administration significantly lowered serum creatinine with no effect on kidney architecture in healthy rats[131], and protected kidneys against I/R injury[132-135]. Treatment with the oil significantly ameliorated gentamicin nephrotoxicity in rats[136-138], sodium nitrite nephrotoxicity[139], diabetic nephropathy[140], attenuated bromobenzene-induced hepatorenal injury[141], significantly normalised physiological parameters and prevented acetaminophen-induced structural changes in kidneys[142], protected against I/R injury of kidneys[143,144], and attenuated the cyclosporine-induced oxidative stress and nephrotoxicity in rats[145]. TQ also protected against acute renal toxicity of gentamicin[146], doxorubicin-induced nephropathy[147], cadmium-induced oxidative stress and nephrotoxicity[148], and ameliorated formation of ethylene glycol-induced renal calculi in rats[149].

Reproductive effects

The diet of male mice supplemented with seeds significantly increased the testosterone level and ameliorated the deleterious effects of heat on spermatogenesis and antioxidant status[150]. Male rats treated with oil also had significantly higher percentage of motile normal and live sperm[151], improved semen

quality and moderated chlorpyrifos-induced reproductive toxicity[152]. Low-dose seeds exerted prominent oestrogenic like effects on reproductive organs of ovariectomised rats[153], and AE significantly increased milk production in rats[154]. However, the VO inhibited the spontaneous and oxytocin induced contractions of rat and guinea pig uterine isolated smooth muscle[155].

Clinical studies

The results of clinical studies are presented in Table 2.

Hyperglycaemia and dyslipidaemia

Supplementation of 94 Saudi patients of either sex with uncontrolled type 2 diabetes and HbA1c > 7%, with 1, 2, and 3 g/day of black seeds powder, an optimal dose of 2 g/day as an adjunct to oral antidiabetic drugs for twelve weeks, had significant reduction in TC, LDL-C, TG, significant increase in HDL-C/LDL-C ratio[156], significant reduction in FBG, HbA1c, and insulin resistance without significantly affecting body weight[157]; and continuing the treatment (2 g/day) for one year also significantly reduced insulin resistance, improved antioxidant defense capacity[158], had a significant reduction in SBP, diastolic blood pressure (DBP), MAP and HR[159], and 30 patients significantly decreased HbA1c, improved LV systolic function, and were protected from diastolic dysfunction compared to 30 placebo-treated patients[160]. In a randomised controlled trial (RCT), hypercholesterolaemic Iranian patients treated with 2 g of black seeds powder daily for four weeks had a significant decrease in TC, LDL-C and highly significant decrease in TG, but no significant effect on FBG[161], and administration of powdered seeds in a dose of 3 g/day along with garlic capsules for eight weeks to 30 healthy postmenopausal Emarati women significantly improved blood oxidants and antioxidants balance[162], whereas 19 postmenopausal hyperlipidaemic Malaysian women and 15 with metabolic syndrome, treated with powdered seeds 1g daily after breakfast for two months showed no significant reduction in body weight but a significant fall in FBG, TC, LDL-C, and TG, and increased HDL-C[163,164]. In a double-blind, crossover RCT, consumption of bread containing black seeds for two months by 27 Iranian patients of either sex with metabolic syndrome did not have any significant effects on TC, LDL-C, TG, HDL, APO-A, APO-B and high sensitive C-reactive protein, compared to those who consumed bread with wheat bran[165]. However, supplementation of powdered seeds (2 g daily) to 10 young sedentary overweight Iranian women coupled with aerobic exercise for eight weeks synergistically lowered TC, LDL-C, TG, and body mass index (BMI), and increased HDL-C, compared to 10 women who were assigned to aerobic training alone[166]. Sixty-nine perimenopausal and menopausal Malaysian women treated with 800 mg of seed powder twice daily for twelve weeks showed significant improvement in the prevalence and severity of menopausal symptoms and LDL-C, but no significant changes in TC, TG and HDL-C[167]. In a double-blind RCT, obese premenopausal Iranian women with BMI between 30 and 35 kg m^2 treated with seed oil (1 g before each meal) and low calorie-diet, or placebo for eight weeks, had significant reduction in body weight and waist circumference, and significant decline in LDL-C and TG levels, compared to placebo[168], increased superoxide dismutase levels, but no significant changes in lipid peroxidation, glutathione peroxidase, and total antioxidant capacity compared to the placebo group[169]. A meta-analysis of 17 RCTs concluded that supplementation with black seeds resulted in an average reduction of 15.6 mg/dL of TC, 14.1 mg/dL of LDL-C, and 20.6 mg/dL of TG. Seed oil lowered TC and LDL-C better than the seed powder; increase in HDL-C levels was, however, observed only with seed powder supplementation[170]. Another meta-analysis of seven RCTs in type 2 diabetics found that black seeds supplementation produced an average reduction of more than 17 mg/dl in FBG, and 22 mg/dl in TC and LDL-C, with no significant effects on TG and HDL-C[171].

Cardiovascular

In a double-blind RCT, healthy Iranian volunteers, aged 34 to 63 years, 2.5 mL of black seed oil twice daily for eight weeks significantly lowered both SBP and DBP from baseline and compared to the placebo group[172], and a seed extract for two months significantly reduced both SBP and DBP, and the TC and LDL-C in mildly hypertensive patients[173]. Meta-analysis of eleven RCTs involving 860 hypertensive or normotensive subjects reported a mean decrease of 7.66 mmHg in SBP and 4.89 mmHg in DBP after a mean treatment duration of 8.3 weeks with black seeds[174]. However, treatment of 30–45 years old Indonesian men with central obesity with 750 mg seeds twice daily for three months, highly significantly reduced body weight and waist circumference, but an insignificant reduction in SBP, DBP, and serum free testosterone, compared to placebo-treated group[175]; similarly, powdered seeds treatment of Iranian patients with Hashimoto's thyroiditis for eight weeks, significantly reduced body weight, BMI, and serum concentrations of thyroid stimulating hormone (TSH), and VEGF[176]. Administration of seeds (5 g daily) for 3–9 consecutive months to children (2–18 years) with brain tumours undergoing chemotherapy, reduced febrile neutropenia episodes to 2.2%, compared to 19.3% in control patients[177].

Inflammation

In a placebo-controlled study, the number of swollen joints and the duration of morning stiffness in 40 Egyptian female patients with rheumatoid arthritis improved after oral treatment with seed oil capsules 450 mg twice daily for a month[178]. Similar results were reported from a double-blind RCT in 42 Iranian female patients with rheumatoid arthritis treated with the oil or placebo for eight weeks, that also reported reduced oxidative stress[179]. However, administration of seed powder 2 g/day in divided doses to 55 Iranian patients with knee osteoarthritis did not significantly differ from

Table 2: Brief characteristics of clinical studies on black seeds (*Nigella sativa L.*)

Study year	# of patients	Form of use and dose	Type of study and targeted subjects	Results	Reference #
2016	860	Powdered seeds and oil for an average 8.3 weeks	Meta-analysis of RCTs, hypertensive and normotensive subjects	Short-term treatment significantly reduced SBP and DBP	Sahebkar *et al.*, 2016b
2016	42	N.S. oil, 1000 mg/day for 8 weeks or placebo	RCT, Anti-inflammatory and antioxidant effects in rheumatoid arthritis subjects	Significant reduction of serum MDA and NO	Hadi *et al.*, 2016
2016	52	Topical application of 600 mg N.S. oil to breasts, twice daily for 2 months	Randomised controlled, comparator study in female patients with cyclic mastalgia	Significant improvement in pain scores	Huseini *et al.*, 2016
2016	51	Seeds supplemented bread for 2 months or placebo	Randomised, double-blind, cross-over clinical study in patients with metabolic syndrome	No significant effects on TG, TC, LDL-C, HDL-C, APO-A, APO-B and high-sensitivity CrP	Mohtashami *et al.*, 2016
2015	60	Powdered seeds 2 g/day for 1 year or placebo	Controlled, nonrandomised study in uncontrolled diabetes patients	Significant reduction in HbA1c Prevented diastolic dysfunction	Bamosa *et al.*, 2015
2015	114	Powdered seeds 2 g/day for 1 year or placebo	Controlled, nonrandomised study in uncontrolled type 2 diabetes patients	Significant reduction in FBG, HbA1c, and improved antioxidant defence system	Kaatabi *et al.*, 2015
2015	50	N.S. oil 3 g/day with a low-calorie diet for 8 weeks or placebo	Double-blind RCT in obese women with BMI 30–35	Significant reduction in body weight	Namazi *et al.*, 2015
2015	90	N.S. oil 3 g/day with a low-calorie diet for 8 weeks or placebo	Double-blind RCT in obese women with BMI 30–35	Significant reduction in body weight and waist circumference, TG and VLDL	Mahdavi *et al.*, 2015
2015	70	Honey-based formulation of N.S. oil (5 ml daily) or placebo	Double-blind RCT in patients with functional dyspepsia	Significant reduction in severity score and the rate of *H. pylori* infection	Mohtashami *et al.*, 2015
2014	37	Powdered seeds 1 g/day for 2 months	Randomised, placebo-controlled, study in hyperlipidaemic menopausal women	Significant reduction in TC, TG, LDL-C, and increase in HDL-C	Ibrahim *et al.*, 2014a
2014	30	Powdered seeds 1 g/day for 2 months	Randomised, placebo-controlled, study in menopausal women with metabolic syndrome	Significant reduction in TC, TG, LDL-C, FBG, and increase in HDL-C	Ibrahim *et al.*, 2014b
2014	69	Powdered seeds 1600 mg/day for 12 weeks	Placebo-controlled, nonrandomised, cross-over study in perimenopausal women aged 45 to 65 years	Significant improvement in menopausal symptoms, and reduction in LDL-C and blood glucose	Latiff *et al.*, 2014
2014	20	Powdered seeds 1 g/day for 8 weeks or placebo	Randomised, double-blind, controlled trial in sedentary overweight females	Significant reduction in TC, TG, LDL-C, BMI, and increase in HDL-C	Farzaneh *et al.*, 2014
2014	68	N.S. oil 2.5 ml/twice daily or Placebo for 2 months	Controlled, nonrandomised study in infertile men with abnormal semen quality	Significant improvement in sperm count, motility and morphology and semen volume	Kolahdooz *et al.*, 2014
2014	48	N.S. seeds (500 mg/day) or Placebo for 4 weeks	Effect of N.S. on mood and cognition in healthy adolescent males	N.S. stabilised mood, decreased anxiety and modulated cognition positively	Bin Sayeed *et al.*, 2014
2014	26	Topical application of N.S. oil or Fish oil on vitiligo	Comparative, nonrandomised, uncontrolled study of N.S. oil and fish oil on vitiligo	N.S. oil more effective than fish oil in reducing vitiligo lesions	Ghorbanibirgani *et al.*, 2014
2014	42	Topical intranasal application of N.S. oil or placebo	Randomised, crossover and controlled trial of geriatric patients with nasal dryness	Significant improvement in nasal dryness, obstruction and crusting by N.S. oil	Oysu *et al.*, 2014
2013	40	Seeds (500 mg/day) or placebo for 9 weeks	Effects of N.S. on memory, attention and cognition in healthy elderly volunteers	N.S. significantly improved memory, attention and cognition	Bin Sayeed *et al.*, 2013
2013	70	N.S. oil 2.5 ml twice daily for 8 weeks or placebo	Double-blind, RCT on blood pressure of normotensive subjects	Significant reduction in SBP and DBP	Fallah Huseini *et al.*, 2013

Table 2: Brief characteristics of clinical studies on black seeds (*Nigella sativa L.*) *continued*

Study year	# of patients	Form of use and dose	Type of study and targeted subjects	Results	Reference #
2013	52	N.S. oil, Betamethasone or Eucerin Cream applied twice daily for 4 weeks	Double-blind, randomised, comparative study in patients with hand eczema	Both N.S. and Betamethasone ointments significantly and rapidly improved eczema symptoms than Eucerin	Yousefi *et al.*, 2013
2013	1	Seeds concoction (10 ml twice daily) for six months	An adult HIV patient	Sero-negative on 187th day on N.S. therapy and remained negative for 24 months without further therapy	Onifade *et al.*, 2013
2013	30	Seeds (450 mg thrice daily) for 3 months	Patients with hepatitis C virus (HCV) infection not eligible for interferon (IFN)-α therapy	Significant improvement in HCV viral load, RBC and platelet counts	Barakat *et al.*, 2013
2013	15 HCV Patients and 15 Controls	Ethanol seed extract (500 mg twice daily) for 1 month	Placebo-controlled, prospective study of HCV patients	Significant improvement in HCV viral load, alpha Fetoprotein, and liver function tests	Abdel-Moneim *et al.*, 2013
2012	94	Powdered seeds 1, 2 and 3 g/day for 12 weeks	Uncontrolled, nonrandomised study in type 2 diabetes patients	Significant decline in TC, TG, and LDL-c, and significant elevation in HDL-c/LDL-c	Kaatabi *et al.*, 2012
2012	88	Powdered seeds 2 g/day for 4 weeks	Randomised, placebo-controlled study in hypercholesterolaemic patients	Significant decline in TC, TG, and LDL-c, but no effect on FBG and HDL-C	Sabzghabaee *et al.*, 2012
2012	40	Oil, 500 mg/twice daily for one month	Placebo-controlled rheumatoid arthritis female subjects	Joints swelling and morning stiffness significantly improved	Gheita and Kenawy, 2012
2011	66	Oil or placebo for 30 days	Double-blinded study of patients with allergic rhinitis	N.S. oil reduced symptoms of allergic rhinitis	Nikakhlagh *et al.*, 2011
2010	39	Powdered seeds 750 mg/twice daily for 3 months	Controlled, nonrandomised study in men with central obesity	Significant reduction in body weight and waist circumference	Datau *et al.*, 2010
2010	94	Powdered seeds 1, 2 and 3 g/day for 3 months	Uncontrolled, randomised study in uncontrolled type 2 diabetes patients	Significant reduction in FBG, 2 hours postprandial blood glucose (2 hPG), HbA1c, and insulin resistance	Bamosa *et al.*, 2010
2010	88	Powdered seeds 1, 2 or 3 g/day + Omeprazole for 4 weeks or Triple Therapy	Comparative, open-label, randomised study in patients with dyspepsia and positive *H. pylori*	Significant improvement in dyspeptic symptoms by N.S. comparable to conventional therapy, but *H. pylori* control better by triple therapy	Salem *et al.*, 2010
2010	15	Seeds boiled extract 50 or 100 mg/kg	Comparative bronchodilatory effects of N.S. and theophylline in asthmatic patients	Potent anti-asthmatic effect of N.S. extract but weaker than theophylline	Boskabady *et al.*, 2010
2010	12 Allergic rhinitis and 8 healthy subjects	Powdered seeds (2 g/day) for 30 days, as adjunct to immunotherapy	Controlled, parallel study on allergic rhinitis patients with sensitivity to house dust mites, and healthy subjects	Significant increase in CD8 Counts and increased phagocytic and intracellular killing activities of PMNs	Isik *et al.*, 2010
2008	-	Seed extract 100 or 200 mg or Placebo twice daily for 8 weeks	Double-blinded RCT in mildly hypertensive patients	Dose-dependent significant reduction in SBP and DBP, and TC and LDL-C	Dehkordi and Kamkhah, 2008
2007	29	Seeds boiled extract 15 mL/kg of 0.1 g% daily for 3 months	Randomised, placebo-controlled study in asthmatic adult patients	Significant improvement in all asthma symptoms, (frequency, symptoms/week, chest wheezing) and PFT	Boskabady *et al.*, 2007
2007	20	Aqueous extract 40 mg/kg/q8h for 4 weeks as adjunct therapy	Double-blinded crossover clinical trial of children (13 months to 13 years old) with refractory epilepsy	Significantly decreased mean frequency of seizures	Akhondian *et al.*, 2007
2003	152	N.S. oil capsule 40 to 80 mg/kg/day	Uncontrolled, German adult patients with allergic diseases	Subjective symptoms improved	Kalus *et al.*, 2003

55 placebo-treated patient in a double-blinded RCT[180]. In a triple-blind, active RCT, topical application of seed oil also provided significant relief from mastalgia associated with menstrual cycle, comparable to topical diclofenac use[181]. Compared to fish oil, application of VO to vitiligo patches for six months significantly improved patches on upper extremities, trunk, head, and neck[182], and VO application twice a day significantly improved hand eczema, similar to the application of betamethasone[183].

GIT

Adult Saudi patients suffering from non-ulcer dyspeptic symptoms and positive for *H. pylori* infection, comparatively treated either with conventional triple antibiotic therapy or with seeds and omeprazole equally improved dyspepsia symptoms but did not show better anti *H. pylori* effect than the triple antibiotic therapy[184]. However, when Iranian patients with functional dyspepsia were treated with a daily dose of seed oil (5 ml) mixed with honey for eight weeks in a double-blind RCT, it significantly improved dyspeptic symptoms and significantly lowered *H. pylori* infection compared to placebo group[185].

Allergy and asthma

Boiled seed extract to 15 asthmatic Iranian patients as an adjunct to asthma therapy further improved asthma symptoms and decreased the usage of rescue inhaler, oral beta-agonists, oral corticosteroid, oral theophylline and the corticosteroid inhaler by the end of three months study period[186]. The onset time of brochodilatory effect was 30 min, and lasted for 150 min, similar to but less than the effect of theophylline[187]. A supplementary dose of seeds (2 g/day) to partly controlled 26 Saudi asthma patients for 12 weeks improved significantly both the peak expiratory flow (PEF) and forced expiratory volume in 1 second (FEV-1) in a single-blind RCT[188], and the seed oil (500 mg twice daily) supplementation to 40 Saudi asthmatics for four weeks, significantly improved mean Asthma Control Test score, significantly reduced blood eosinophils, and improved FEV-1 in a double-blind RCT[189]. Oral VO treatment in a dose of 40 to 80 mg/kg/day improved subjective feeling score over the course of the treatment, in a total of 152 German patients with allergic diseases, such as allergic rhinitis, bronchial asthma, and atopic eczema[190]. In a double-blind, prospective study, 66 Iranian patients with allergic rhinitis treated with VO or placebo for 30 days, had improvement in their nasal mucosal congestion, nasal itching, runny nose, sneezing attacks, turbinate hypertrophy, and mucosal pallor in the treatment group[191]. Similarly, 12 Turkish patients sensitive to house dust mites with allergic rhinitis receiving immunotherapy supplemented with seed (2 g/day orally) for 30 days showed a significant increase in CD8 counts, and significantly increased phagocytic and intracellular killing activities of polymorphonuclear leukocytes compared to 12 patients receiving only specific immunotherapy[192]. Also, in a prospective, crossover RCT of 42 Turkish geriatric patients with nasal dryness and related symptoms, two weeks' topical application of VO significantly improved nasal dryness, obstruction and crusting[193].

HIV and HCV

In a dramatic case of a 46-year-old Nigerian sero-positive HIV patient with multiple papular pruritic lesions, 10 ml concoction of seeds twice daily was allegedly administered by a herbalist that resulted in clearance of his pruritic lesions by 20th day and became seronegative after six months treatment, and remained so for twenty-four months follow up without further treatment[194]. The seed oil (450 mg tid) administered to 30 Egyptian patients with positive hepatitis C virus (HCV) infection with chronic liver disease or liver cirrhosis, not eligible for interferon (IFN)-α therapy, significantly improved the HCV viral load, improved clinical symptoms, oxidative stress, and reduced blood glucose in those with diabetes[195].

Miscellaneous

Oral administration of 500 mg powdered seeds twice daily to 20 healthy elderly Bangladeshi volunteers for nine weeks significantly enhanced memory, attention and cognition[196], and stabilised mood, decreased anxiety and modulated cognition in 20 healthy adolescent male volunteers compared to equal numbers of placebo-treated individuals[197]. In a double-blinded crossover clinical trial, addition of aqueous black seed extract (40 mg/kg thrice daily) for four weeks as an adjunct to anticonvulsant therapy in 20 children aged 13 months to 13 years old with refractory epilepsy, significantly decreased the frequency of seizures[198]. In a double-blind RCT, 34 Iranian infertile men with abnormal semen quality treated with 2.5 ml of seed oil twice daily for two months significantly improved sperm morphology, count, motility and semen volume, compared to 34 men treated with placebo[199].

CYP450 and drug–drug interactions

Black seeds aqueous extract significantly inhibited *in vitro* CYP2D6 and CYP3A4 in human liver microsomes and in healthy human volunteers[200], and seven days' administration of seeds to rats also significantly inhibited the mRNA and protein expression levels of CYP2C11[201]. Co-administration of black seeds significantly reduced phenytoin elimination in dogs[202], reduced AUC, Cmax and half-life of sildenafil in beagle dogs[203], significantly decreased the Cmax and AUC of cyclosporine[204], but did not affect the pharmcokinetics of carbamazepine in rabbits[205].

Human adverse effects/toxicity

Although not many cases of adverse effects or toxicity are reported, a few deserve to be mentioned here, which resolved after discontinuing the use of oil without any sequelae. Use of topical preparations containing black seed oil caused allergic contact dermatitis in German patients[206,207], and a 53-year-old French woman developed systematic erythematous plaques with vesicles and

bullous lesions after ingesting and topically using black seed oil for two weeks[208].

Animal toxicity

Black seeds supplemented the diet of Sprague-Dawley rats up to a dose of 1g/kg for four weeks did not cause any signs of liver dysfunction[209]. However, oral administration of AE to male Sprague-Dawley rats for 14 consecutive days was reported to significantly increase serum alanine aminotransferase concentrations with no histopathological changes in hepatocytes[210], but an oral dose of 6 g/kg/day for 14 consecutive days to mice caused some degenerative changes in hepatocytes[211], LD50 (i.p.) of the AE is reported to be 4.23 g/kg[154], Oral and i.p. LD50s of the fixed oil in mice were reported to be 28.8 ml/kg and 2.06 ml/kg, respectively; the oral dose of 2 ml/kg fixed oil for 12 weeks to rats did not cause any histopathological changes in heart, liver, kidneys and pancreas,[54] and fixed oil orally to mice in a dose of 10 ml/kg for 15 days was also nonlethal[41].

Discussion

From the experimental and clinical studies, it is apparent that black seeds exert a broad spectrum of pharmacological and clinical effects that could directly or indirectly affect a disease outcome or overall health status. Blood glucose and lipid profile appear to be the most significantly affected parameters. An improvement in lipid profile, blood glucose and/or blood pressure is potentially likely to help in controlling the most common diseases of our time. However, the observations of the clinical studies indicate that the optimal daily dose of black seeds is 2 g/day and use of a lesser dose does not offer the full benefits; the dose of VO or extract should correspond to the optimal dose of the seeds. Secondly, the duration of most clinical studies and the number of patients involved are limited, and standard control groups are sometimes lacking. Also, there is a paucity of follow-up clinical studies to reproduce the clinical outcomes. Studies in more diverse populations, of longer duration involving significantly higher number of patients would be helpful in determining the reproducibility and the lasting impact of beneficial effects. Sometimes, a lower dose used over a longer period of time may offer the same benefits as a higher dose used for shorter periods. Moreover, the chemical composition of seeds used in clinical studies were either not determined or not reported. As has been mentioned earlier, the seeds vary remarkably in their chemical contents, both qualitatively and quantitatively, which should affect the clinical outcomes, if one relies on the constituents–effects relationship. Until more detailed randomised, controlled clinical trials are conducted, the jury will be out for a definitive answer about the potential usefulness of black seeds, but the trend points to positive health benefits.

Inhibition of CYP2D6 and CYP3A4 by black seeds, the two enzymes most commonly involved in the metabolism of prescription drugs, is likely to increase the possibility of a drug–drug interaction, resulting in adverse effects and toxicity of other prescription drugs metabolised by these enzymes. The significant decrease in the Cmax and AUC of cyclosporine, if true in humans, could negatively affect the intended immunosuppression in patients where black seeds are used concurrently with cyclosporin.

Declaration of interest

The author has no conflicts of interest to report.

References

1. Elkhayat ES, Alorainy MS, El-Ashmawy IM, Fat'hi S. Potential antidepressant constituents of *Nigella sativa* seeds. Pharmacogn Mag 2016;12(Suppl 1):S27–31.
2. Dymock W, Warden CJH, Hooper D. 1890. Pharmacographia Indica. Reprinted Karachi, Pakistan: The Institute of Health and Tibbi Research, Hamdard National Foundation, 1972, vol. i, 28.
3. Zohary D, Hopf M. Domestication of Plants in the Old World. 3rd edn. Oxford University Press, 2001, p. 206.
4. Salih B, Sipahi T, Dönmez EO. Ancient nigella seeds from Boyali Höyük in north-central Turkey. J Ethnopharmacol 2009;124(3):416–20.
5. Beheshti F, Khazaei M, Hosseini M. Neuropharmacological effects of *Nigella sativa*. Avicenna J Phytomed 2016;6(1):104–16.
6. Khory RN, Katrak NN. Materia Medica of India and Their Therapeutics. Delhi-52, Neeraj Publishing House, 1984, pp. 16–17.
7. Ali BH, Blunden G. Pharmacological and toxicological properties of *Nigella sativa*. Phytother Res 2003;17(4):299–305.
8. Tahraoui A, El-Hilaly J, Israili ZH, Lyoussi B. Ethnopharmacological survey of plants used in the traditional treatment of hypertension and diabetes in south-eastern Morocco (Errachidia province). J Ethnopharmacol 2007;110(1):105–17.
9. Otoom SA, Al-Safi SA, Kerem ZK, Alkofahi A. The use of medicinal herbs by diabetic Jordanian patients. J Herb Pharmacother 2006;6(2):31–41.
10. Hosseinzadeh H, Parvardeh S. Anticonvulsant effects of thymoquinone, the major constituent of *Nigella sativa* seeds, in mice. Phytomedicine 2004;11(1):56–64.
11. Ahmad A, Husain A, Mujeeb M *et al.* Physico-chemical and phytochemical standardization with HPTLC fingerprinting of *Nigella sativa L*. seeds. Pak J Pharm Sci 2014;27(5):1175–82.
12. Asif M, Jabeen Q, Abdul Majid AM, Atif M. Diuretic activity of aqueous extract of *Nigella sativa* in albino rats. Acta Pol Pharm 2015;72(1):129–35.
13. Ali Z, Ferreira D, Carvalho P, Avery MA, Khan IA. Nigellidine-4-O-sulfite, the first sulfated indazole-type alkaloid from the seeds of *Nigella sativa*. J Nat Prod 2008;71(6):1111–12.
14. Yuan T, Nahar P, Sharma M *et al.* Indazole-type alkaloids from *Nigella sativa* seeds exhibit antihyperglycemic effects via AMPK activation in vitro. J Nat Prod 2014;77(10):2316–20.
15. Morikawa T, Xu F, Kashima Y, Matsuda H, Ninomiya K, Yoshikawa M. Novel dolabellane-type diterpene alkaloids with lipid metabolism promoting activities from the seeds of *Nigella sativa*. Org Lett 2004;6(6):869–72.
16. Morikawa T, Xu F, Ninomiya K, Matsuda H, Yoshikawa M. Nigellamines A3, A4, A5, and C, new dolabellane-type diterpene alkaloids, with lipid metabolism-promoting activities from the Egyptian medicinal food black cumin. Chem Pharm Bull (Tokyo) 2004;52(4):494–97.
17. Mehta BK, Mehta P, Gupta M. A new naturally acetylated triterpene saponin from *Nigella sativa*. Carbohydr Res 2009b;344(1):149–51.
18. Kumara SS, Huat BT. Extraction, isolation and characterisation of antitumor principle, alpha-hederin, from the seeds of *Nigella sativa*. Planta Med 2001;67(1):29–32.

19. Mehta BK, Pandit V, Gupta M. New principles from seeds of *Nigella sativa*. Nat Prod Res 2009;23(2):138–48.
20. Tiruppur Venkatachallam SK, Pattekhan H, Divakar S, Kadimi US. Chemical composition of *Nigella sativa L.* seed extracts obtained by supercritical carbon dioxide. J Food Sci Technol 2010;47(6):598–605.
21. Kiralan M. Volatile compounds of black cumin seeds (*Nigella sativa L.*) from microwave-heating and conventional roasting. J Food Sci 2012;77(4):C481–84.
22. Ait Mbarek L, Ait Mouse H, Elabbadi N *et al.* Anti-tumor properties of blackseed (*Nigella sativa L.*) extracts. Braz J Med Biol Res 2007;40(6):839–47.
23. Nickavar B, Mojab F, Javidnia K, Amoli MA. Chemical composition of the fixed and volatile oils of *Nigella sativa L.* from Iran. Z Naturforsch [C] 2003;58(9–10):629–31.
24. Mahmoudvand H, Sepahvand A, Jahanbakhsh S, Ezatpour B, Ayatollahi Mousavi SA. Evaluation of antifungal activities of the essential oil and various extracts of *Nigella sativa* and its main component, thymoquinone against pathogenic dermatophyte strains. J Mycol Med 2014;24(4):e155–61.
25. Ghourchian A, Hajimehdipoor H, Ara L *et al.* Essential oil and fixed oil content of *Nigella sativa* after a traditional medicine processing — A comparative study. Biol Forum 2016;8:120–25.
26. Majdalawieh AF, Fayyad MW. Recent advances on the anti-cancer properties of *Nigella sativa*, a widely used food additive. J Ayurveda Integr Med 2016;7(3):173–80.
27. Al-Johar D, Shinwari N, Arif J *et al.* Role of *Nigella sativa* and a number of its antioxidant constituents towards azoxymethane-induced genotoxic effects and colon cancer in rats. Phytother Res 2008;22(10):1311–23.
28. Khan N, Sultana S. Inhibition of two stage renal carcinogenesis, oxidative damage and hyperproliferative response by *Nigella sativa*. Eur J Cancer Prev 2005;14(2):159–68.
29. Mabrouk GM, Moselhy SS, Zohny SF *et al.* Inhibition of methylnitrosourea (MNU) induced oxidative stress and carcinogenesis by orally administered bee honey and *Nigella* grains in Sprague-Dawley rats. J Exp Clin Cancer Res 2002;21(3): 341–46.
30. Salomi MJ, Nair SC, Panikkar KR. Inhibitory effects of *Nigella sativa* and saffron (*Crocus sativus*) on chemical carcinogenesis in mice. Nutr Cancer 1991;16(1):67–72.
31. Salim EI, Fukushima S. Chemopreventive potential of volatile oil from black cumin (*Nigella sativa L.*) seeds against rat colon carcinogenesis. Nutr Cancer 2003;45(2):195–202.
32. Linjawi SA, Khalil WK, Hassanane MM, Ahmed ES. Evaluation of the protective effect of *Nigella sativa* extract and its primary active component thymoquinone against DMBA-induced breast cancer in female rats. Arch Med Sci 2015;11(1):220–29.
33. Farah IO, Begum RA. Effect of *Nigella sativa* (*N. sativa L.*) and oxidative stress on the survival pattern of MCF-7 breast cancer cells. Biomed Sci Instrum 2003;39:359–64.
34. Kaseb AO, Chinnakannu K, Chen D *et al.* Androgen receptor and E2F-1 targeted thymoquinone therapy for hormone-refractory prostate cancer. Cancer Res 2007;67(16):7782–88.
35. Badary OA, Al-Shabanah OA, Nagi MN, Al-Rikabi AC, Elmazar MM. Inhibition of benzo(a)pyrene-induced forestomach carcinogenesis in mice by thymoquinone. Eur J Cancer Prev 1999;8(5):435–40.
36. Badary OA, Gamal El-Din AM. Inhibitory effects of thymoquinone against 20-methyl-cholanthrene-induced fibrosarcoma tumorigenesis. Cancer Detect Prev 2001;25(4):362–68.
37. Yi T, Cho SG, Yi Z *et al.* Thymoquinone inhibits tumor angiogenesis and tumor growth through suppressing AKT and extracellular signal-regulated kinase signaling pathways. Mol Cancer Ther 2008;7(7):1789–96.
38. Rahmani AH, Alzohairy MA, Khan MA, Aly SM. Therapeutic implications of black seed and its constituent thymoquinone in the prevention of cancer through inactivation and activation of molecular pathways. Evid Based Complement Alternat Med 2014;724658.
39. Meddah B, Ducroc R, El Abbes Faouzi M *et al. Nigella sativa* inhibits intestinal glucose absorption and improves glucose tolerance in rats. J Ethnopharmacol 2009;121(3):419–24.
40. Meral I, Yener Z, Kahraman T, Mert N. Effect of *Nigella sativa* on glucose concentration, lipid peroxidation, anti-oxidant defence system and liver damage in experimentally-induced diabetic rabbits. J Vet Med A Physiol Pathol Clin Med 2001;48(10):593–99.
41. Zaoui A, Cherrah Y, Alaoui K, Mahassine N, Amarouch H, Hassar M. Effects of *Nigella sativa* fixed oil on blood homeostasis in rat. J Ethnopharmacol 2002;79(1):23–26.
42. Kanter M, Coskun O, Korkmaz A, Oter S. Effects of *Nigella sativa* on oxidative stress and beta-cell damage in streptozotocin-induced diabetic rats. Anat Rec A Discov Mol Cell Evol Biol 2004;279(1):685–91.
43. Sultan MT, Butt MS, Karim R *et al.* Effect of *Nigella sativa* fixed and essential oils on antioxidant status, hepatic enzymes, and immunity in streptozotocin induced diabetes mellitus. BMC Complement Altern Med 2014;14:193.
44. Fararh KM, Atoji Y, Shimizu Y, Takewaki T. Isulinotropic properties of *Nigella sativa* oil in Streptozotocin plus Nicotinamide diabetic hamster. Res Vet Sci 2002;73(3):279–82.
45. Fararh KM, Atoji Y, Shimizu Y, Shiina T, Nikami H, Takewaki T. Mechanisms of the hypoglycaemic and immuno-potentiating effects of *Nigella sativa L.* oil in streptozotocin-induced diabetic hamsters. Res Vet Sci 2004;77(2):123–29.
46. Kanter M, Meral I, Yener Z, Ozbek H, Demir H. Partial regeneration/proliferation of the beta-cells in the islets of Langerhans by *Nigella sativa L.* in streptozotocin-induced diabetic rats. Tohoku J Exp Med 2003;201(4):213–19.
47. Kanter M, Akpolat M, Aktas C. Protective effects of the volatile oil of *Nigella sativa* seeds on beta-cell damage in streptozotocin-induced diabetic rats: a light and electron microscopic study. J Mol Histol 2009;40(5–6):379–85.
48. El-Dakhakhny M, Mady N, Lembert N, Ammon HP. The hypoglycemic effect of *Nigella sativa* oil is mediated by extrapancreatic actions. Planta Med 2002;68(5):465–66.
49. Kocyigit Y, Atamer Y, Uysal E. The effect of dietary supplementation of *Nigella sativa L.* on serum lipid profile in rats. Saudi Med J 2009;30(7):893–96.
50. Muneera KE, Majeed A, Naveed AK. Comparative evaluation of *Nigella sativa* (Kalonji) and simvastatin for the treatment of hyperlipidemia and in the induction of hepatotoxicity. Pak J Pharm Sci 2015;28(2):493–98.
51. Al-Rasheed N, Al-Rasheed N, Bassiouni Y, Faddah L, Mohamad AM. Potential protective effects of *Nigella sativa* and *Allium sativum* against fructose-induced metabolic syndrome in rats. J Oleo Sci 2014;63(8):839–48.
52. Al-Naqeep G, Al-Zubairi AS, Ismail M, Amom ZH, Esa NM. Antiatherogenic potential of *Nigella sativa* seeds and oil in diet-induced hypercholesterolemia in rabbits. Evid Based Complement Alternat Med 2011;213628.
53. El-Saleh SC, Al-Sagair OA, Al-Khalaf MI. Thymoquinone and *Nigella sativa* oil protection against methionine-induced hyperhomocysteinemia in rats. Int J Cardiol 2004;93(1):19–23.
54. Zaoui A, Cherrah Y, Mahassini N, Alaoui K, Amarouch H, Hassar M. Acute and chronic toxicity of *Nigella sativa* fixed oil. Phytomedicine 2002;9(1):69–74.
55. Al-Ghamdi MS. The anti-inflammatory, analgesic and antipyretic activity of *Nigella sativa*. J Ethnopharmacol 2001;76(1):45–48.
56. Bashir MU, Qureshi HJ, Saleem T. Comparison of anti-inflammatory activity of *Nigella sativa* and diclofenac sodium in albino rats. J Ayub Med Coll Abbottabad 2015;27(3):523–26.
57. Bashir MU, Qureshi HJ. Analgesic effect of *Nigella sativa* seeds extract on experimentally induced pain in albino mice. J Coll Physicians Surg Pak 2010;20(7):464–67.

58. Abdel-Fattah AM, Matsumoto K, Watanabe H. Antinociceptive effects of *Nigella sativa* oil and its major component, thymoquinone, in mice. Eur J Pharmacol 2000;400(1):89–97.
59. Hajhashemi V, Ghannadi A, Jafarabadi H. Black cumin seed essential oil, as a potent analgesic and anti-inflammatory drug. Phytother Res 2004;18(3):195–99.
60. Houghton PJ, Zarka R, de las Heras B, Hoult JR. Fixed oil of *Nigella sativa* and derived thymoquinone inhibit eicosanoid generation in leukocytes and membrane lipid peroxidation. Planta Med 1995;61(1):33–36.
61. Abbas AT, Abdel-Aziz MM, Zalata KR, Abd Al-Galel Tel-D. Effect of dexamethasone and *Nigella sativa* on peripheral blood eosinophil count, IgG1 and IgG2a, cytokine profiles and lung inflammation in murine model of allergic asthma. Egypt J Immunol 2005;12(1):95–102.
62. El Mezayen R, El Gazzar M, Nicolls MR, Marecki JC, Dreskin SC, Nomiyama H. Effect of thymoquinone on cyclo-oxygenase expression and prostaglandin production in a mouse model of allergic airway inflammation. Immunol Lett 2006;106(1):72–81.
63. Tekeoglu I, Dogan A, Ediz L, Budancamanak M, Demirel A. Effects of thymoquinone (volatile oil of black cumin) on rheumatoid arthritis in rat models. Phytother Res 2007;21(9):895–97.
64. Mansour M, Tornhamre S. Inhibition of 5-lipoxygenase and leukotriene C4 synthase in human blood cells by thymoquinone. J Enzyme Inhib Med Chem 2004;19(5):431–36.
65. Morsi NM. Antimicrobial effect of crude extracts of *Nigella sativa* on multiple antibiotics-resistant bacteria. Acta Microbiol Pol 2000;49(1):63–74.
66. Khan MA, Ashfaq MK, Zuberi HS, Mahmood MS, Gilani AH. The *in vivo* antifungal activity of the aqueous extract from *Nigella sativa* seeds. Phytother Res 2003;17(2):183–86.
67. Bakathir HA, Abbas NA. Detection of the antibacterial effect of *Nigella sativa* ground seeds with water. Afr J Tradit Complement Altern Med 2011;8(2):159–64.
68. Hannan A, Saleem S, Chaudhary S, Barkaat M, Arshad MU. Antibacterial activity of *Nigella sativa* against clinical isolates of methicillin resistant *Staphylococcus aureus*. J Ayub Med Coll Abbottabad 2008;20(3):72–74.
69. Hassan W, Noreen H, Khalil S *et al.* Ethanolic extract of *Nigella sativa* protects Fe(II) induced lipid peroxidation in rat's brain, kidney and liver homogenates. Pak J Pharm Sci 2016;29(1): 231–37.
70. Toama MA, El-Alfy TS, El-Fatatry HM. Antimicrobial activity of the volatile oil of *Nigella sativa* Linneaus seeds. Antimicrob Agents Chemother 1974;6(2):225–26.
71. Islam SK, Ahsan M, Hassan CM, Malek MA. Antifungal activities of the oils of *Nigella sativa* seeds. Pak J Pharm Sci 1989;2:25–28.
72. Emeka LB, Emeka PM, Khan TM. Antimicrobial activity of *Nigella sativa L.* seed oil against multi-drug resistant *Staphylococcus aureus* isolated from diabetic wounds. Pak J Pharm Sci 2015;28(6):1985–90.
73. Oyero OG, Toyama M, Mitsuhiro N *et al.* Selective inhibition of hepatitis c virus replication by Alpha-zam, a *Nigella sativa* seed formulation. Afr J Tradit Complement Altern Med 2016;13(6):144–48.
74. El Shenawy NS, Soliman MF, Reyad SI. The effect of antioxidant properties of aqueous garlic extract and *Nigella sativa* as anti-schistosomiasis agents in mice. Rev Inst Med Trop Sao Paulo 2008;50(1):29–36.
75. Salem ML, Hossain MS. Protective effect of black seed oil from *Nigella sativa* against murine cytomegalovirus infection. Int J Immunopharmacol 2000;22(9):729–40.
76. Al-Hariri MT, Yar T, Bamosa AO, El-Bahai MN. Effects of two-months *Nigella sativa* supplementation on cardiac hemodynamics and adrenergic responsiveness. J Pak Med Assoc 2009;59(6):363–68.
77. El-Bahai MN, Al-Hariri MT, Yar T, Bamosa AO. Cardiac inotropic and hypertrophic effects of *Nigella sativa* supplementation in rats. Int J Cardiol 2009;131(3):e115–e117.
78. Yar T, El-Hariri M, El-Bahai MN, Bamosa AO. Effects of *Nigella sativa* supplementation for one month on cardiac reserve in rats. Indian J Physiol Pharmacol 2008;52(2):141–48.
79. Al-Asoom LI, Al-Shaikh BA, Bamosa AO, El-Bahai MN. Effect of *Nigella sativa* supplementation to exercise training in a novel model of physiological cardiac hypertrophy. Cardiovasc Toxicol 2014;14(3):243–50.
80. Al-Asoom LI. Coronary angiogenic effect of long-term administration of *Nigella sativa*. BMC Complement Altern Med 2017;17:308.
81. Boskabady MH, Shafei MN, Parsaee H. Effects of aqueous and macerated extracts from *Nigella sativa* on guinea pig isolated heart activity. Pharmazie 2005;60(12):943–48.
82. Shafei MN, Boskabady MH, Parsaee H. Effect of aqueous extract from *Nigella sativa L.* on guinea pig isolated heart. Indian J Exp Biol 2005;43(7):635–39.
83. Seif AA. *Nigella sativa* attenuates myocardial ischemic reperfusion injury in rats. J Physiol Biochem 2013;69(4):937–44.
84. Al-Jishi SA, Abuo Hozaifa B. Effect of *Nigella sativa* on blood hemostatic function in rats. J Ethnopharmacol 2003;85(1):7–14.
85. Hebi M, Zeggwagh N, Hajj L, Bouhali BE, Eddouks M. Cardiovascular effect of *Nigella sativa L.* aqueous extract in normal rats. Cardiovasc Hematol Disord Drug Targets 2016;16(1):47–55.
86. Jaarin K, Foong WD, Yeoh MH *et al.* Mechanisms of the antihypertensive effects of *Nigella sativa* oil in L-NAME-induced hypertensive rats. Clinics (Sao Paulo) 2015;70(11):751–57.
87. el Tahir KE, Ashour MM, al-Harbi MM. The cardiovascular actions of the volatile oil of the black seed (*Nigella sativa*) in rats: elucidation of the mechanism of action. Gen Pharmacol 1993;24(5):1123–31.
88. Cherkaoui-Tangi K, Israili ZH, Lyoussi B. Vasorelaxant effect of essential oil isolated from *Nigella sativa L.* seeds in rat aorta: Proposed mechanism. Pak J Pharm Sci 2016;29(1):1–8.
89. Al-Naggar TB, Gómez-Serranillos MP, Carretero ME, Villar AM. Neuropharmaco-logical activity of *Nigella sativa L.* extracts. J Ethnopharmacol 2003;88(1):63–68.
90. Bano F, Ahmed A, Parveen T, Haider S. Anxiolytic and hyperlocomotive effects of aqueous extract of *Nigella sativa L.* seeds in rats. Pak J Pharm Sci 2014;27(5 Spec no):1547–52.
91. Perveen T, Haider S, Zuberi NA, Saleem S, Sadaf S, Batool Z. Increased 5-HT levels following repeated administration of *Nigella sativa L.* (black seed) oil produce antidepressant effects in rats. Sci Pharm 2013;82(1):161–70.
92. Sahak MK, Mohamed AM, Hashim NH, Hasan Adli DS. *Nigella sativa* oil enhances the spatial working memory performance of rats on a radial arm maze. Evid Based Complement Alternat Med 2013;180598.
93. Abdel-Zaher AO, Abdel-Rahman MS, Elwasei FM. Blockade of nitric oxide over-production and oxidative stress by *Nigella sativa* oil attenuates morphine-induced tolerance and dependence in mice. Neurochem Res 2010;35(10):1557–65.
94. Ezz HS, Khadrawy YA, Noor NA. The neuroprotective effect of curcumin and *Nigella sativa* oil against oxidative stress in the pilocarpine model of epilepsy: a comparison with valproate. Neurochem Res 2011;36(11):2195–204.
95. Ilhan A, Gurel A, Armutcu F, Kamisli S, Iraz M. Antiepileptogenic and antioxidant effects of *Nigella sativa* oil against pentylenetetrazol-induced kindling in mice. Neuropharmacology 2005;49(4):456–64.
96. Hosseinzadeh H, Parvardeh S, Asl MN, Sadeghnia HR, Ziaee T. Effect of thymoquinone and *Nigella sativa* seeds oil on lipid peroxidation level during global cerebral ischemia-reperfusion injury in rat hippocampus. Phytomedicine 2007;14(9):621–27.

97. Kanter M, Coskun O, Kalayci M, Buyukbas S, Cagavi F. Neuroprotective effects of *Nigella sativa* on experimental spinal cord injury in rats. Hum Exp Toxicol 2006;25(3):127–33.
98. Kanter M. Effects of *Nigella sativa* and its major constituent, thymoquinone on sciatic nerves in experimental diabetic neuropathy. Neurochem Res 2008;33(11):87–96.
99. Noor NA, Aboul Ezz HS, Faraag AR, Khadrawy YA. Evaluation of the antiepileptic effect of curcumin and *Nigella sativa* oil in the pilocarpine model of epilepsy in comparison with valproate. Epilepsy Behav 2012;24(2):199–206.
100. Vafaee F, Hosseini M, Hassanzadeh Z *et al.* The effects of *Nigella sativa* hydro-alcoholic extract on memory and brain tissues oxidative damage after repeated seizures in rats. Iran J Pharm Res 2015;14(2):547–57.
101. Seghatoleslam M, Alipour F, Shafieian R *et al.* The effects of *Nigella sativa* on neural damage after pentylenetetrazole induced seizures in rats. J Tradit Complement Med 2015;6(3):262–68.
102. Hosseini M, Mohammadpour T, Karami R, Rajaei Z, Sadeghnia HR, Soukhtanloo M. Effects of the hydro-alcoholic extract of *Nigella sativa* on scopolamine-induced spatial memory impairment in rats and its possible mechanism. Chin J Integr Med 2015;21(6):438–44.
103. Beheshti F, Hosseini M, Shafei MN *et al.* The effects of *Nigella sativa* extract on hypothyroidism-associated learning and memory impairment during neonatal and juvenile growth in rats. Nutr Neurosci 2016;1–11.
104. Eltony SA, Elgayar SA. Histological study on effect of *Nigella sativa* on aged olfactory system of female albino rat. Rom J Morphol Embryol 2014;55(2):325–34.
105. Malik T, Hasan S, Pervez S, Fatima T, Haleem DJ. *Nigella sativa* oil reduces extrapyramidal symptoms (EPS)-like behavior in haloperidol-treated rats. Neurochem Res 2016;41(12): 3386–98.
106. Kanter M. Protective effects of *Nigella sativa* on the neuronal injury in frontal cortex and brain stem after chronic toluene exposure. Neurochem Res 2008;33(11):2241–49.
107. Kanter M. *Nigella sativa* and derived thymoquinone prevents hippocampal neuro-degeneration after chronic toluene exposure in rats. Neurochem Res 2008;33(3):579–88.
108. Bukhari MH, Khalil J, Qamar S *et al.* Comparative gastroprotective effects of natural honey, *Nigella sativa* and cimetidine against acetylsalicylic acid induced gastric ulcer in albino rats. J Coll Physicians Surg Pak 2011;21(3):151–56.
109. El-Dakhakhny M, Barakat M, El-Halim MA, Aly SM. Effects of *Nigella sativa* oil on gastric secretion and ethanol induced ulcer in rats. J Ethnopharmacol 2000;72(1–2):299–304.
110. Kanter M, Demir H, Karakaya C, Ozbek H. Gastroprotective activity of *Nigella sativa L.* oil and its constituent, thymoquinone against acute alcohol-induced gastric mucosal injury in rats. World J Gastroenterol 2005;11(42):6662–66.
111. El-Abhar HS, Abdallah DM, Saleh S. Gastroprotective activity of *Nigella sativa* oil and its constituent, thymoquinone, against gastric mucosal injury induced by ischaemia/ reperfusion in rats. J Ethnopharmacol 2003;84(2–3):251–58.
112. Isik F, Tunali Akbay T, Yarat A *et al.* Protective effects of black cumin (*Nigella sativa*) oil on TNBS-induced experimental colitis in rats. Dig Dis Sci 2011;56(3):721–30.
113. Farrag AR, Mahdy KA, Abdel Rahman GH, Osfor MM. Protective effect of *Nigella sativa* seeds against lead-induced hepatorenal damage in male rats. Pak J Biol Sci 2007;10(17):2809–16.
114. Hassan AS, Ahmed JH, Al-Haroon SS. A study of the effect of *Nigella sativa* (Black seeds) in isoniazid (INH)-induced hepatotoxicity in rabbits. Indian J Pharmacol 2012;44(6):678–82.
115. Jaswal A, Sharma M, Raghuvanshi S *et al.* Therapeutic efficacy of *Nigella sativa Linn.* against antituberculosis drug-induced hepatic injury in Wistar Rats. J Environ Pathol Toxicol Oncol 2016;35(1):59–71.
116. Develi S, Evran B, Betül Kalaz E, Koçak-Toker N, Erata GÖ. Protective effect of *Nigella sativa* oil against binge ethanol-induced oxidative stress and liver injury in rats. Chin J Nat Med 2014;12(7):495–99.
117. Pourbakhsh H, Taghiabadi E, Abnous K *et al.* Effect of *Nigella sativa* fixed oil on ethanol toxicity in rats. Iran J Basic Med Sci 2014;17(12):1020–31.
118. Al-Ghamdi MS. Protective effect of *Nigella sativa* seeds against carbon tetrachloride-induced liver damage. Am J Chin Med 2003;31(5):721–28.
119. Jaswal A, Shukla S. Therapeutic efficacy of *Nigella sativa Linn.* seed extract against CCl4 induced hepatic injury in Wistar rats. Indian J Exp Biol 2015;53(1):44–50.
120. Türkdoğan MK, Ozbek H, Yener Z, Tuncer I, Uygan I, Ceylan E. The role of *Urtica dioica* and *Nigella sativa* in the prevention of carbon tetrachloride-induced hepatotoxicity in rats. Phytother Res 2003;17(8):942–46.
121. Al-Seeni MN, El Rabey HA, Zamzami MA, Alnefayee AM. The hepatoprotective activity of olive oil and *Nigella sativa* oil against CCl4 induced hepatotoxicity in male rats. BMC Complement Altern Med 2016;16(1):438.
122. Meral I, Kanter M. Effects of *Nigella sativa L.* and *Urtica dioica L.* on selected mineral status and hematological values in CCl4-treated rats. Biol Trace Elem Res 2003;96(1–3):263–70.
123. El-Dakhakhny M, Mady NI, Halim MA. *Nigella sativa L.* oil protects against induced hepatotoxicity and improves serum lipid profile in rats. Arzneimittelforschung 2000;50(9):832–36.
124. Ibrahim ZS, Ishizuka M, Soliman M *et al.* Protection by *Nigella sativa* against carbon tetrachloride-induced downregulation of hepatic cytochrome P450 isozymes in rats. Jpn J Vet Res 2008;56(3):119–28.
125. Al-Suhaimi EA. Hepatoprotective and immunological functions of *Nigella sativa* seed oil against hypervitaminosis A in adult male rats. Int J Vitam Nutr Res 2012;82(4):288–97.
126. Yildiz F, Coban S, Terzi A *et al. Nigella sativa* relieves the deleterious effects of ischemia reperfusion injury on liver. World J Gastroenterol 2008;14(33):5204–09.
127. Kanter M, Meral I, Dede S *et al.* Effects of *Nigella sativa L.* and *Urtica dioica L.* on lipid peroxidation, antioxidant enzyme systems and some liver enzymes in CCl4-treated rats. J Vet Med A Physiol Pathol Clin Med 2003;50(5):264–8. Erratum in: J Vet Med A Physiol Pathol Clin Med 2003;50(7):383.
128. Kanter M, Coskun O, Budancamanak M. Hepatoprotective effects of *Nigella sativa L.* and *Urtica dioica L.* on lipid peroxidation, antioxidant enzyme systems and liver enzymes in carbon tetrachloride-treated rats. World J Gastroenterol 2005;11(42):6684–88.
129. Türkdoğan MK, Ağaoğlu Z, Yener Z, Sekeroğlu R, Akkan HA, Avci ME. The role of antioxidant vitamins (C and E), selenium and *Nigella sativa* in the prevention of liver fibrosis and cirrhosis in rabbits: new hopes. Dtsch Tierarztl Wochenschr 2001;108(2):71–73.
130. Mansour MA, Ginawi OT, El-Hadiyah T, El-Khatib AS, Al-Shabanah OA, Al-Sawaf HA. Effects of volatile oil constituents of *Nigella sativa* on carbon tetrachloride-induced hepatotoxicity in mice: evidence for antioxidant effects of thymoquinone. Res Commun Mol Pathol Pharmacol 2001;110(3–4):239–51.
131. Dollah MA, Parhizkar S, Izwan M. Effect of *Nigella sativa* on the kidney function in rats. Avicenna J Phytomed 2013;3(2):152–58.
132. Caskurlu T, Kanter M, Erboga M, Erboga ZF, Ozgul M, Atis G. Protective effect of *Nigella sativa* on renal reperfusion injury in rat. Iran J Kidney Dis 2016;10(3):135–43.
133. Mousavi G. Study on the effect of black cumin (*Nigella sativa Linn.*) on experimental renal ischemia-reperfusion injury in rats. Acta Cir Bras 2015;30(8):542–50.
134. Mousavi G, Mohajeri D. Effect of ground black seeds (*Nigella sativa L.*) on renal tubular cell apoptosis induced by ischemia/reperfusion injury in the rats. Iran J Basic Med Sci 2014;17(12):1032–35.

135. Yildiz F, Coban S, Terzi A *et al.* Protective effects of *Nigella sativa* against ischemia-reperfusion injury of kidneys. Ren Fail 2010;32(1):126–131.
136. Ali BH. The effect of *Nigella sativa* oil on gentamicin nephrotoxicity in rats. Am J Chin Med 2004;32(1):49–55.
137. Saleem U, Ahmad B, Rehman K, Mahmood S, Alam M, Erum A. Nephroprotective effect of vitamin C and *Nigella sativa* oil on gentamicin-associated nephrotoxicity in rabbits. Pak J Pharm Sci 2012;25(4):727–30.
138. Yaman I, Balikci E. Protective effects of *Nigella sativa* against gentamicin-induced nephrotoxicity in rats. Exp Toxicol Pathol 2010;62(2):183–90.
139. Al-Gayyar MM, Hassan HM, Alyoussef A, Abbas A, Darweish MM, El-Hawwary AA. *Nigella sativa* oil attenuates chronic nephrotoxicity induced by oral sodium nitrite: Effects on tissue fibrosis and apoptosis. Redox Rep 2016;21(2):50–60.
140. Yusuksawad M, Chaiyabutr N. Restoration of renal hemodynamics and functions during black cumin (*Nigella sativa*) administration in streptozotocin-induced diabetic rats. J Exp Pharmacol 2011;4:1–7.
141. Hamed MA, El-Rigal NS, Ali SA. Effects of black seed oil on resolution of hepato-renal toxicity induced by bromobenzene in rats. Eur Rev Med Pharmacol Sci 2013;17(5):569–81.
142. Ahmed OG, El-Mottaleb NA. Renal function and arterial blood pressure alterations after exposure to acetaminophen with a potential role of *Nigella sativa* oil in adult male rats. J Physiol Biochem 2013;69(1):1–13.
143. Bayrak O, Bavbek N, Karatas OF *et al. Nigella sativa* protects against ischaemia/reperfusion injury in rat kidneys. Nephrol Dial Transplant 2008;23(7):2206–12.
144. Havakhah S, Sadeghnia HR, Hajzadeh MA *et al.* Effect of *Nigella sativa* on ischemia-reperfusion induced rat kidney damage. Iran J Basic Med Sci 2014;17(12):986–92.
145. Uz E, Bayrak O, Uz E *et al. Nigella sativa* oil for prevention of chronic cyclosporine nephrotoxicity: an experimental model. Am J Nephrol 2008;28(3):517–22.
146. Sayed-Ahmed MM, Nagi MN. Thymoquinone supplementation prevents the development of gentamicin-induced acute renal toxicity in rats. Clin Exp Pharmacol Physiol 2007;34(5–6):399–405.
147. Badary OA, Abdel-Naim AB, Abdel-Wahab MH, Hamada FM. The influence of thymoquinone on doxorubicin-induced hyperlipidemic nephropathy in rats. Toxicology 2000;143(3): 219–26.
148. Erboga M, Kanter M, Aktas C *et al*. Thymoquinone ameliorates cadmium-induced nephrotoxicity, apoptosis, and oxidative stress in rats is based on its anti-apoptotic and anti-oxidant properties. Biol Trace Elem Res 2016;170(1):165–72.
149. Hadjzadeh MA, Mohammadian N, Rahmani Z, Rassouli FB. Effect of thymoquinone on ethylene glycol-induced kidney calculi in rats. Urol J 2008;5(3):149–55.
150. Mohajeri D, Kaffashi Elahi R. Effects of *Nigella sativa* on heat-induced testis damage in mouse. Bratisl Lek Listy 2015;116(4):264–69.
151. Cho Ping N, Hashim NH, Hasan Adli DS. Effects of *Nigella sativa* (*Habbatus sauda*) oil and nicotine chronic treatments on sperm parameters and testis histological features of rats. Evid Based Complement Alternat Med 2014;218293.
152. Mosbah R, Yousef MI, Maranghi F, Mantovani A. Protective role of *Nigella sativa* oil against reproductive toxicity, hormonal alterations, and oxidative damage induced by chlorpyrifos in male rats. Toxicol Ind Health 2016;32(7):1266–77.
153. Parhizkar S, Latiff LA, Parsa A. Effect of *Nigella sativa* on reproductive system in experimental menopause rat model. Avicenna J Phytomed 2016;6(1):95–103.
154. Hosseinzadeh H, Tafaghodi M, Mosavi MJ, Taghiabadi E. Effect of aqueous and ethanolic extracts of *Nigella sativa* seeds on milk production in rats. J Acupunct Meridian Stud 2013;6(1):18–23.
155. Aqel M, Shaheen R. Effects of the volatile oil of *Nigella sativa* seeds on the uterine smooth muscle of rat and guinea pig. J Ethnopharmacol 1996;52(1):23–26.
156. Kaatabi H, Bamosa AO, Lebda FM, Al Elq AH, Al-Sultan AI. Favorable impact of *Nigella sativa* seeds on lipid profile in type 2 diabetic patients. J Family Community Med 2012;19(3):155–61.
157. Bamosa AO, Kaatabi H, Lebdaa FM, Elq AM, Al-Sultanb A. Effect of *Nigella sativa* seeds on the glycemic control of patients with type 2 diabetes mellitus. Indian J Physiol Pharmacol 2010;54(4):344–54.
158. Kaatabi H, Bamosa AO, Badar A *et al*. *Nigella sativa* improves glycemic control and ameliorates oxidative stress in patients with type 2 diabetes mellitus: placebo controlled participant blinded clinical trial. PLoS One 2015;10(2):e0113486.
159. Badar A, Kaatabi H, Bamosa A *et al*. Effect of *Nigella sativa* supplementation over a one-year period on lipid levels, blood pressure and heart rate in type-2 diabetic patients receiving oral hypoglycemic agents: nonrandomized clinical trial. Ann Saudi Med 2017;37(1):56–63.
160. Bamosa A, Kaatabi H, Badar A *et al*. *Nigella sativa*: A potential natural protective agent against cardiac dysfunction in patients with type 2 diabetes mellitus. J Family Community Med 2015;22(2):88–95.
161. Sabzghabaee AM, Dianatkhah M, Sarrafzadegan N, Asgary S, Ghannadi A. Clinical evaluation of *Nigella sativa* seeds for the treatment of hyperlipidemia: a randomized, placebo controlled clinical trial. Med Arch 2012;66(3):198–200.
162. Mostafa RM, Moustafa YM, Mirghani Z, AlKusayer GM, Moustafa KM. Antioxidant effect of garlic (*Allium sativum*) and black seeds (*Nigella sativ*a) in healthy post-menopausal women. SAGE Open Med 2013;1:2050312113517501.
163. Ibrahim RM, Hamdan NS, Mahmud R *et al.* A randomised controlled trial on hypolipidemic effects of *Nigella sativa* seeds powder in menopausal women. J Transl Med 2014;12:82.
164. Ibrahim RM, Hamdan NS, Ismail M *et al.* Protective effects of *Nigella sativa* on metabolic syndrome in menopausal women. Adv Pharm Bull 2014;4(1):29–33.
165. Mohtashami A, Mahaki B, Azadbakht L, Entezari MH. Effects of bread with *Nigella sativa* on lipid profiles, apolipoproteins and inflammatory factor in metabolic syndrome patients. Clin Nutr Res 2016;5(2):89–95.
166. Farzaneh E, Nia FR, Mehrtash M, Mirmoeini FS, Jalilvand M. The Effects of 8-week *Nigella sativa* supplementation and aerobic training on lipid profile and VO2 max in sedentary overweight females. Int J Prev Med 2014;5(2):210–16.
167. Latiff LA, Parhizkar S, Dollah MA, Hassan ST. Alternative supplement for enhancement of reproductive health and metabolic profile among perimenopausal women: a novel role of *Nigella sativa*. Iran J Basic Med Sci 2014;17(12):980–85.
168. Mahdavi R, Namazi N, Alizadeh M, Farajnia S. Effects of *Nigella sativa* oil with a low-calorie diet on cardiometabolic risk factors in obese women: a randomized controlled clinical trial. Food Funct 2015;6(6):2041–48.
169. Namazi N, Mahdavi R, Alizadeh M, Farajnia S. Oxidative stress responses to *Nigella sativa* oil concurrent with a low-calorie diet in obese women: A randomized, double-blind controlled clinical trial. Phytother Res 2015;29(11):1722–28.
170. Sahebkar A, Beccuti G, Simental-Mendía LE, Nobili V, Bo S. *Nigella sativa* (black seed) effects on plasma lipid concentrations in humans: A systematic review and meta-analysis of randomized placebo-controlled trials. Pharmacol Res 2016;106:37–50.
171. Daryabeygi-Khotbehsara R, Golzarand M, Ghaffari MP, Djafarian K. *Nigella sativa* improves glucose homeostasis and serum lipids in type 2 diabetes: A systematic review and meta-analysis. Complement Ther Med 2017;35:6–13.
172. Fallah Huseini H, Amini M, Mohtashami R *et al*. Blood pressure lowering effect of *Nigella sativa L.* seed oil in healthy volunteers: a randomized, double-blind, placebo-controlled clinical trial. Phytother Res 2013;27(12):1849–53.

173. Dehkordi FR, Kamkhah AF. Antihypertensive effect of *Nigella sativa seed* extract in patients with mild hypertension. Fundam Clin Pharmacol 2008;22(4):447–52.
174. Sahebkar A, Soranna D, Liu X *et al*. A systematic review and meta-analysis of randomized controlled trials investigating the effects of supplementation with *Nigella sativa* (black seed) on blood pressure. J Hypertens 2016;34(11):2127–35.
175. Datau EA, Wardhana, Surachmanto EE, Pandelaki K, Langi JA, Fias. Efficacy of *Nigella sativa* on serum free testosterone and metabolic disturbances in central obese male. Acta Med Indones 2010;42(3):130–34.
176. Farhangi MA, Dehghan P, Tajmiri S, Abbasi MM. The effects of *Nigella sativa* on thyroid function, serum Vascular Endothelial Growth Factor (VEGF) — 1, Nesfatin-1 and anthropometric features in patients with Hashimoto's thyroiditis: a randomized controlled trial. BMC Complement Altern Med 2016;16(1):471.
177. Mousa HFM, Abd-El-Fatah NK, Darwish OA, Shehata SF, Fadel SH. Effect of *Nigella sativa* seed administration on prevention of febrile neutropenia during chemotherapy among children with brain tumors. Childs Nerv Syst 2017;33(5):793–800.
178. Gheita TA, Kenawy SA. Effectiveness of *Nigella sativa* oil in the management of rheumatoid arthritis patients: a placebo-controlled study. Phytother Res 2012;26(8):1246–48.
179. Hadi V, Kheirouri S, Alizadeh M, Khabbazi A, Hosseini H. Effects of *Nigella sativa* oil extract on inflammatory cytokine response and oxidative stress status in patients with rheumatoid arthritis: a randomized, double-blind, placebo-controlled clinical trial. Avicenna J Phytomed 2016;6(1):34–43.
180. Salimzadeh A, Ghourchian A, Choopani R, Hajimehdipoor H, Kamalinejad M, Abolhasani M. Effect of an orally formulated processed black cumin, from Iranian traditional medicine pharmacopoeia, in relieving symptoms of knee osteoarthritis: A prospective, randomized, double-blind and placebo-controlled clinical trial. Int J Rheum Dis 2017;20(6):691–701.
181. Huseini HF, Kianbakht S, Mirshamsi MH, Zarch AB. Effectiveness of topical *Nigella sativa* seed oil in the treatment of cyclic mastalgia: a randomized, triple-blind, active, and placebo-controlled clinical trial. Planta Med 2016;82(4):285–88.
182. Ghorbanibirgani A, Khalili A, Rokhafrooz D. Comparing *Nigella sativa* oil and fish oil in treatment of vitiligo. Iran Red Crescent Med J 2014;16(6):e4515.
183. Yousefi M, Barikbin B, Kamalinejad M *et al.* Comparison of therapeutic effect of topical *Nigella* with Betamethasone and Eucerin in hand eczema. J Eur Acad Dermatol Venereol 2013;27(12):1498–1504.
184. Salem EM, Yar T, Bamosa AO *et al.* Comparative study of *Nigella sativa* and triple therapy in eradication of *Helicobacter Pylori* in patients with non-ulcer dyspepsia. Saudi J Gastroenterol 2010;16(3):207–14.
185. Mohtashami R, Huseini HF, Heydari M *et al.* Efficacy and safety of honey based formulation of *Nigella sativa* seed oil in functional dyspepsia: A double blind randomized controlled clinical trial. J Ethnopharmacol 2015;175:147–52.
186. Boskabady MH, Javan H, Sajady M, Rakhshandeh H. The possible prophylactic effect of *Nigella sativa* seed extract in asthmatic patients. Fundam Clin Pharmacol 2007;21(5):559–66.
187. Boskabady MH, Mohsenpoor N, Takaloo L. Antiasthmatic effect of *Nigella sativa* in airways of asthmatic patients. Phytomedicine 2010;17(10):707–13.
188. Salem AM, Bamosa AO, Qutub HO *et al.* Effect of *Nigella sativa* supplementation on lung function and inflammatory mediatorsin partly controlled asthma: a randomized controlled trial. Ann Saudi Med 2017;37(1):64–71.
189. Koshak A, Wei L, Koshak E *et al. Nigella sativa* supplementation improves asthma control and biomarkers: A randomized, double-blind, placebo-controlled trial. Phytother Res 2017;31(3):403–409.
190. Kalus U, Pruss A, Bystron J, Smekalova A, Lichius JJ, Kiesewetter H. Effect of *Nigella sativa* (black seed) on subjective feeling in patients with allergic diseases. Phytother Res 2003;17(10):1209–14.
191. Nikakhlagh S, Rahim F, Aryani FH, Syahpoush A, Brougerdnya MG, Saki N. Herbal treatment of allergic rhinitis: the use of *Nigella sativa*. Am J Otolaryngol 2011;32(2):402–407.
192. Işik H, Cevikbaş A, Gürer US *et al.* Potential adjuvant effects of *Nigella sativa* seeds to improve specific immunotherapy in allergic rhinitis patients. Med Princ Pract 2010;19(3):206–11.
193. Oysu C, Tosun A, Yilmaz HB, Sahin-Yilmaz A, Korkmaz D, Karaaslan A. Topical *Nigella sativa* for nasal symptoms in elderly. Auris Nasus Larynx 2014;41(3):269–72.
194. Onifade AA, Jewell AP, Adedeji WA. *Nigella sativa* concoction induced sustained seroreversion in HIV patient. Afr J Tradit Complement Altern Med 2013;10(5):332–35.
195. Barakat EM, El Wakeel LM, Hagag RS. Effects of *Nigella sativa* on outcome of hepatitis C in Egypt. World J Gastroenterol 2013;19(16):2529–36.
196. Bin Sayeed MS, Asaduzzaman M, Morshed H, Hossain MM, Kadir MF, Rahman MR. The effect of *Nigella sativa Linn.* seed on memory, attention and cognition in healthy human volunteers. J Ethnopharmacol 2013;148(3):780–86.
197. Bin Sayeed MS, Shams T, Fahim Hossain S *et al. Nigella sativa L.* seeds modulate mood, anxiety and cognition in healthy adolescent males. J Ethnopharmacol 2014;152(1):156–62.
198. Akhondian J, Parsa A, Rakhshande H. The effect of *Nigella sativa L.* (black cumin seed) on intractable pediatric seizures. Med Sci Monit 2007;13(12):CR555–59.
199. Kolahdooz M, Nasri S, Modarres SZ, Kianbakht S, Huseini HF. Effects of *Nigella sativa L.* seed oil on abnormal semen quality in infertile men: a randomized, double-blind, placebo-controlled clinical trial. Phytomedicine 2014;21(6):901–05.
200. Al-Jenoobi FI, Al-Thukair AA, Abbas FA *et al.* Effect of black seed on dextromethorphan O- and N-demethylation in human liver microsomes and healthy human subjects. Drug Metab Lett 2010;4:51–55.
201. Korashy HM, Al-Jenoobi FI, Raish M *et al.* Impact of herbal medicines like *Nigella sativa*, *Trigonella foenum-graecum*, and *Ferula asafoetida*, on Cytochrome P450 2C11 gene expression in rat liver. Drug Res (Stuttg) 2015;65(7):366–72.
202. Alkharfy KM, Al-Jenoobi FI, Al-Mohizea AM, Al-Suwayeh SA, Khan RM, Ahmad A. Effects of *Lepidium sativum*, *Nigella sativa* and *Trigonella foenum-graceum* on phenytoin pharmacokinetics in beagle dogs. Phytother Res 2013;27(12):1800–04.
203. Al-Mohizea AM, Ahad A, El-Maghraby GM, Al-Jenoobi FI, Alkharfy KM, Al-Suwayeh SA. Effects of *Nigella sativa*, *Lepidium sativum* and *Trigonella foenum-graecum* on sildenafil disposition in beagle dogs. Eur J Drug Metab Pharmacokinet 2015;40(2):219–24.
204. Al-Jenoobi FI, Al-Suwayeh SA, Muzaffar I *et al.* Effects of *Nigella sativa* and *Lepidium sativum* on cyclosporine pharmacokinetics. Biomed Res Int 2013;953520.
205. Alkharfy KM, Al-Jenoobi FI, Alam MA *et al. Lepidium sativum* but not *Nigella sativa* affects carbamazepine disposition in an animal model. Drug Metab Lett 2013;7(1):47–51.
206. Steinmann A, Schätzle M, Agathos M, Breit R. Allergic contact dermatitis from black cumin (*Nigella sativa*) oil after topical use. Contact Dermatitis 1997;36(5):268–69.
207. Zedlitz S, Kaufmann R, Boehncke WH. Allergic contact dermatitis from black cumin (*Nigella sativa*) oil-containing ointment. Contact Dermatitis 2002;46(3):188.
208. Gelot P, Bara-Passot C, Gimenez-Arnau E, Beneton N, Maillard H, Celerier P. [Bullous drug eruption with *Nigella sativa* oil]. [Article in French] Ann Dermatol Venereol 2012;139(4):287–91.
209. Dollah MA, Parhizkar S, Latiff LA, Bin Hassan MH. Toxicity effect of *Nigella sativa* on the liver function of rats. Adv Pharm Bull 2013;3(1):97–102.
210. Tennekoon KH, Jeevathayaparan S, Kurukulasooriya AP, Karunanayake EH. Possible hepatotoxicity of *Nigella sativa* seeds and *Dregea volubilis* leaves. J Ethnopharmacol 1991;31(3):283–89.
211. Vahdati-Mashhadian N, Rakhshandeh H, Omidi A. An investigation on LD50 and subacute hepatic toxicity of *Nigella sativa* seed extracts in mice. Pharmazie 2005;60(7):544–47.

Australian Journal of Herbal and Naturopathic Medicine **2018** 30(4)

Comparative effects of mistletoe extracts in combination with 5-Fluorouracil on viability of IEC-6 and Caco-2 intestinal epithelial cells

Zahra Lotfollahi Hons[a], Suzanne Mashtoub PhD[a,b,c], Gordon S Howarth PhD[a,b,d], Lauren C Chartier Hons[a,b], Ljubov Simson PhD[e], Ker Y Cheah PhD[b,f] and Susan EP Bastian PhD[f]

[a] Discipline of Physiology, Adelaide Medical School, The University of Adelaide, Adelaide, SA, Australia
[b] Gastroenterology Department, Women's and Children's Hospital, North Adelaide, SA, Australia
[c] School of Medicine, The University of Western Australia; Fiona Stanley Hospital, Murdoch, WA, Australia
[d] School of Animal and Veterinary Sciences, The University of Adelaide, Roseworthy Campus, SA, Australia
[e] Faculty of Applied Sciences, University of Canberra, Canberra, ACT, Australia
[f] School of Agriculture, Food and Wine, The University of Adelaide, Adelaide, SA, Australia

Contact information

Professor Gordon S Howarth, School of Animal and Veterinary Sciences, The University of Adelaide
Roseworthy Campus, SA 5371, Australia,
Email gordon.howarth@adelaide.edu.au

Abstract

Background: Colorectal cancer is the second most common cancer in Western countries. Mistletoe extract has been used for decades as a complementary cancer therapy in Europe to improve patient general condition, vitality, pain and appetite.

Aim: To examine the effect of mistletoe extracts *(Quercus, Fraxini and Mali)* on the viability of colon cancer and normal intestinal cells.

Materials/methods: Cell viability of IEC-6 (non-transformed) and Caco-2 (colon cancer) cells was determined by MTT assay for mistletoe extracts *(Quercus, Fraxini and Mali;* aqueous; 1–100 µg/mL) alone and in combination with 5-Flurorouracil (5-FU). $p < 0.05$ was considered significant.

Results: IC_{50} values on Caco-2 cells for *Fraxini, Mali* and *Quercus* were 42.7, 65.5 and 84.4 µg/mL, respectively. IC_{50} values for *Fraxini, Mali* and *Quercus* on IEC-6 cells were 71.74, 65.52 and 84.39 µg/mL, respectively. *Fraxini* (50 µg/mL), when combined with 5-FU (5 µM), significantly increased the potency of 5-FU on IEC-6 cells compared to *Fraxini* (50 µg/mL) alone ($p<0.05$). *Quercus* was less effective than *Fraxini* at reducing Caco-2 cell viability. However, effects on IEC-6 cells were also less pronounced. *Mali* was the least effective extract on both cell lines.

Conclusion: *Fraxini* was the most potent mistletoe extract at decreasing colon cancer cell viability.

Keywords: Cell culture, chemotherapy, gastrointestinal diseases, mistletoe extracts.

Introduction

Colorectal cancer is the second most prevalent cause of cancer-related death in Western countries[1,2]. Chemotherapy, particularly 5-Fluorouracil (5-FU), is used for the most advanced stages (III and IV) of colon cancer[1,2]. Unfortunately, the indiscriminate mode of action of 5-FU not only targets cancer cells but also kills the rapidly proliferating cells in human body such as cells lining the gastrointestinal tract (enterocytes)[3-5]. Enterocyte damage results in severe side effects, including mucositis[5,6]. Mucositis is characterised by inflammation and ulceration of mucosal tissue in the gastrointestinal tract[5,6]. The severity of 5-FU-related side-effects can result in the cessation of chemotherapy[7]. Therefore, new therapeutic agents with specific toxicity to colon cancer cells are desirable, without exacerbating the undesirable impact of 5-FU on the normal healthy intestine cells.

Mistletoe is a semi-parasitic plant, which grows on several types of tree such as oak (*Quercus*), pine (*Pinus*), apple (*Mali*) and ash (*Fraxini*)[8,9]. Mistletoe extract (ME; *Viscum album L.*) was introduced in 1920 by Rudolf Steiner as an anticancer substance[9,10]. Aqueous MEs have been used for several decades as an adjunctive complementary cancer therapy in Europe[8,9,11]. A recent review concluded that mistletoe therapy resulted in long-term disease stability, improvements in patient general condition, vitality, strength, pain, sleep, and appetite. Furthermore, chemotherapy was better tolerated and patients displayed improved emotional and mental condition following mistletoe therapy[12]. The therapeutic efficacy is attributed primarily to the mistletoe lectins[13]. Other cytotoxic components of ME include viscotoxin and alkaloids[8,14]. The composition of ME varies depending on factors such as the host tree, the extraction technique and the manufacturing process[8,15].

Mistletoe lectin (ML) is a heterodimeric glycoprotein which belongs to the class of ribosome-inactivating protein type II. ML comprises a toxic chain A (N-glycosidase enzyme) and chain B (galactoside-recognising lectin)[14]. It has been proposed that ML induces tumour death as chain B of ML binds to the cell surface and then chain A inhibits protein synthesis. The glycoprotein expression on the membrane of tumour cells differs from normal cells and has a higher binding affinity for mistletoe lectins. Therefore, ME toxicity to normal cells is less pronounced indicating selective toxicity to tumour cells[16,17]. However, to date the comparative effects of MEs on normal and transformed intestinal epithelial cells have not been investigated.

In the current study, we examined MEs from three different tree species (ash, oak and apple) for their potential to induce cell death in normal (IEC-6) and transformed (Caco-2) colonic epithelial cells and whether the three extracts differentially had an impact on 5-FU induced toxicity.

Materials and methods

Materials

Dimethyl sulfoxide (DMSO) (Sigma Aldrich), 3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide (MTT) and tissue culture solutions including Dulbecco's Modified Eagle Medium (DMEM), foetal calf serum (FCS), phosphate buffer saline (PBS), antibodies and trypelExpress were from Gibco BRL, Life Technologies Pty Ltd. DBL 5-Fluorouracil for injection was purchased from Mayne Pharma Pty Ltd, Mulgrave, Victoria, Australia. The vented tissue culture flasks (75 cm^2) were from Gibco BRL, Life Technologies Pty Ltd and sterile 96-well tissue culture plates were purchased from Greiner Bio-one. The CO_2 incubator was from SANYO (Japan) and the spectrophotometer was from Dynatech (Germany).

Cell culture

The IEC-6 and Caco-2 cell lines were obtained from the American Type Culture Collection (ATCC, Manassas, USA). Both cell lines were maintained at 37°C in a humidified atmosphere of 5% CO_2, and 90% relative humidity in DMEM supplemented with 10% (v/v) FCS and 1% (v/v) antibiotics (penicillin, gentamicin and streptomycin). The cells were grown in 75 cm^2 vented tissue culture flasks. Culture medium was changed every two days and cells were passaged when they achieved 80–90% confluency.

Mistletoe extracts, *Viscum album*

Ampoules of ME (aqueous; 20 mg/ml; ABNOBA, Viscum) including *Quercus*, *Fraxini* and *Mali* dissolved in physiological saline were kindly provided by A/Prof Ljubov Simson. These samples were initially obtained from ABNOBA Pty Ltd (Germany). The extracts were prepared from mistletoe plant material using a proprietary press developed by ABNOBA. Table 1 presents the lectin and viscotoxin content (µg/mL) of the MEs.

MTT positive control

Grape seed extract (GSE) was used as a positive control as it was shown to be non-toxic to IEC-6 cells in low doses and to protect against 5-FU[18]. Also, GSE (100 µg/mL) decreased Caco-2 cell viability by 20%[18].

Cell viability assay/MTT

MTT assay was used to determine the Caco-2 (passage number: 25–30) and IEC-6 (passage number: 16–19) cell viability[16] according to a previously described method[19], described further[18]. After 24hrs and 48hrs incubation of IEC-6 and Caco-2 cells with the DMEM, respectively, the DMEM was replaced with 100 µl of each ME (1–100 µg/mL)[12] followed by 48 hrs addition of MTT reagent and reading at 570 nm. For the experiments, which examined the effect of ME combined with 5-FU on cell viability, DMEM was replaced with 90 µL of each ME mixed with 10 µL of 5-FU.

Statistical analysis

All data are expressed as mean ± SEM. Statistical analyses were performed using XLSTAT Version 2012.6.03 (Addinsoft) and GraphPad Prism 6. The data for IC_{50} calculation and percentage of cell viability were analysed using one-way ANOVA and two-way ANOVA respectively with a Tukey's *post hoc* test. $P < 0.05$ was considered statistically significant.

Results

$IC_{50\ values}$ of MEs on IEC-6 and Caco-2 cells

The IC_{50} of *Quercus* on IEC-6 cells was significantly higher than on Caco-2 cells (84.39 and 64.54 µg/mL respectively; $p < 0.05$; Figure 1). Similarly, the IC_{50} of *Fraxini* was significantly higher on IEC-6 cells compared to Caco-2 cells (71.74 and 42.66 µg/mL respectively; $p < 0.01$; Figure 1). However, the IC_{50} for *Mali* was significantly lower when administered to IEC-6 cells compared to Caco-2 cells (65.52 and 86.75 µg/mL respectively; $p < 0.05$).

Effects of *Quercus* combined with 5-FU on viability of IEC-6 and Caco-2 cells

Efficacy of 5-FU was confirmed as the viability of 5-FU-treated cells decreased significantly by 30% for IEC-6 cells ($p < 0.05$; Figures 2A, 3A and 4A) and 50% for Caco-2 cells ($p < 0.05$; Figures 2B, 3B and 4B). *Quercus* (50µg/mL and 100 µg/mL; 10 µg/mL, 50 µg/mL and 100µg/mL, respectively) significantly decreased cell viability of IEC-6 and Caco-2 cells compared to cell controls ($p < 0.05$; Figures 2A and 2B). In IEC-6 cells, the combination of 5-FU (5 µM) with 100 µg/mL of *Quercus* increased the potency of 5-FU on IEC-6 cells ($p < 0.05$; Figure 2A). Importantly in the context of colon cancer treatment, *Quercus* (50 and 100 µg/mL) combined with 100 µM of 5-FU significantly decreased Caco-2 cell viability compared to 5-FU only treated cells ($p < 0.05$; Figure 2B).

Effects of *Fraxini* combined with 5-FU on viability of IEC-6 and Caco-2 cells

Fraxini (10, 50 and 100 µg/mL) significantly decreased viability of IEC-6 and Caco-2 cells compared to cell controls ($p < 0.05$; Figures 3a and 3b). In IEC-6 cells, *Fraxini* (50 µg/mL) combined with 5-FU (5 µM) significantly increased 5-FU potency compared to IEC-6 cells independently treated with either *Fraxini* or 5-FU (30%, $p < 0.05$, Figure 3A). After 48 hours, treatment of Caco-2 cells with the combination of *Fraxini* (50 and 100 µg/mL) and 5-FU (100 µM), resulted in significantly fewer viable cells (49% and 46% respectively) compared to cells exposed to 5-FU alone (71%, $p < 0.05$). Nonetheless, there was no significant difference between the viability of Caco-2 cells treated with *Fraxini* (50 and 100 µg/mL) compared to *Fraxini* in combination with 5-FU (100 µM; Figure 3B).

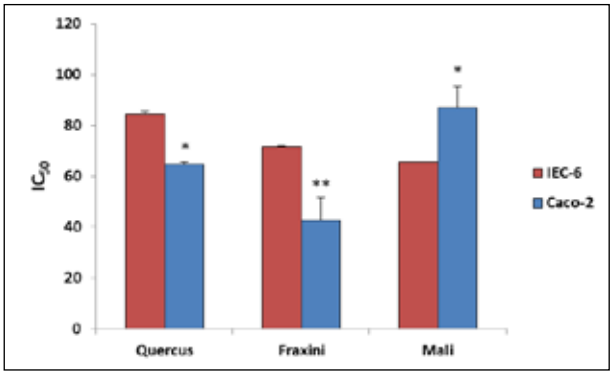
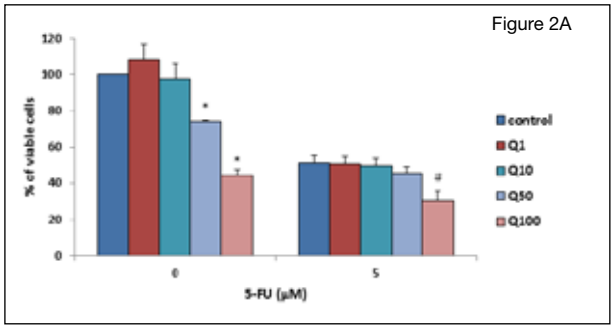


Figure 1: IC_{50} values of Quercus, Fraxini and Mali for IEC-6 and Caco-2 cells. IC50s were calculated by MTT assay after 48hrs incubation. Data are expressed as mean (IC50) ± SEM of triplicate wells from two independent experiments. * indicates $p < 0.05$, ** indicates $p < 0.01$ compared to IEC-6 treated with same ME.



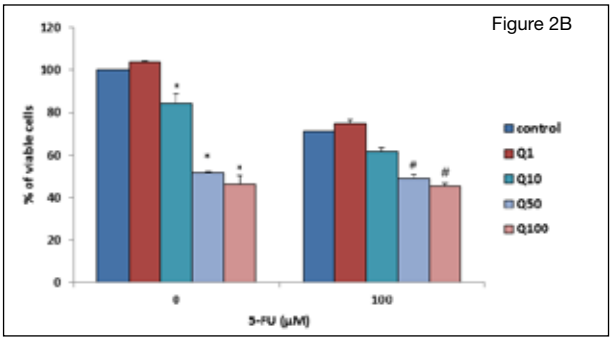
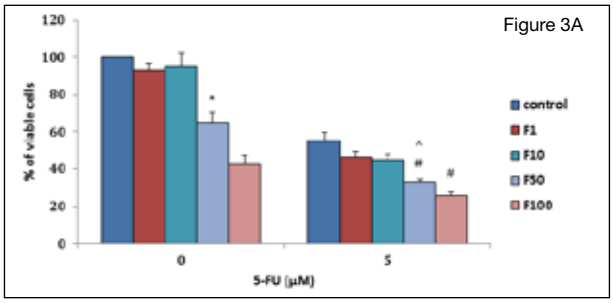


Figure 2: Effects of *Quercus* (1–100 µg/ml) and 5-FU (µM) in combination on viability of IEC-6 (A) and Caco-2 cells (B) after 48hrs. Data are expressed as mean (percentage of cell viability relative to serum free only treated cells) ± SEM of triplicate wells from 2–3 independent experiments. * indicates $p < 0.05$ compared to cell control (0 5-FU); # indicates $p < 0.05$ compared to cell control (5 and 100 µM 5-FU).

Effects of *Mali* combined with 5-FU on viability of IEC-6 and Caco-2 cells

Mali (50 µg/mL and 100 µg/mL) significantly decreased viability of IEC-6 and Caco-2 cells compared to cell controls ($p < 0.05$; Figures 4a and 4b). The combination of *Mali* (50 µg/mL and 100 µg/mL) with 5-FU (5 µM) significantly decreased IEC-6 cell viability compared to cells treated with 5-FU alone ($p < 0.05$, Figure 4A). Nevertheless, in IEC-6 cells, there was no significant difference in viability between *Mali* (50 µg/mL) and the combination of *Mali* and 5-FU (5 µM; Figure 4A). After 48hrs treatment of Caco-2 cells with *Mali* (50 and 100 µg/mL) and 5-FU (100 µM), cell viability was decreased (47% and 44% respectively) compared to cells exposed to 5-FU alone (77%). However, there was no significant difference between the viability of Caco-2 cells treated with *Mali* (50 and 100 µg/mL) compared to *Mali* in combination with 5-FU (100 µM; Figure 4B).



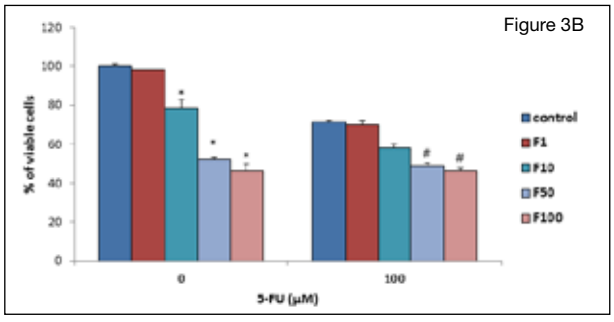
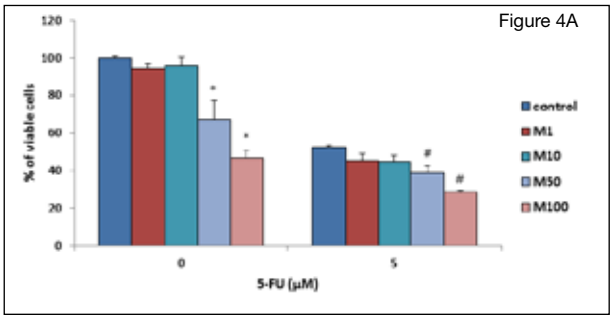


Figure 3: Effects of *Fraxini* (1–100 µg/ml) combined with 5-FU (µM) on viability of IEC-6 (A) and Caco-2 cells (B) after 48hrs. Data are expressed as mean (percentage of cell viability relative to serum free only treated cells) ± SEM of triplicate wells from 2–3 independent experiments. * indicates $p < 0.05$ compared to cell control (0 5-FU); # indicates $p < 0.05$ compared to cell control (5 and 100µM 5-FU); ^ indicates $p < 0.05$ between cells treated with 50 µg/ml of *Fraxini* and cells treated with *Fraxini* + 5µM of 5-FU.

Discussion

Despite advances in chemical-based medications, cancer patients are inclined to use naturally-sourced toxins due to adverse effects associated with chemotherapy and radiation[20]. We investigated the effects of three different MEs (*Quercus*, *Fraxini* and *Mali*), alone and in combination with 5-FU, on the viability of normal and



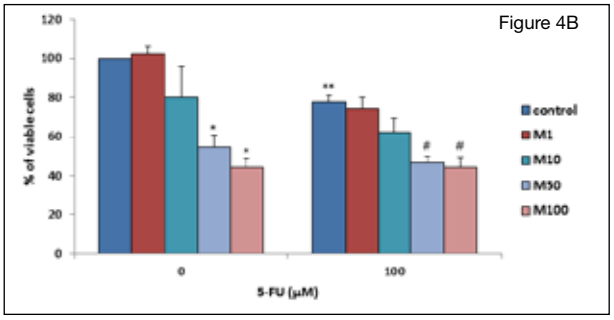


Figure 4: Effects of *Mali* (1–100 µg/ml) and 5-FU (µM) in combination on viability of IEC-6 (A) and Caco-2 cells (B) after 48 hours. Data are expressed as mean (percentage of cell viability relative to serum free only treated cells) ± SEM of triplicate wells from 2–3 independent experiments. * and ** indicate $p < 0.05$ and $p < 0.01$ compared to cell control (0 5-FU); # indicates $p < 0.05$ compared to cell control (5 and 100 5-FU µM).

transformed intestinal epithelial cells. *Fraxini* was the most potent ME on Caco-2 cells; however, it significantly increased the potency of 5-FU chemotherapy on IEC-6 cells compared to *Fraxini* alone (50 µg/mL). With the exception of *Fraxini*, none of the MEs had a significant impact on IEC-6 cell viability when combined with 5-FU, compared to IEC-6 cells treated with 5-FU alone. Furthermore, *Fraxini* was the only ME to increase 5-FU efficacy on Caco-2 cells above that of the same concentration of ME alone.

In the current study, *Fraxini* had the greatest effect on Caco-2 cells compared to *Mali* and *Quercus*. This presumably reflected the highest concentration of lectin and viscotoxin in *Fraxini* compared to *Quercus* and *Mali* (Table 1). In a study conducted by Ding and colleagues[21], the anti-proliferative activity of Fraxini was shown to be almost 10 and 6 times stronger than that of Iscador M and Iscador Q, respectively, in both Hep3B and HepG2 cells. Furthermore, *Quercus* (with slightly higher lectin and viscotoxin levels; as shown in Table 1) was more toxic than *Mali* when applied to Caco-2 cells.

Table 1: Main active constituents (lectin and viscotoxin) in *Quercus, Fraxini* and *Mali*.

Mistletoe extract	Lectin (µg/mL)	Viscotoxin (µg/mL)
Quercus	6.37	50.33
Fraxini	10.38	61.0
Mali	6.02	45.95

Lectin and viscotoxin are believed to be the main biological active components of MEs contributing to their potency to cancer cells[13,22]. These active components are reported to show anti-neoplastic effects by different mechanism of action including cell cycle arrest, induction of apoptosis, altering tumour angiogenesis and anti-inflammatory effects[21-24]. However, the exact underlying mechanism of action of MEs is not clearly understood yet[24].

It is proposed that 5-FU and MEs induce cell death through distinct molecular mechanisms of action, which include inhibition of protein synthesis, activation of apoptotic cascades, such as p-53 independent apoptosis and inhibition of telomerase[15,16,25]. Engdal (2009) demonstrated that MEs (Iscador M series II) inhibited the p-glycoprotein function of Caco-2 cells[26]. On the other hand, 5-FU induces cell death by interfering with thymidylate synthase (TS) enzyme function and consequently inhibition of DNA synthesis[27]. The MTT assay, employed in the current study, was limited in terms of distinguishing apoptosis from necrosis of cells treated with MEs. Future assays which measure direct DNA binding and determine cell apoptosis, such as ^{3}H-thymidine and Edu-IT, respectively, are recommended.

Another interesting observation was the greater efficacy of *Mali* on IEC-6 cells compared to Caco-2 cells. Previous *in vitro* studies have mainly focused on the impact of MEs on tumour cell lines and not the normal human cell lines. Further experiments are necessary to investigate the factors influencing the higher potency of *Mali* on normal intestine cells compared to colon cancer cells.

Apart from possessing the lowest IC_{50}, *Fraxini* (50 µg/mL combined with 5-FU) significantly potentiated 5-FU on IEC-6 cells compared to the same concentration of *Fraxini* alone. The study by Cazacu and his colleagues (2003) showed that mistletoe therapy (Isorel) as an adjunct to 5-FU chemotherapy after surgery improved survival rate and also alleviated chemotherapy side-effects (digestive and/or hematological toxicity) of colon cancer patients[16].

In the current study, none of the MEs protected the normal intestinal cells from 5-FU toxicity. Similar results were obtained in a recent study done by Weissenstein and colleagues, as *Viscum album* extraction did not inhibit chemotherapy-induced toxicity on 5 different cell lines[22]. In clinical settings, internal factors, such as the

immune system; a purported target of ME could have contributed to alleviation of chemotherapy side-effects after mistletoe therapy[13,22,28]. Clearly these factors were absent in the present *in-vitro* study.

The most likely explanation for IC_{50} variations in *Quercus, Fraxini* and *Mali* is due to different magnitudes of toxic components in each. The aforementioned extracts originated from mistletoe bushes, which grow on different host trees and possess distinct compositions resulting in varying magnitudes of toxicity[8,15]. Also, as mentioned previously lectins can induce apoptosis through different pathways, some being more effective than others in specific cell lines, therefore resulting in different toxicity values for different cell lines[29].

Recently, Huber and colleagues conducted a maximum tolerable dose and safety investigation of intravenous mistletoe application[30]. ME (200, 400, 700, 1200 or 2000mg) infusions were administered once weekly for 3 weeks in advanced cancer patients. A dose-limiting toxicity was not reached and no serious adverse events or suspected unexpected serious adverse events occurred as a result of mistletoe treatment. Furthermore, it was concluded that weekly infusions of ME at a starting dose of up to 2000mg is well-tolerated; however, at 2000mg dose, there is a minor risk of fever or allergic reaction[30]. These results support the safety of mistletoe therapy in advanced cancer patients.

In summary, the current study indicated that all MEs significantly reduced viability of colon cancer cells (Caco-2), while not inhibiting 5-FU efficacy. However, all MEs differentially reduced IEC-6 cell viability. Importantly *Quercus* and *Fraxini* at lower doses exhibited greater toxicity to Caco-2 cells when compared to IEC-6 cells. These observations support previous studies describing ME as an 'anticancer' substance and warrant future studies in animal models of colon cancer. Interestingly, both *Quercus* and *Fraxini* potentiated the effects of 5-FU at higher doses. On the basis of the impact on IEC-6 and Caco-2 cells displayed in this study, *Quercus* would likely be the preferred candidate for further clinical development. MEs could represent a promising new adjunct to conventional chemotherapy regimens. Further studies are required to determine the optimal host tree species, optimal dosing regimen and the specific bioactive factors responsible.

Acknowledgements

This study was not funded or sponsored by any organisation, grants or any other resources. The full raw data are available and we are able to provide them in Excel format if requested by the journal. The authors have no other acknowledgements to disclose.

Declaration of interest

The authors have no conflicts of interest to report.

References

1. André T, Boni C, Mounedji-Boudiaf L *et al.* Oxaliplatin, Fluorouracil, and Leucovorin as adjuvant treatment for colon cancer. N Engl J Med 2004;350:2343–2351.
2. Markle B, May EJ, Majumdar APN. Do nutraceutics play a role in the prevention and treatment of colorectal cancer? Cancer Metastasis Rev 2010;29:395–404.
3. Fata F, Ron IG, Kemeny N *et al.* 5-fluorouracil-induced small bowel toxicity in patients with colorectal carcinoma. Cancer 1991;86:1129–1134.
4. Mauger CA, Butler RN, Geier MS *et al.* Probiotic effects on 5-fluorouracil-induced mucositis assessed by the sucrose breath test in rats. Dig Dis Sci 2007;52:612–619.
5. Wright TH, Yazbeck R, Lymn KA *et al.* The herbal extract, Iberogast, improves jejunal integrity in rats with 5-Fluorouracil (5-FU)-induced mucositis. Cancer Biol Ther 2009;8:923–929.
6. Bowen JM, Gibson RJ, Cummins AG *et al.* Intestinal mucositis: the role of the Bcl-2 family, p53 and caspases in chemotherapy-induced damage. Support Care Cancer 2006;14:713–731.
7. Lindsay RJ, Geier MS, Yazbeck R *et al.* Orally administered emu oil decreases acute inflammation and alters selected small intestinal parameters in a rat model of mucositis. Br J Nutr 2010;104:513–519.
8. Kelter G, Schierholz JM, Fischer IU *et al.* Cytotoxic activity and absence of tumor growth stimulation of standardized mistletoe extracts in human tumor models in vitro. Anticancer Res 2007;27:223–233.
9. Mabed M, El-Helw L, Shamaa S. Phase II study of viscum fraxini-2 in patients with advanced hepatocellular carcinoma. Br J Cancer 2004;90:65–69.
10. Beuth J, Schneider B, Schierholz JM. Impact of complementary treatment of breast cancer patients with standardized mistletoe extract during aftercare: a controlled multicenter comparative epidemiological cohort study. Anticancer Res 2008;28:523–527.
11. Elsasser-Beile U, Leiber C, Wetterauer U *et al.* Adjuvant intravesical treatment with a standardized mistletoe extract to prevent recurrence of superficial urinary bladder cancer. Anticancer Res 2005;25:4733–4736.
12. Kienle GS, Mussler M, Fuchs D *et al.* Intravenous mistletoe treatment in integrative cancer care: A qualitative study exploring the procedures, concepts, and observations of expert doctors. Evid Based Complement Alternat Med 2016;4628287.
13. Heinzerling L, Von Baehr V, Liebenthal C *et al.* Immunologic effector mechanisms of a standardized mistletoe extract on the function of human monocytes and lymphocytes *in vitro*, *ex vivo*, and *in vivo*. J Clin Immunol 2006;26:347–359.
14. Duong Van Huyen J-P, Delignat S, Bayry J *et al.* Interleukin-12 is associated with the *in vivo* anti-tumor effect of mistletoe extracts in B16 mouse melanoma. Cancer Letters 2006;243:32–37.
15. Eggenschwiler J, Von Balthazar L, Stritt B *et al.* Mistletoe lectin is not the only cytotoxic component in fermented preparations of *Viscum album* from white fir (*Abies pectinata*). BMC Complement Alter Med 2007;7:14.
16. Cazacu M, Oniu T, Lungoci C *et al.* The influence of isorel on the advanced colorectal cancer. Cancer Biother Radiopharm 2003;18:27–34.
17. Guo Q, Xia B, Zhang F *et al.* Tetraspanin CO-029 inhibits colorectal cancer cell movement by deregulating cell-matrix and cell–cell adhesions. PLoS ONE 2012;7:e38464.
18. Cheah KY, Howarth GS, Yazbeck R *et al.* Grape seed extract protects IEC-6 cells from chemotherapy-induced cytotoxicity and improves parameters of small intestinal mucositis in rats with experimentally-induced mucositis. Cancer Biol Ther 2009;8: 382–390.
19. Huynh-Delerme C, Huet H, Noël L *et al.* Increased functional expression of P-glycoprotein in Caco-2 TC7 cells exposed long-term to cadmium. Toxicol in Vitro 2005;19:439–47.
20. Molassiotis A, Scott JA, Kearney N *et al.* Complementary and alternative medicine use in breast cancer patients in Europe. Support Care Cancer 2005;14:260–267.
21. Ding X, Cartwright C, Tan L *et al.* Abstract 3206: Mistletoe extract inhibits the proliferation of human hepatocellular carcinoma cells by induction of apoptosis and downregulation of c-MYC. Cancer Research 2014;74:3206–3206.

22. Weissenstein U, Kunz M, Urech K *et al.* Interaction of standardized mistletoe (*Viscum album*) extracts with chemotherapeutic drugs regarding cytostatic and cytotoxic effects in vitro. BMC Complement and Altern Med 2014;14:1–9.
23. Von Schoen-Angerer T, Wilkens J, Kienle GS *et al.* High-dose *Viscum album* extract treatment in the prevention of recurrent bladder cancer: A retrospective case series. Perm J 2015;19:76–83.
24. Saha C, Hegde P, Friboulet A *et al. Viscum album*-mediated COX-2 inhibition implicates destabilization of COX-2 mRNA. PLoS One 2015;10:e0114965.
25. Hostanska K, Vuong V, Rocha S *et al.* Recombinant mistletoe lectin induces p53-independent apoptosis in tumour cells and cooperates with ionising radiation. Br J Cancer 2003;88:1785–1792.
26. Engdal S, Nilsen OG. *In vitro* inhibition of CYP3A4 by herbal remedies frequently used by cancer patients. Phytother Res 2009;23:906–912.
27. Miyazaki K, Shibahara T, Sato D *et al.* Influence of chemotherapeutic agents and cytokines on the expression of 5-fluorouracil-associated enzymes in human colon cancer cell lines. J Gastroenterol 2006;41:140–50.
28. Lee JY, Kim JY, Lee YG *et al. In vitro* immunoregulatory effects of Korean mistletoe lectin on functional activation of monocytic and macrophage-like cells. Biol Pharm Bull 2007;30:2043–2051.
29. Yau T, Dan X, Ng CC *et al.* Lectins with potential for anti-cancer therapy. Molecules 2015;20:3791–3810.
30. Huber R, Schlodder D, Effertz C *et al.* Safety of intravenously applied mistletoe extract — results from a phase I dose escalation study in patients with advanced cancer. BMC Complement Altern Med 2017;17:465.

Australian Journal of Herbal and Naturopathic Medicine **2018** 30(4)

The naturopathic management of interstitial cystitis: A case study

Amy Taylor BHSc Naturopathy (final year student), BJourn[1], David Casteleijn MHSc Herbal Medicine[1,2], and Sophia Gerontakos BHSc Naturopathy[1]

[1]Endeavour College of Natural Health, Fortitude Valley Brisbane, QLD, Australia

[2]University of Technology Sydney, Faculty of Health, Australian Research Centre in Complementary and Integrative Medicine, Ultimo, NSW, Australia

Contact information

Amy Taylor, Endeavour College of Natural Health, Level 2, 269 Wickham St, Fortitude Valley, Brisbane, QLD 4006
Email amy.elyn@hotmail.com

Abstract

This case study describes the treatment of a middle-aged woman with symptoms of increased urgency and frequency of urination, and incomplete voiding, consistent with the diagnosis of interstitial cystitis (IC). Treatment was underpinned by holistic philosophy and incorporated the naturopathic principle, 'treat the whole person'. Aggravating and sustaining factors, including chronic stress, nervous system dysregulation and inflammation, were identified and addressed using herbal medicine. The client reported alleviation of symptoms within two weeks of naturopathic treatment, as well as improvements of other characteristic symptoms, including sleep onset and quality, increased energy levels, resolution of restless legs, and decreased oedema in her feet and ankles. This case demonstrates the efficacy of naturopathy for treatment of symptoms associated with IC.

Keywords: Bladder, cystitis, interstitial cystitis, bladder pain, herbal medicine, naturopathy.

Introduction

Interstitial cystitis (IC) is a chronic inflammatory condition of the urinary tract with a common clinical presentation of urinary frequency, urgency and pain[1]. Specific aetiology is unknown; however, there is some semblance to urinary tract infection (UTI) but without presence of bacteria on urine cultures, and without response to antibiotic therapy[2]. IC is commonly associated with thinning of the bladder epithelium[3], altered nerve signalling[4] and increased apoptosis in urothelial cells[3].

Associations between clinical characteristics of IC, and mood and personality traits were explored in a study in 2016[5]. Researchers found a significant correlation between the pain of IC and anxiety, depression and interpersonal problems, highlighting the potential benefits of a holistic naturopathic approach[5]. Persons with IC commonly present with associated anxiety and depression[5], and report a decrease in quality of life and social wellbeing[6]. A holistic naturopathic approach includes consideration of all aspects that may impact on a person's health and wellbeing, including pathology, physiology, as well as social and environmental factors.

In IC, the intensity of urinary symptoms such as urgency and pain are positively correlated with increased stress levels[7], and sympathetic nervous system (SNS) dominance is a common underlying mechanism of pathogenesis[3].

The following case study illustrates the whole person implications in the naturopathic management of a 54-year-old woman with symptoms associated with chronic IC. Alleviation of symptoms occurred following naturopathic treatment that incorporated methods to reduce chronic stress and inflammation.

Case description

The client, Rose*, is a 54-year-old female. Rose initially presented to the Wellnation student clinic in March 2018 with lower urinary tract symptoms (increased urinary urgency and frequency), and anxiety. She explained a need to urinate over 15 times per day and was experiencing incomplete voiding. There was no marked increase in urine volume (polyuria). She would pass urine only to feel a sense of urgency to urinate again less than five minutes later. Pain was present only if she had to 'hold on'. These symptoms began 2–3 years prior and Rose could not identify any specific event occurring at that time. Since onset she had gradually reduced her water intake down to 1–2 glasses per day. She believed water was exacerbating her symptoms. Rose had not sought treatment from her general practitioner (GP) and noted she had experienced 1–2 incidents of cystitis over 20 years previously; however, she felt her current symptoms were different and unrelated.

Rose described delayed sleep onset (1–3 hours) due to ruminating thoughts, and disturbed sleep due to nocturia and restless legs. She woke unrefreshed approximately three times per week. Oedema was present in her ankles and lower legs.

Rose's urinary tract symptoms were causing her increased anxiety and fear. She had modified some of her behaviours to accommodate her symptoms, avoiding places without easy access to a bathroom and always assessing the proximity of the nearest bathroom in new places. Her symptoms resulted in her feeling anxious and fearful about new situations. Rose described depressive-type symptoms of lethargy and feeling unmotivated and unenthusiastic. She was experiencing increased stress at work, and occasional constipation, which she associated with an increase in consecutive shifts. Rose had worked full time in a high-stress, 24/7, shift work environment for many years. Her diet consisted of processed and refined foods, and she described a habit of snacking on junk foods, which included sweets and potato chips. She said she frequently felt hungry between meals, particularly at work. She had identified that citrus, hot and spicy foods, and soft drink aggravated her symptoms. Rose was not taking any medications and had no reports from pathology testing. Her blood pressure was 125/85.

Case assessment

Rose's work environment was noted as a cause of ongoing emotional and mental stress. Shift work and chronic stress may result in sympathetic nervous system (SNS) dominance, and occasional constipation, which in Rose's case occurred specifically when she was at work. SNS dominance is defined as prolonged activation of the hypothalamus-pituitary-adrenal (HPA) axis and may result in a myriad of imbalances, including cortisol dysregulation[8]. Shift work (specifically sleep loss and circadian disruption) is a physiological stressor, and may disturb cortisol regulation, and contribute to systemic inflammation[9].

Inflammatory cascades are self-perpetuating and in instances of chronic inflammation treatments aimed at breaking inflammatory feedback mechanisms may be relevant[10]. There is a range of causative mechanisms of chronic inflammatory states; however, stress is noted as a common predisposing, exacerbating and sustaining factor[10].

Therefore, although Rose's symptoms presented in the urinary tract, it was considered they were being exacerbated and sustained by her chronic stress and complicated by chronic systemic inflammation. Holistic naturopathic treatment incorporated these aspects as foundational aims of treatment, and utilised herbal medicine to reduce symptoms, provide stress relief and reduce inflammation, and improve Rose's vitality and capacity to incorporate healthy behavioural and lifestyle changes.

Treatment

Herbal medicine was targeted to support healthy nervous system function, and to reduce inflammation. Behavioural recommendations included improved sleep hygiene and stress-reducing self-administered rituals (baths, aromatherapy, reading, breathing exercises, warm showers, and limited screen time before bed) aimed to promote relaxation. An increase in water consumption was also recommended, along with a decreased dietary intake of aggravating foods (spicy food, citrus and soft drinks).

The liquid herbal formula (Table 1) was created and Rose was instructed to take 7.5 mL twice daily in a small amount of water. The herbal tablet formula (Table 2) was prescribed at 4 tablets per day (2 x am, 2 x pm) with food.

Table 1: Liquid herbal formula

Herbal mix 1 — liquid herbal formula			
Botanical	***Common***	**Actions**	**Dose**
Hypericum perforatum	St John's Wort	Anxiolytic, antidepressant[11,12]	20 mL
Eleutherococcus senticosus	Siberian Ginseng	Adaptogen, immunomodulator, tonic[13]	30 mL
Scutellaria lateriflora	Skullcap	Nervine, spasmolytic[14]	20 mL
Schisandra chinensis	Schisandra	Adaptogen, antioxidant[15]	30 mL
Crocus sativus	Saffron	Serotonergic, anti-inflammatory, antioxidant, antidepressant, neuroprotective[16,17]	10 mL
Dose			
7.5 mL twice daily (am/pm) in a small amount of water			110 mL

Table 2: Tablet herbal formula

Herbal mix 2 — tablet herbal formula (per tablet)			
Botanical	***Common***	**Actions**	**Dose**
Boswellia serrata	Boswellia	Anti-inflammatory[18]	1.9 g
Curcuma longa	Turmeric	Anti-inflammatory, Antioxidant, anti-neoplastic neuroprotective[19]	2.0 g
Apium graveolens	Celery	Antioxidant, anti-inflammatory, anti-spasmolytic[20]	1.0 g
Zingiber officinale	Ginger	Anti-inflammatory, digestive stimulant[21]	300 mg
Dose			
Two tablets twice daily (am/pm) with food			

Treatment outcome

Rose reattended the clinic in March 2018, two weeks after her initial appointment. She reported increased energy levels and vitality and described a marked reduction in the frequency and urgency of her urinary symptoms. Sleep onset and quality had also improved, and she was no longer experiencing restless legs. The oedema in her feet and ankles had decreased. Rose felt

more energetic and capable of managing daily events and noted a significant benefit from the 'unwinding' routine before sleep.

Discussion

The unknown aetiology of IC can be a cause for confusion in identifying mechanistic treatment strategies[22]. Numerous suggestions for the pathophysiologic mechanisms in IC have been proposed, including epithelial dysfunction, mast cell activation, and neurogenic inflammation[8]. Although it remains unclear whether altered neural mechanisms and inflammation are causative, or resultant, they are an important consideration in treatment as neural upregulation plays a role in the chronic presentations of urgency and frequency in urinary tract symptoms[8]. A naturopathic approach that incorporates multifactorial aspects of the individual case can facilitate consideration of the 'whole person' and simultaneously treat several potential aetiologies of chronic IC.

Lower urinary tract symptoms may also be associated with diabetes mellitus type 2 (DM2) and given the client's urinary tract symptoms, dietary habits, and sedentary lifestyle[23], this differential diagnosis was an important consideration. Although Rose described increased urinary frequency and urgency, she did not describe polyuria (increased urine volume) commonly associated with DM2[23]. Rose's symptoms included a lack of thirst and decreased water intake, as well as normal range blood pressure. In DM2 there is commonly increased thirst and water intake, and elevated blood pressure[24]. Therefore, although undiagnosed, the client's symptom profile was considered to correlate more closely with those of IC, and short-term treatment aimed to address these symptoms and their underlying drivers.

There is a positive association between stress and abnormalities of the HPA axis, and an increase in the occurrence of bladder symptoms[8], as well as an increase in intensity and severity of existing symptoms, such as pain and urgency[7]. Chronic stress has been linked with a wide array of adverse health outcomes, many of which are chronic in nature, and complicated by chronic inflammation[25]. Supporting the nervous system to reduce excessive stress and anxiety to improve mood and potentially inflammation was pivotal in the successful management of this case.

Antidepressants are sometimes utilised in the medical treatment of IC based on the inhibition of the synaptic reuptake of serotonin and norepinephrine. This treatment may also improve mood, and pain perception[2]. The tricyclic amitriptyline is commonly recommended and has been effective in reducing symptoms of pain and urgency intensity[26]. Amitriptyline decreases mast cell activity and blocks H1-histaminergic receptors; however, its use is also commonly associated with significant side effects of dry mouth, dizziness and gastrointestinal complaints in up to 88% of patients[2]. Research indicates low acceptability in the use of amitriptyline, with higher dropout rates than comparators, despite findings of superior effect[27,28]. Many people prefer treatment options with fewer side effects.

Herbal medicines with demonstrated efficacy for reducing depression and improving mood may provide a natural alternative without the side effects of pharmaceuticals for treatment of IC. The action of *Crocus sativus* may increase synaptic cleft serotonin concentration and reduce neuroinflammation[16] and *Hypericum perforatum* has been found to have clinical efficacy comparable to selective serotonin reuptake inhibitors (SSRIs) in symptoms of depression[29]. Adaptogens, or herbal medicines that may improve resilience such as *Eleutherococcus senticosus* and *Schisandra chinensis,* were used traditionally in herbal medicine to regulate stress response and provide additional benefits of increasing concentration, performance, and endurance in fatigue states[13,30]. *Scutellaria lateriflora* was traditionally used as a nervine and anxiolytic; however, its additional anti-inflammatory, spasmolytic, antimicrobial, and antioxidant actions, may all have further benefited this client[31].

The prescription of Herbal Mix 1 aimed to reduce symptoms by supporting the client's nervous system, regulating neural pathways and stress responses to improve mood, decrease anxiety and increase the client's sense of control over her body and health.

Herbal Mix 2 was prescribed to reduce urgency and frequency of urinary tract symptoms.

Inhibition of inflammatory cascades and mast cell stabilisation associated with extracts from *Boswellia serrata*[32] may reduce inflammation and halt complex feedback loops associated with IC[10], decreasing urinary urgency and frequency and inhibiting the formation of oxygen radicals capable of causing further inflammation and damage to the urinary tract[18,33]. *Zingiber officinale* and *Curcuma longa* also inhibit multiple pro-inflammatory pathways particularly relevant in IC and possess sound safety profiles[34].

The holistic nature of naturopathy in case analysis and treatment decisions and its consideration of the interdependence of all body systems including exacerbating and compensating factors, is well suited to the treatment of complex and chronic disease states. The broad scope of the naturopathic consult and resultant treatment plan (a unique treatment approach informed by individual case characteristics) may provide treatment at multiple levels at the same time. In this case, herbal medicines, dietary changes and the inclusion of self-care rituals and behaviours were used to empower and engage the client, reduce inflammation and stress, balance nervous system physiology, and improve urinary tract symptoms and risks of progressive disease.

This case study provides an example of the potential benefits of a naturopathic approach and the importance of treating the nervous system and applying stress

management, as well as effective treatment of symptoms in people with complex and chronic physical conditions.

Limitations of this case include uncertainty regarding exactly which parts of the complex treatment correlate with the therapeutic effects. Naturopathic treatment involves extended consultations, active listening and validation of the client's concerns. This incidental counselling may provide therapeutic effect, which has not been directly accounted for in this case study. Another limitation is the short time frame of the case management. This case spans two weeks of treatment and long-term effectiveness has not been described; nevertheless, the significant alleviation of symptoms and the subsequent improvement in quality of life in that time warrant dissemination and discussion.

This case highlights the broad-ranging actions of herbal medicines, from their many and varied constituents, potentially providing benefits at multiple levels, addressing symptomology and interdependent aspects of pathophysiology, which remain poorly understood.

Conclusion

This case describes the benefits of treating symptoms of IC with naturopathy where the treatment strategy was underpinned by naturopathic philosophy and addressed the complex interplay of aspects relevant in a person with chronic disease. Despite the multifactorial aetiology of IC, herbal medicine addressed the presenting complaints and associated conditions in a prompt and timely manner.

Note

* The patient's name has been changed to preserve her anonymity.

References

1. Ogawa T *et al.* Current and emerging drugs for interstitial cystitis/bladder pain syndrome (IC/BPS). Expert Opin Emerg Drugs 2015;20(4):555–70.
2. Davis N, Brady C, Creagh T. Interstitial cystitis/painful bladder syndrome: epidemiology, pathophysiology and evidence-based treatment options. Eur J Obstet Gynecol Reprod Biol 2014;175:30–37.
3. Ke Q-S, Kuo H-C. Pathophysiology of interstitial cystitis/bladder pain syndrome. Tzu Chi Medical Journal 2015;27(4):139–144.
4. Mullins C *et al.* Novel research approaches for interstitial cystitis/bladder pain syndrome: thinking beyond the bladder. Transl Androl Urol 2015;4(5):524.
5. Chen W-C, Lee M-H, Wu H-C. Relationship among symptoms, mood, and personality traits in patients with interstitial cystitis/bladder pain syndrome. Urol Sci 2017;28(3):147–151.
6. Vasudevan V, Moldwin R. Addressing quality of life in the patient with interstitial cystitis/bladder pain syndrome. Asian J Urol 2017;4(1):50–54.
7. Lai H *et al.* Correlation between psychological stress levels and the severity of overactive bladder symptoms. BMC Urol 2015;15(1):14.
8. Nazif O, Teichman JM, Gebhart G, Neural upregulation in interstitial cystitis. Urology 2007;69(4):S24–S33.
9. Reynolds AC *et al.* The shift work and health research agenda: considering changes in gut microbiota as a pathway linking shift work, sleep loss and circadian misalignment, and metabolic disease. Sleep Med Rev 2017;34:3–9.
10. Okin D, Medzhitov R Evolution of inflammatory diseases. Curr Biol 2012;22(17):R733–40.
11. Clement K *et al.* St John's wort and the treatment of mild to moderate depression: a systematic review. Holist Nurs Pract 2006;20(4):197–203.
12. Müller WE. Current St John's wort research from mode of action to clinical efficacy. Pharmacol Res 2003;47(2):101–109.
13. Provino R. The role of adaptogens in stress management. Australian Journal of Medical Herbalism 2010;22(2):41.
14. Brock C *et al.* American skullcap (*Scutellaria lateriflora*): A randomised, double-blind placebo-controlled crossover study of its effects on mood in healthy volunteers. Phytother Res 2014;28(5):692–698.
15. Panossian A, Wikman G. Pharmacology of Schisandra chinensis Bail: an overview of Russian research and uses in medicine. J Ethnopharmacol 2008;118(2):183–212.
16. Khazdair MR *et al.* The effects of *Crocus sativus* (saffron) and its constituents on nervous system: A review. Avicenna J Phytomed 2015;5(5):376.
17. Akhondzadeh S *et al. Crocus sativus L*. in the treatment of mild to moderate depression: a double-blind, randomized and placebo-controlled trial. Phytother Res 2005;19(2):148–151.
18. Iram F, Khan SA, Husain A. Phytochemistry and potential therapeutic actions of Boswellic acids: A mini-review. Asian Pac J Trop Biomed 2017;7(6):513–523.
19. Fadus MC *et al.* Curcumin: An age-old anti-inflammatory and anti-neoplastic agent. J Tradit Complement Med 2017;7(3):339–346.
20. Al-Asmari AK, Athar MT, Kadasah SG. An updated phytopharmacological review on medicinal plant of Arab region: *Apium graveolens linn.* Pharmacogn Rev 2017;11(21):13.
21. Kooti W, Daraei N. A review of the antioxidant activity of celery (*Apium graveolens L*). J Evid Based Complementary Altern Med 2017;22(4):1029–1034.
22. Hsieh C-H *et al.* Treatment of interstitial cystitis in women. Taiwan J Obstet Gynecol 2012;51(4):526–532.
23. Egan AM, Dinneen SF. What is diabetes? Medicine 2014;42(12):679–681.
24. Barrett T. Type 2 diabetes mellitus: incidence, management and prognosis. Paediatr Child Health 2013;23(4):163–167.
25. Miller GE, Cohen S, Ritchey AK. Chronic psychological stress and the regulation of pro-inflammatory cytokines: a glucocorticoid-resistance model. Health Psychol 2002;21(6):531.
26. van Ophoven A *et al.* A prospective, randomized, placebo controlled, double-blind study of amitriptyline for the treatment of interstitial cystitis. J Urol 2004;172(2):533–536.
27. Cipriani A *et al.* Comparative efficacy and acceptability of 21 antidepressant drugs for the acute treatment of adults with major depressive disorder: a systematic review and network meta-analysis. Lancet 2018;391(10128):1357–1366.
28. Van Ophoven A, Hertle L. Long-term results of amitriptyline treatment for interstitial cystitis. J Urol 2005;174(5):1837–1840.
29. Ng QX, Venkatanarayanan N, Ho CYX, Clinical use of *Hypericum perforatum* (St John's wort) in depression: A meta-analysis. J Affect Disord 2017;210:211–221.
30. Cicero A *et al.* Effects of Siberian ginseng (*Eleutherococcus senticosus maxim.*) on elderly quality of life: a randomized clinical trial. Arch Gerontol Geriatr 2004;38:69–73.
31. Upton R, Dayu R. Skullcap *Scutellaria lateriflora L.*: an American nervine. J Herb Med 2012;2(3):76–96.
32. Ammon H. Modulation of the immune system by *Boswellia serrata* extracts and boswellic acids. Phytomedicine 2010;17(11):862–867.
33. Sant GR *et al.* The mast cell in interstitial cystitis: role in pathophysiology and pathogenesis. Urology 2007;69(4):S34–S40.
34. Aggarwal BB, Harikumar KB. Potential therapeutic effects of curcumin, the anti-inflammatory agent, against neurodegenerative, cardiovascular, pulmonary, metabolic, autoimmune and neoplastic diseases. Int J Biochem Cell Biol 2009;41(1):40–59.

Australian Journal of Herbal and Naturopathic Medicine **2018** 30(4)

A synopsis of the 2018 International Congress on Integrative Medicine and Health

Bradley Leech BHSc Nut Med (Hons)[a,b], Jessica Bayes BHSc Nut Med (Hons)[b]

[a] University of Technology Sydney, Faculty of Health, Australian Research Centre in Complementary and Integrative Medicine, Ultimo, NSW, Australia

[b] Endeavour College of Natural Health, Office of Research, Fortitude Valley, QLD, Australia

Contact information

Bradley Leech, University of Technology Sydney, Faculty of Health, Australian Research Centre in Complementary and Integrative Medicine, 15 Broadway, Ultimo, NSW, 2007, Australia

Email Bradgleech@gmail.com

Abstract

The 2018 International Congress on Integrative Medicine and Health (ICIMH) took place in Baltimore, America, and focused on the advancement of integrative health through research, education, clinical practice and policy. The Congress served as a platform to share novel research in integrative medicine and health. Major themes of the 2018 ICIMH include the mechanism of action behind traditional and complementary therapies, mindfulness and non-pharmaceutical pain management techniques. Throughout the Congress, trends were seen suggesting a new paradigm of health care is necessary, moving away from solely conventional medicine to an integrative approach, utilising manual therapies, mindfulness practice and dietary interventions in conjunction with conventional care. Delegates who attended the 2018 ICIMH networked with international researchers and health care practitioners, forming new relationships and strengthening pre-existing partnerships.

Introduction

The 2018 International Congress on Integrative Medicine and Health (ICIMH), organised by the Academic Consortium for Integrative Medicine and Health, was held 8–11 May 2018 in Baltimore, Maryland, America. The focus of the 2018 ICIMH was to advance integrative health through research, education, clinical practice and policy.

The 2018 ICIMH attracted many health care practitioners, researchers, educators and policy advocates from over 25 countries. During the Congress, hundreds of poster presentations and over 70 concurrent symposia, panel discussions and oral presentations were held. While a wide range of topics were discussed and presented at this Congress, a number of trends were apparent, including the mechanism of action behind traditional and complementary therapies, mindfulness and non-pharmaceutical pain management techniques. Validation of traditional therapies such as Ayurveda, traditional Chinese medicine, acupuncture, yoga, tai chi and mindfulness were seen throughout many of the poster sessions and a key focus for presenters. One other theme seen at the 2018 ICIMH was the need for a new paradigm, moving away from solely pharmaceutical treatments to an integrative approach, utilising manual therapies, mindfulness practices and dietary interventions, in conjunction with conventional care. Numerous researchers highlighted the importance of an integrative approach in clinical practice for the management of chronic diseases such as Alzheimer's disease, cancer, autoimmune conditions and debilitating symptoms such as pain.

Highlights from the oral presentations

Helene Langevin (*Osher Center for Integrative Medicine*) received a standing ovation after presenting the preliminary results of her research on connective tissue. Her recent research into the mechanism of action behind stretching and the reduction of acute inflammation had the audience in captured attention. Most insightful were the preliminary results from animal models that suggest stretching may reduce cancer growth (manuscript accepted for publication). Further human studies are necessary to translate these results into human application.

The 2018 ICIMH reminded us of the importance of the microbiome, intestinal integrity and inflammation for optimal health, with Alessio Fasano (*Center for Celiac Research and Treatment*) giving an engaging and informative presentation on chronic inflammatory diseases and their relationship to the health of the gastrointestinal system.

Steven Woolf (*VCU Center on Society and Health*) discussed numerous topics surrounding population health. One of which is the fact that an individual's postcode (location) can influence their life expectancy[1]. Steven Woolf's presentation illustrates why an integrative approach requires practitioners to consider not only one's genetic make-up but also their postcode during a health assessment.

The role of nutrition and the anti-inflammatory diet in paediatric medicine was discussed by Diane Barsky (*Children's Hospital of Philadelphia*). The anti-inflammatory diet, which combines aspects from both the Mediterranean and Asian diets, can be used to improve health and prevent chronic disease[2]. An emphasis was placed on the importance of implementing good eating habits from a young age in order to prevent chronic disease in later life. Practical advice on how to incorporate these changes into a household setting in conjunction with healthy lifestyle habits was also discussed.

Further adding to the theme of mind–body medicine was the symposium on "Interoception, Meditation, and Pain", which explored the ability of the brain to consciously sense the physiological state of the body. The importance of developing interoception skills was discussed in relation to reporting clinical outcomes. The take-home message from this symposium was that meditative practices often employ techniques to heighten and enhance the interoceptive experience, which can be useful when treating patients with chronic pain.

Suzanne Grant and Jennifer Hunter (*National Institute of Complementary Medicine*) spoke about the integrative oncology services available in the Australian health care system[3]. The services were compared with other Western countries including Canada, America and the United Kingdom. Through this comparison, Australia appears to be lagging behind in providing integrative oncology services in the health care system. The presenters further discussed the barriers to overcome to incorporate traditional and complementary medicine into the health care system of Australia.

Poster sessions

The poster sessions provided a unique platform for researchers to engage with other academics and health care practitioners. Attendees were able to ask questions and participate in active discussions with poster presenters in order to gain further insights into the latest integrative health research. This approach allowed for a personalised delivery and provided an atmosphere of collaboration, bridging the gap between health care practitioners and researchers.

The poster presentations followed similar underlying themes to the oral presentations, with a focus on mindfulness practice, non-pharmaceutical pain management and the importance of adopting an integrative approach to health care. A range of different professions were represented among the various posters, including acupuncture, herbal medicine, manual therapies, naturopathy and nutrition. A considerable amount of research on yoga and breathing techniques was also presented during the poster sessions. The comprehensive display of poster presentations, coupled with the unique presentation platform, highlighted the importance of partnerships between different health care practitioners and also the role of integrative health in a wider health care setting.

Australian researchers

Australian researchers and academics from the *University of Technology Sydney*, *Endeavour College of Natural Health* and *Western Sydney University* presented on numerous advancements in the area of integrative medicine, including intestinal permeability in clinical practice, folate absorption, integrative oncology, naturopathic education, Australian complementary and integrative practitioner specialisations and disclosure of complementary medicine use in chronic disease. These research topics highlight a small portion of the research currently being conducted within Australia. The Australian delegates who attended the 2018 ICIMH networked with international researchers and health care practitioners, forming new relationships and strengthening pre-existing partnerships.

Concluding remarks

The major take-home message from the 2018 ICIMH includes the significance and effectiveness of traditional therapies on optimal health and their potential application within the health care system here in Australia. There is also a trend in the research community to validate and understand the mechanism of actions of Ayurveda, traditional Chinese medicine, acupuncture, yoga, tai chi and mindfulness. Furthering the understanding of the 'how' behind traditional and complementary medicine provides the foundations to incorporate these therapies into a larger health care setting.

We look forward to attending the 14th International Congress on Complementary Medicine Research (ICCMR) hosted by Endeavour College of Natural Health in Brisbane 7–10 May 2019. The theme of the Congress is *Pathways and Partnerships*, bringing together researchers and practitioners from an array of disciplines within complementary medicine.

Acknowledgement

BL would like to acknowledge the support of the Australian Government Research Training Program Scholarship.

Conflict of interest

None.

References

1. Woolf SH, Aron L, Chapman DA *et al.* The Health of the States Summary Report: How US States Compare in Health Status and the Factors that Shape Health: Center on Society and Health, Virginia Commonwealth University, 2016.
2. Nowson CA, Service C, Appleton J, Grieger JA. The impact of dietary factors on indices of chronic disease in older people: A systematic review. J Nutr Health Aging 2018;22(2):282–296.
3. Grant SJ, Hunter J, Bensoussan A, Delaney GP. Guidance for establishing an integrative oncology service in the Australian healthcare setting — a discussion paper. Support Care Cancer Feb 2018;26(2):471–481.

Reviews of medical journal articles

Jodie Tester

These abstracts are brief summaries of articles in recent issues of medical journals. Articles selected are of a general nature for the information of practitioners of naturopathy and herbal medicine. A dominant theme is often present throughout the journals, which will be reflected in the reviews.

Dairy consumption and cardiovascular disease and mortality

Dehghan M, Mente A, Rangarajan S *et al.* Association of dairy intake with cardiovascular disease and mortality in 21 countries from five continents (PURE): a prospective cohort study. *Lancet* 2018;392:2288–97

Cardiovascular disease is the leading cause of mortality worldwide, with an estimated 80% of the burden from low- and middle-income countries. Dietary guidelines commonly recommend minimising the consumption of whole-fat dairy products for cardiovascular disease (CVD) prevention as they are a source of saturated fats and presumed to adversely affect blood lipids and increased CVD and mortality. The evidence for this is limited. Furthermore, the beneficial compounds of dairy and dairy fat may also influence health outcomes. The current study aimed to assess the associations between total dairy intake and specific types of dairy products with mortality and major cardiovascular disease.

The Prospective Urban Rural Epidemiology (PURE) study is a large, multinational cohort study of individuals aged 35–70 years, enrolled from 21 countries in five continents. Dietary intakes of dairy products were recorded using country-specific food frequency questionnaires (FFQ). Dairy consumption was considered as whole-fat or low-fat, with products comprised including milk, yoghurt, and cheese. Case-report forms were used to record data for major cardiovascular events and mortality during follow-up. The primary outcome was the composite of mortality or major cardiovascular events (defined as death from cardiovascular causes, non-fatal myocardial infarction, stroke, or heart failure). In the present study, outcome events until 14 July 2018 were included.

A total of 153,220 participants completed the FFQ, of which some were excluded due to implausible energy intakes and/or history of CVD at baseline. In total, 136,384 individuals were included in the study. During the median follow-up period of 9.1 years, 10,576 (7.7%) composite events were recorded (deaths n=6796 and major cardiovascular events n=5855). Total dairy intake was higher in Europe and North America, the middle East, and South America than in other regions. There were inverse associations between total dairy consumption and mortality or major CVD events. A higher intake of total dairy (>2 serves per day compared to no intake) was associated with lower risk of composite outcome, non-cardiovascular mortality, cardiovascular mortality, major CVD, and stroke. The risk of stroke was markedly lower with higher dairy consumption. No association with myocardial infarction was observed. Higher intake of milk and yoghurt was associated with lower risk of the composite outcome, whereas cheese intake as not significantly associated with the composite outcome.

Authors concluded their findings support the dairy product consumption might be beneficial for mortality and CVD, especially stroke. Being a large, multinational cohort study, the findings have relevance across a broader population and are strengthened by the population size. Limitations include the use of FFQ and potential errors in measurement recording or data collection. Furthermore, as an observational study, the findings do not confirm causality. The findings provide clinically relevant information regarding dairy consumption and cardiovascular risk profiles, with the suggestion that consumption of dairy products should not be discouraged.

Low FODMAP diet and infantile colic

Iacovou M, Craig SS, Yelland GW, Barrett JS, Gibson PR, Muir JG. Randomised clinical trial: reducing the intake of dietary FODMAPs of breastfeeding mothers is associated with a greater improvement of the symptoms of infantile colic than for a typical diet. *Ailment Pharmacol Ther* 2018;48:1061–1073

Infantile colic is a common medical condition estimated to affect approximately 14–30% of infants. It is a common cause of emergency department presentations, as well as being associated with cessation of breastfeeding and post-natal depression. Colic is defined as per the Wessel criteria as paroxysmal crying episodes lasting for >3h/d, >3d/week and for ≥3 weeks. While infantile colic spontaneously resolved by about 3–4 months and despite a lack of evidence of therapeutic diets, many breastfeeding mothers will avoid 'windy' foods in attempt to improve colic. The current study aimed to investigate the effects of a maternal low-FODMAP (fermentable, oligosaccharides, disaccharides, monosaccharides, and polyols) diet compared to a typical-Australian diet on infant crying and fussing durations of infants with colic.

The study was a randomised, double-blind, crossover study conducted between 2014 and 2016. Infants who were exclusively breastfed, were aged ≤9 weeks and who met the Wessel criteria for infantile colic were included. Mothers were provided with a 10-day low-FODMAP or typical-Australian diet, then alternated to the other dietary intervention without a washout period. Infants without colic were used as a control group who maintained a habitual diet. The primary endpoint was

change in duration of infant crying and fussing at end of dietary intervention. Secondary endpoints included duration of infant feeding, sleeping and periods of awake and content, maternal wellbeing, and faecal and breast milk indices were measured.

Of the 180 pairs of mothers and colicky infants assessed for eligibility, only 17 were recruited, largely due to not meeting inclusion criteria. Of these, 14 were randomised to treatment and 13 completed both dietary arms and had evaluable data. At baseline, crying-fussy times of infants with colic was 269 minutes, compared to 91 minutes in infants without colic. Crying-fussy duration was significantly reduced by a median of 23% during the low-FODMAP diet compared with 20% during the typical-Australian diet. No difference was observed regarding dietary intervention order. No significant changes in breast milk or infant faecal indices were noted. Maternal anxiety and stress reduced with the typical-Australian diet, but remained stable on the low-FODMAP diet.

The study provides interesting findings with a low-FODMAP diet being associated with a clinically significant improvement in crying-fussing durations of infants with colic compared with a typical-Australian diet. These findings should be considered with recognition of the small sample size; however, given the small duration of intervention, the study suggests there may be a benefit in trialling a low-FODMAP diet in breastfeeding mothers who have an infant with colic. Future research to understand the mechanism of effect, and any wider implications on maternal wellbeing and infant behaviour would be valuable.

ART-conceived adolescents and arterial hypertension

Meister TA, Rimoldi SF, Soria R *et al.* Association of assisted reproductive technologies with arterial hypertension during adolescence. *J Am Coll Cardiol* 2018;72(11):1267–74

The use of assisted reproductive technologies (ART) has grown considerably over the past decades and now accounts for 2–5% of births in developed countries. Over six million persons have been conceived by ART worldwide. There is evidence that ART alters the cardiovascular phenotype in apparently healthy children and mice, including premature vascular ageing. The long-term outcomes of altered cardiovascular phenotype in children are unknown. The authors hypothesised that vascular alterations may persist in ART-conceived adolescents and that hypertension may be the first detectable clinically relevant endpoint.

Five years after the initial study into cardiovascular phenotyping in ART-conceived humans, participants were invited to participate in the follow-up. The current study reassessed vascular function in 54 young, apparently healthy, ART-conceived adolescents and in 43 age- and sex-matched controls and evaluated 24-ambilatory blood pressure (BP). Vascular function, arterial stiffness and structural alterations were also measured and documented. The control group involved school friends of the ART-conceived children, with the rationale to best match for potential confounding factors including socio-economic background, physical activity, and interests in sport. All participants were born at term, had a normal birth weight and none of the pregnancies were complicated by pre-eclampsia.

Body mass index was comparable in ART and control participants, and serum markers were comparable between groups and within normal limits. None of the participants had structural heart disease as assessed by echocardiography. Premature vascular ageing persisted in the ART-conceived adolescents at the five-year follow-up. This seemed to be related to endothelial dysfunction, increased vascular stiffness, and altered vascular wall morphology. Importantly, 24-h systolic as well as diastolic BP was markedly higher in the ART group than control. Of the ART participants, 8 of the 52 met the criteria for diagnosis of arterial hypertension (>130/80mmHG and/or >95th percentile) whereas only one of the control participants fulfilled the criteria. BP variability was also markedly higher in ART-conceived subjects that in control subjects.

ART has allowed for millions of infertile couples to have children; however, the long-term health effects should also be taken into account. Increasing evidence suggests altered cardiovascular phenotypes and the results of this study demonstrates persistent premature vascular ageing into adolescence that progresses to increased arterial BP. The long-term consequences on cardiovascular risk and outcomes remain unknown. The study includes only a relatively small number of ART-conceived adolescents, so whether this is representative of a broader ART-population is unclear. Ongoing follow-up studies, and larger population studies will provide further insight in the future.

Omega-3 supplementation during pregnancy reduces preterm births

Middleton P, Gomersall JC, Gould JF, Shepherd E, Olsen SF, Makrides M. Omega-3 fatty acid addition during pregnancy. *Cochrane Database Syst Rev* 2018; Issue 11:CD003402. Available from: doi:10.1002/14651858.CD003402.pub.3

Preterm birth (born before 37 weeks' gestation) is a leading cause of disability and death in the first five years of life. Omega-3 long-chain polyunsaturated fatty acids (LCPUFA) is a commonly used supplement and dietary component during all stages of life. Previous research has found a higher intake of foods containing omega-3 LCPUFA, such as fish, during pregnancy to be associated with longer gestation and improved perinatal outcomes. The aim of the current review was to assess the effects of omega-3 LCPUFA, as supplements or as dietary additions, during pregnancy on maternal, perinatal, and neonatal outcomes and longer term outcomes for mother and child. A Cochrane review on omega-3 intake during pregnancy was previously undertaken in 2006, and this review is an update on the previous publication.

In August 2018, the authors completed a literature search with 70 randomised controlled trials (RCTs) included in the review, with a total of 19,927 women at low, mixed, or high risk of poor pregnancy outcomes. Studies compared omega-3 interventions as food or supplements to placebo or no intervention. The quality of evidence included varied from high to very low, with overall mixed level of bias. Most trials were conducted in middle-upper or high-income countries, with a large number of trials including women at increased risk of preterm birth.

Preterm birth (<37 weeks) and early preterm birth (<34 weeks) were significantly lower in women who received omega-3 LCPUFA compared with no omega-3. Prolonged gestation (>42 weeks) possibly increased from 1.6% to 2.6% women in those who received omega-3 compared to those who did not. Outcome perinatal data demonstrated a possible reduced risk of perinatal death and possible reduction in neonatal care admissions. A reduced risk of low birthweight babies but possible increase in large-for-gestational age babies was reported for omega-3 supplementation. Insufficient evidence was available to assess maternal outcomes such as post-term induction, maternal serious adverse events, maternal admission into intensive care, or postnatal depression.

The authors concluded that omega-3 LCPUFA supplementation during pregnancy to be an effective strategy for reducing the incidence of preterm birth, noting that it may increase the incidence of post-term pregnancies. A sufficient evidence base has been established for omega-3 supplementation during pregnancy, compared to placebo or no intervention; however, additional studies into varying types of omega-3 PUFA, timing and dose optimisation may further understanding and best clinical practice.

Mediterranean diet and stroke risk

Paterson KE, Myint K, Jennings A *et al.* Mediterranean diet reduces risk of incident stroke in a population with varying cardiovascular disease risk profiles. *Stroke* 2018 49:2415–2420.

Stroke is a leading cause of health burden, contributing to a significant amount of disability and mortality. It is estimated that up to 90% of stroke risk is preventable and attributable to modifiable factors including diet. The Mediterranean diet (MD) is a well-researched diet and has been demonstrated to be beneficial for numerous health outcomes, including stroke prevention. Previous studies on the MD and stroke have focused on high-risk cardiovascular disease (CVD) groups or without stratification of CVD risk factors. The current study aimed to look at the role of MD in a broader population of middle and older aged people, and to better understand whether any associations differ between men and women, the associations between different CVD risk categories, and the individual components of the MD with stroke risk.

The prospective study involved participants from the European Prospective Investigation into Cancer study in the UK, which included a baseline cohort of 23,232 men and women aged 40 to 77 years. Participants completed a health and lifestyle questionnaire and attended a health examination. The adherence to an MD diet was established using seven-day diet diaries completed with consideration of protective items including fruit and nuts, vegetables, fish, moderate alcohol intake, and higher unsaturated: saturated fat intake. Stroke incidence (fatal and non-fatal) was recorded and linked through hospital records and death certification information. Risk of incident stroke was calculated in the whole population, and also stratification by sex and CVD profile, using the Framingham risk score.

Of the 22,232 participants with a mean age of 59.1 years, 2009 incident strokes occurred during the follow-up period of 17.0 ± 4.6 years (mean). Risk of stroke was significantly reduced with greater adherence to the MD across the whole study population. After stratification by sex, these associations remained in women, but not men. In women, even a moderate adherence to MD was associated with a significant reduction in stroke risk, but again, this was not observed in men. Higher adherence to the MD was associated with reduced stroke risk, particularly in participants at high cardiovascular risk as based on Framingham risk score. No significant risk reduction was observed in the subgroup with low cardiovascular risk. Individual components of the MD that were associated with the risk reduction included vegetables and moderate alcohol intake.

Strengths of the study include the prospective design, long-term follow-up, consideration of a number of other stroke risk factors, and being representative of a larger population; however, it should be noted that the population was predominantly wide and may not be generalisable to different populations. Limitations include that dietary assessment was only undertaken at baseline, not accounting for atrial fibrillation as a risk factor.

The study provides interesting findings that are relevant at a population-based level, demonstrating that greater adherence to the MD was associated with lower risk of stroke. When considering sex stratification, only women were found to have MD associated with significantly reduced stroke risk, although the risk for men was down trending. While there are a variety of benefits that gave been associated with the MD, assessing the effect across different sub-groups of population enables the understanding of where it may be of most benefit.

Genetics, lifestyle and stroke risk

Rutten-Jacobs LAC, Larsson SC, Malik R *et al.* Genetic risk, incident stroke, and the benefits of adhering to a healthy lifestyle: cohort study of 306 473 UK Biobank participants. BMJ 2018;363:k4168. Available from: doi:10.1136/bmj.k4168

Stroke is a leading cause of morbidity and mortality worldwide and is associated with a number of risk factors, including diet and lifestyle. A role in genetics has also been established from twin studies, family history studies

and, more recently, genome-wide association studies. Our understanding about whether adhering to a healthy lifestyle could attenuate the effect of genetic background on risk of incident stroke is currently unknown. The present study investigated whether a weighted genetic risk score correlated with a stroke incident in a large population, and whether adherence to a healthy lifestyle influenced this association.

The prospective, population-based cohort study used data from the UK Biobank, which includes participants aged 40–69 years and collects extensive health data from a variety of methods. The main outcomes and exposures of interest in this study were imputed genetic data, incident stroke, and lifestyle factors. Inclusion criteria included all who were classified as white British, without a history of stroke or myocardial infarction, and with complete data on lifestyle. Genetic data was used and a polygenic risk score was derived using data from MEGASTROKE, a large meta-analysis of genome-wide association studies. Adherence to a healthy lifestyle was determined on the basis of four factors: non-smoker, healthy diet, body mass index (BMI) <30kg/m^2, and regular physical exercise. The main outcome was incident of stroke and associations with genetic risk and lifestyle factors.

Complete data was available for 306,473 participants with a median follow-up period of 7.1 years. Over this period, 2077 incident fatal or non-fatal strokes were reported as first incident vascular event or death. The risk of incident stoke was 20% and 35% higher for intermediate and high genetic risk scores, respectively compared to those at low genetic risk. These associations were independent of lifestyle profile. The genetic risk score was significantly associated with systolic blood pressure, diastolic blood pressure, lipid lowering drugs, and diabetes, but not with BMI. Unfavourable lifestyle was defined as one or less of the favourable factors was associated with a 66% increased risk of stroke compared with a favourable lifestyle (defined as three or more favourable factors). A high genetic risk combined with an unfavourable lifestyle profile was associated with more than a twofold increase risk of stroke compared with a low genetic risk and favourable lifestyle.

Strengths of the study include its large sample size, allowing for thorough investigation of genetics and lifestyle factors in detail. Limitations include the narrow range of lifestyle and behavioural changes after baseline not being well accounted for. Furthermore, the study population was limited and may not be generalisable to a broader population. The study provides interesting and relevant findings, highlighting the risk of genetics and lifestyle in stroke risk, and also highlights the potential of healthy lifestyle interventions to reduce risk, across even high genetic risk profile.

Brain age from electroencephalogram of sleep

Sun H, Paizao L, Oliva JT, Goparaju B, Cavalho DZ, van Leeuwen KG *et al*. Brain age from the electroencephalogram of sleep. *Neurobiol Aging* 2019. Available online: doi:10.1016/j.neurobiolaging.2018.10.016

Human sleep patterns are known to undertake robust and predictable changes with age that are observable in overall sleep architecture and electroencephalogram (EEG) oscillations/waveforms. Changes include earlier to sleep and earlier waking, shorter sleep duration, increased sleep fragmentation, and altered rapid eye movement (REM) sleep and non-REM sleep. On EEG, older persons exhibit changes in slow wave during deep sleep, changes in spindle amplitude, density and duration, and changes in the coupling of spindles and slow oscillations. The changes on EEG may be considered as representing a 'brain age' (BA), with the potential to act as an ageing biomarker by comparing BA variation between individuals of the same chronological age. The authors of the current study aimed to develop a model to predict to predict BA based on two large sleep EEG data.

The retrospective analysis included data sets from two large studies: the Massachusetts General Hospital (MGH) sleep lab data set (n=2532); and the Sleep Heart Health Study (SHHS) (n=1974). Participants in the MGH study were aged 18–80 years (median 50), while participants in the SHHS study were aged 40–80 years, with a median age 58 years at first visit and 64 years at second visit. Participants included underwent diagnostic polysomnogram (PSG) and had a clinical diagnosis available from five years before to one year after the PSG from the hospital database. Patients were defined as having significant neurological or psychiatric disease if they had at least one neurological or psychiatric diagnosis during the same period. To predict age, authors extracted a number of features from the sleep EEG and created a model to predict BA comparing against chronological age.

In total, 2532 participants were included in the analysis, of whom 167 had significant neurological or psychiatric disease. The model prediction tool obtained a mean absolute deviation of 7.6 years between BA and chronological age in healthy participants from the MGH data set. Advancing age was tracked using a subset of participants from the SHHS data set that had two visits over average 5.2 years, that was associated with an average 5.4-year increase in BA. The study reported a mean excess BA in participants with significant neurological or psychiatric disease of four years relative to healthy controls. Participants with hypertension and diabetes were also associated with an excess BA.

The authors note that EEG-based BA may offer some potential advantages to assessing aging in that it reflects functional changes, is participant friendly, and is readily repeatable to assess intervention effect. Furthermore, it was suggested the study presents preliminary findings that excess BA to chronological age reflects underlying brain pathology. Further research is required to understand the role of EEG-BA as a potential biomarker of brain age and health.

Reviews of articles on medicinal herbs

Dr Wendy McLean

These abstracts are brief summaries of articles which have appeared in recent issues of herbal medicine journals, some of which may be held in the NHAA library.

Saffron therapy for the treatment of mild/moderate age-related macular degeneration

Broadhead GK, Grigg JR, McCluskey P, Hong T, Schlub T E, Chang AA. Saffron therapy for the treatment of mild/moderate age-related macular degeneration: a randomised clinical trial. *Graefes Arch Clin Exp Ophthalmol* 2018;1–10.

Age-related macular degeneration (AMD) is the most common cause of irreversible visual loss in the developed world. Despite recent advances in the treatment of AMD, therapeutic options are limited. Dietary modification and nutritional augmentation with supplements trialled in the Age Related Eye Diseases Study (AREDS) remain the basis of AMD management. Saffron (*Crocus sativus*) is a well-known Middle Eastern spice that has antioxidant and anti-inflammatory properties that may be effective for AMD. In the current study, the efficacy of saffron for AMD is investigated.

A prospective, randomised, placebo-controlled, double-blind, crossover trial including 100 participants attending a single tertiary retinal clinic was conducted between January 2013 and March 2015. Inclusion criteria were age ≥50 years, moderate severity of AMD (defined as AREDS grade 2 or 3) affecting at least one eye and best corrected visual acuity (BCVA) better than 55. Early Treatment of Diabetic Retinopathy Study (ETDRS). The diagnosis of AMD was confirmed by both dilated retinal examination and dilated retinal fundus photography.

Participants were randomised to one of two arms, either 20 mg saffron or placebo for 90 days. After initial three-month period, all participants crossed over into the other arm and received treatment with the other intervention (saffron or placebo, as appropriate) for a further three months. Participants were requested to maintain their normal diet and continue on any supplements (including AREDS-based therapies). Participants underwent monthly ophthalmic examination for: (a) intraocular pressure (IOP) monitoring via Goldmann Applanation Tonometry, (b) adverse event monitoring, (c) standardised BCVA assessment in ETDRS letters and (d) colour fundus photography. Additionally, at the baseline, three- and six-month visits, participants also underwent flicker perimetry (FP), microperimetry (MP), SD-OCT, FAF, lens grading using AREDS lens assessment criteria and multifocal electroretinogram (mfERG) assessment.

Of the 100 participants, 97 completed both the initial and crossover phases of the study. Fifty-one participants were male (52.6%), the mean age of participants was 73.9 years. Seventy-one participants (73.2%) were already consuming AREDS supplements and were evenly distributed in the saffron and placebo groups. Saffron significantly improved mean BCVA 0.69 letters ($p = 0.001$) and reduced mean-pooled mfERG latency by 0.17 ms ($p = 0.04$) compared to placebo. Amongst participants on AREDS supplements, mean BCVA improved 0.73 letters ($p = 0.006$) and mean pooled mfERG response density improved 2.8% ($p = 0.038$). There was no significant difference in adverse event occurrence between groups ($p > 0.10$).

Oral saffron supplementation modestly improved visual function in participants with AMD, including those using AREDS supplements. Some limitations apply to this study: namely the small sample size, short follow-up period and lack of reliable biomarkers available to test the response of AMD to saffron. However, given the increasing prevalence of AMD, and its propensity to lead to irreversible vision loss, further, longer duration studies are justified to investigate role of saffron in treating AMD.

Effects of *Rhodiola rosea* supplementation on mental performance, physical capacity and oxidative stress biomarkers in healthy men

Jówko E, Sadowski J, Długołęcka B, Gierczuk D, Opaszowski B, Cieśliński I. Effects of *Rhodiola rosea* supplementation on mental performance, physical capacity, and oxidative stress biomarkers in healthy men. *J Sport Health Sci* 2016;7:473–480

The adaptogenic properties of *Rhodiola rosea* (Rhodiola) are attributed to the biologically active substances in its root including flavonoids and phenolic glycosides (salidrosides and rosavins). These compounds potentially enhance physical and mental performance and reduce fatigue. The mechanism of Rhodiola's adaptogenic effect are thought to be due to regulation of the hypothalamus-pituitary-adrenal (HPA) axis and key mediators of the stress response including cortisol. In addition, Rhodiola has demonstrated its ability to scavenge free radicals shown *in vitro* and in animal studies. Strenuous exercise increases oxidative stress in the body, and exercise-induced oxidative stress in blood, liver and skeletal muscle has been shown in animal studies to be reduced by Rhodiola.

The results of studies to date investigating the adaptogenic effects of Rhodiola in humans have been ambiguous, and may in part be due to doses used and the length of clinical trials. The majority of human studies have used low-dose extracts (100–200 mg) while higher doses (≥600 mg) have only been used for short durations

(4–7 days). Therefore, the aim of the current study was to investigate the effects of higher dose Rhodiola (600 mg for 4 weeks) on select parameters of mental performance, physical capacity, hormonal profile and exercise-induced oxidative stress and muscle damage biomarkers in healthy, physically active male students during an examination period.

Twenty-six male physical education students were enrolled in the study. All students were non-smokers, engaged in high-performance sport, did not regularly drink alcohol, did not have recent infections or joint or bone injuries, and had not taken supplements at least two months prior to the study. The recruited students were randomly assigned to the treatment group (200 mg capsules of Rhodiola, 3 times per day) or placebo for a four-week period. There was no significant difference of anthropometric characteristics of participants (age: 20.9 ± 0.2 and 20.5 ± 0.3 years, height: 184.7 ± 2.1 and 182.1 ± 2.2 cm, body mass: 81.1 ± 3.0 and 79.1 ± 2.8 kg; for placebo and Rhodiola groups, respectively) at baseline. The content of salidroside in the Rhodiola tablet was found to average 4.7 mg of salidroside per tablet (1.1% of tablet weight on average). All participants were 100% compliant with the study protocol.

Psychomotor tests (the Vienna Test System) were undertaken prior to and following four-week supplementation. Incremental cycle ergometer tests were also performed before and after four weeks of supplementation. Biochemical markers were measured before and during testing, including oxygen uptake heart rate, lactate threshold, and VO_{2peak}. Blood samples were analysed before and after supplementation to assess biochemical markers of oxidative stress (erythrocyte superoxide dismutase (SOD), lipid hydroperoxides (LHs), total antioxidant capacity (TAC), muscle damage (creatine kinase) and concentrations of hormones (testosterone, cortisol and growth hormone).

In total, 26 healthy males completed the trial. Rhodiola ingestion shortened reaction time and total response time in psychomotor testing, and significantly increased psychomotor performance compared to placebo (16.0% vs. 6.8%; $p<0.05$). No changes in endurance exercise capacity and hormonal profile were observed after Rhodiola ingestion. Rhodiola ingestion significantly increased plasma total antioxidant capacity (1.34±0.06 mmol/L to 1.63±0.06 mmol/L), but did not affect other measured parameters. The researchers concluded that chronic Rhodiola ingestion does not affect physical performance but can improve some psychomotor function (simple and choice reaction time) in young, healthy and physically active men.

Several mechanisms of action have been proposed for improving the cognitive and/or neural performance following Rhodiola ingestion. Results of animal studies suggest the anti-fatigue effect of Rhodiola may be due to effects on cortisol secretion; however, the results of the current study did not show any significant differences in salivary cortisol for Rhodiola and placebo in healthy males after exercise. Some studies also suggest Rhodiola's effect on cognitive performance and endurance exercise capacity may be through its ability to stimulate the synthesis, transport and receptor activity of opioid receptors and peptides such as β-endorphins. The results of the current study showed no changes in exercise capacity biomarkers and do not support this hypothesis; however, the authors acknowledge the time between Rhodiola ingestion and testing may have been too long to affect physical performance. It has been proposed that Rhodiola may have antioxidant properties and while the results of the current study did show an increase in resting plasma TAC, these did not correlate with changes in endogenous antioxidants such as uric acid or albumin, and did not change biomarkers of oxidative stress and muscle damage. Therefore, the authors concluded that Rhodiola extract improved mental performance, but changes did not seem to be related to changes in cortisol or antioxidant activity, and specific mechanisms responsible for these effects still need to be elucidated.

This meta-analysis has several limitations, including the small sample sizes of the included studies, the inclusion of studies with combined therapy (where garlic was administered with conventional medicines or other supplements) and heterogeneity of studies. Further large and well-designed randomised controlled studies are warranted to further investigate these findings.

Green tea as a safe treatment for non-alcoholic fatty liver disease

Mansour-Ghanaei F, Hadi A, Pourmasoumi M, Joukar F, Golpour S, Najafgholizadeh A. Green tea as a safe alternative approach for non-alcoholic fatty liver treatment: A systematic review and meta-analysis of clinical trials. *Phytother Res* 2018;32(10), 1876–1884.

Recent evidence suggests that plant-derived natural products, such as flavonoids and polyphenols, may be promising in non-alcoholic fatty liver disease (NAFLD) management by reducing liver enzymes and acting as an antioxidant and anti-inflammatory. Green tea, derived from leaves of *Camilla sinensis*, is rich in polyphenols including catechin, epicatechin gallate and epigallocatechin-3-gallate and has been used in traditional medicine for the treatment of metabolic disorders, including hypertension, obesity and cardiovascular disease. Clinical trials investigating green tea for NAFLD have produced conflicting results, so the aim of the current meta-analysis was to collate and evaluate the effect of green tea supplementation on metabolic outcomes in patients with NAFLD.

Databases including MEDLINE, Google Scholar and ISI Web of Knowledge were searched. The review included clinical trials that included patients with a history of NAFLD or NASH and evaluated the use of green tea supplementation in any dosage or form. Studies were excluded if green tea was used as an adjuvant or trial periods were less than two weeks. The meta-analysis was completed using a random-effects model.

In total, six studies met the inclusion criteria and were included in qualitative assessment. Four articles were included in the meta-analysis. In the six studies, 256

participants were included with a mean age of 55 years. The duration of randomised controlled trials ranged between 84 and 180 days, and the mean dosage of green tea intervention was approximately 700 mg/day. All studies were parallel controlled trials and were published between 2006 and 2017. NAFLD was diagnosed and confirmed by ultrasonography, liver biopsy and liver enzyme levels. AST and ALT were the main biomarkers in all studies, and biomarkers of insulin resistance and serum lipids were also reported.

The meta-analysis of data from four studies indicated significant effects of green tea supplementation in altering alanine and aspartate blood concentrations. Likewise, a favourable effect of green tea administration was observed on body mass index, triacylglycerol, total cholesterol and low density lipoprotein cholesterol, whereas no significant effect was detected on high-density lipoprotein cholesterol concentrations and homeostasis model assessment of insulin resistance. Based on animal and human studies, the authors propose several therapeutic mechanisms of action for green tea for NAFLD including its antioxidant, hypolipidaemic and anti-inflammatory effects. Green tea was found to be safe, as serious adverse effects were not reported among the studies included. Green tea supplementation up to 1.944 mg/day has been demonstrated to not cause adverse effects.

There were a number of limitations to this meta-analysis, including the small number of eligible trials, and the heterogeneity among trials in regard to study design, dosage and duration of intervention. To better understand the efficacy of green tea for NAFLD, long-term intervention trials with varying dosage supplementation are required.

Saffron *(Crocus sativus)* versus duloxetine for the treatment of patients with fibromyalgia

Shakiba M, Moazen-Zadeh E, Noorbala AA. *et al.* (2018). Saffron (Crocus sativus) versus duloxetine for treatment of patients with fibromyalgia: A randomized double-blind clinical trial. *Avicenna J Phytomed* 2018;8(6), 513.

Fibromyalgia is a chronic debilitating disorder that affects 1–10% of the population, mostly women. The disorder is characterised by chronic pain accompanied by other symptoms including fatigue, sleep disturbances, anxiety/depression, cognitive dysfunction and other somatic symptoms. Major depressive disorder is common in fibromyalgia suffers, affecting 22–90% of these individuals. This is not surprising, given the aberrant neurobiological findings which are common to the pathogenesis of both conditions. Lowered serotonin and norepinephrine levels have been identified as possible mechanisms of pathophysiology of both chronic pain and mental symptoms in fibromyalgia, potentially complicated by decreased antioxidant capacity. Conventional medications, such as the norepinephrine serotonin reuptake inhibitor (NSRI)

duloxetine, are recommended as standard therapy to reduce symptoms in fibromyalgia patients; however, limited efficacy, poor tolerability and low adherence has been reported, suggesting a clinical need for treatment options in addition to pharmaceutical antidepressants. Therefore, interest remains in alternative therapies that are better tolerated and have greater efficacy. Clinical trials have demonstrated that saffron (*Crocus sativus*) has antidepressant and anti-anxiety effects, and animal studies have shown promising effects for chronic pain. The aim of the current study was to compare the efficacy and safety of saffron with duloxetine in treatment of patients with fibromyalgia.

The study was a double-blind randomised control trial that compared saffron against duloxetine at an outpatient rheumatology clinic, Imam Khomeini Hospital, Tehran, Iran. Participants were men and women who were diagnosed with fibromyalgia based on the American College of Rheumatology (ACR) 2010 criteria and had a pain score of over 40 out of 100 based on the visual analogue scale (VAS). Exclusion criteria included co-morbid rheumatologic diseases, inflammatory/infectious/autoimmune arthritis, co-morbid neuropsychiatric disorders except depressive disorders, pain due to traumatic injuries, drug history of duloxetine or saffron use, current use of psychoactive medications, recent use of muscle relaxants, steroids, opioid analgesics, benzodiazepines, anti-epileptics, or injective analgesics. Subjects were given either one capsule of saffron (15 mg) or duloxetine (30 mg) for the first week, and two capsules per day from week 2 to week 8. Primary outcomes included differences between groups for the mean scores of the Hamilton Rating Scale for Depression (HRSD), Fibromyalgia Impact Questionnaire (FIQ), and Brief Pain Inventory (BPI) pain score. Secondary outcomes were changes in pain Visual Analogue Scale (VAS), Hospital Anxiety and Depression (HADS) subscales. Time of treatment interaction effect was assessed for all scales.

Fifty-four patients were recruited into the study, with 27 participants randomly assigned to each treatment group and 23 (per group) completing the trial. Baseline characteristics were comparable between groups, with the exception of marital status. Primary and secondary outcomes were also comparable at baseline. At the end of the trial, all scores had decreased in both groups. There were no significant differences detected between groups for any scales in terms of score changes from baseline to endpoint (p-values: 0.182–0.900) nor in terms of time of treatment interactions (p values: 0.209–0.964). No significant difference was detected between the two arms for the adverse events.

The authors conclude that saffron was equivalent to duloxetine for treating mental and physical symptoms of fibromyalgia. They propose the potential mechanisms of action underlying saffron's effect on fibromyalgia involve its anti-inflammatory and antioxidant effects, as well as its effects on the serotonergic system. There are several limitations to this study, including narrow inclusion criteria, and the short study period. As a comparative effectiveness study, a placebo arm was not included in the study design and future research to elucidate the size of the treatment effect for saffron in people with fibromyalgia is needed. A strength of the study was that it demonstrated equivalency for a low dose of saffron for this expensive herbal medicine. This study provided preliminary evidence on comparable efficacy of saffron and duloxetine in treatment of fibromyalgia. Given the outcomes of the study, future clinical trials that investigate the mechanism of therapeutic effect are warranted, and the safety of saffron as an adjuvant to other pharmaceutical medications should also be investigated.

A comparison between the effects of ginger, pyridoxine (vitamin B6) and placebo for the treatment of the first trimester nausea and vomiting of pregnancy (NVP)

Sharifzadeh F, Kashanian M, Koohpayehzadeh J, Rezaian F, Sheikhansari N, Eshraghi N. A comparison between the effects of ginger, pyridoxine (vitamin B6) and placebo for the treatment of the first trimester nausea and vomiting of pregnancy (NVP). *J Matern Fetal Neonatal Med* 2018;31(19), 2509–2514.

Sixty to eighty per cent of women experience nausea and vomiting of pregnancy (NVP) or morning sickness in the first half of pregnancy. Although NVP has been linked to better pregnancy outcomes, it can have significant impacts on the social and daily functioning of women, and 10% of pregnant women require medication. The cause of NVP is still unknown but the relationship between human chorionic gonadotropin (HCG) and oestrogen has been proposed and pregnant women's preferences for natural medicines have been reported. Accordingly, interest is high for natural therapies that provide relief for NVP and are safe and well tolerated. Two natural interventions previously explored include vitamin B6 and ginger. Ginger is a competitive antagonist for H3 receptors and it has clinical effect a few days after treatment for approximately 80% of women. The aim of the current study was to compare the effects of ginger to vitamin B6 and placebo for the treatment of NVP.

The study was a triple-blind random and consecutive control trial including women who were 6–16 weeks' pregnant, had mild to moderate NVP and presenting to casualty from September 2012 to January 2015 to the Akbarabadi Teaching Hospital, Tehran, Iran. Exclusion criteria included NVP needing hospitalisation, lack of acceptance of herbal medicine, gastrointestinal or other pathological disorders, or allergy to ginger or vitamin B6. Subjects were allocated to one of three groups: ginger (500 mg), B6 (40 mg) or placebo twice daily for four days. The primary endpoint was changes in the Rhodes Score at four days.

In total, 77 women completed the study (28 in the ginger group, 26 in the Vitamin B6 group and 23 in the placebo group). At the beginning of the trial, the women did not show significant difference in age, parity, gestational age, level of education or severity of symptoms. An endpoint, a statistically significant improvement, was found for both B6 and ginger compared to placebo for reducing intensity frequency and distress of nausea, vomiting and

retching. In a per group analysis of change over time, ginger was more effective for reducing severity of nausea and amount of vomiting, and B6 was more effective for reducing retching and distress of vomiting. A limitation of this study was that the method of allocating participants to the intervention groups (B6, ginger and placebo) was not clearly explained. The researchers state group allocation was conducted using a mixture of consecutive assignment and randomisation. Consecutive assignment means that the confounding characteristics of the participants, such as parity, term of pregnancy, age, reproductive history were not controlled and that the outcomes may not be explained by the treatments alone.

In previous systematic reviews, ginger has been found to be an effective and safe medication to reduce the frequency and severity of nausea and vomiting in NVP. However, it is important to note that a maximum safe dose in pregnancy is not clear, and while the current and previous research indicates it is as effective as B6 in reducing symptoms of NVP, more studies are required. Future research is required to assess optimal dose for each symptom of NVP.

Effect of garlic supplementation on serum C-reactive protein

Taghizadeh M, Hamedifard Z, Jafarnejad S. Effect of garlic supplementation on serum C-reactive protein level: A systematic review and meta-analysis of randomized controlled trials. *Phytother Res* 2018.

Garlic is one of the oldest known medicinal plants, and it contains several bioactive constituents, such as allicin, which are known to have anticoagulant, antithrombotic, antioxidant, hypoglycemic, hypocholesterolaemic and hypotensive effects. In addition, it is shown that garlic has antimicrobial, anticarcinogenic, anti-asthmatic, immunomodulatory and prebiotic effects. Allcin and other sulphur-containing compounds are believed to be responsible for the anti-atherosclerotic effects of garlic, while the non-sulphur-containing compounds, including polyphenols, are believed to have antioxidant properties that may provide benefit for inflammatory and metabolic disorders. The aim of the review was to assess the anti-inflammatory benefits of different forms of garlic using C-reactive protein (CRP) levels.

Databases including Scopus, PubMed, Cochrane Library and Google Scholar were searched through to January 2018. The review included randomised controlled trials that investigated garlic in any form (raw, aged, powder, tablet and capsule) and CRP. Studies were excluded if they were not randomised or did not have a control group, lacked enough data for statistical pooling or did not report CRP values. Predefined subgroup analyses were conducted including duration of study, intervention dose, baseline CRP and quality of studies. Weighted random effect meta-regressions were performed to determine the effects of potential moderators like garlic dosage and baseline CRP. The funnel plot test was used to assess publication bias.

In total, nine studies were included for meta-analysis, ranging from 2 to 48 weeks, with a median of 12 weeks. Sample size ranged from five to 28 subjects, with a total sample size of 363 (183 in intervention groups and 180 in control groups). Garlic dosing ranged from 250 to 3,600 mg/day with the median of 2,100 mg/day. The age of participants ranged from 25 to 75 years, and five out of nine studies involved patients with chronic disease (TD2M, or coronary artery disease). Baseline CRP varied from 0.21 to 6.7 mg/L with a median of 2.4 mg/L. Six studies were classified as high methodological quality.

Compared with the controls, garlic intake significantly reduced the concentrations of serum CRP by 0.8 mg/L (p=0.02) with the evidence of heterogeneity among studies. Heterogeneity included variations in interventions (aged garlic powder, allicin with or without other co-supplements such as arginine, B12, B6, or CoQ10), and subject characteristics (for example, race, lifestyle, chronic disease). Subgroup and meta-regression analyses revealed supplementation with garlic produced a significant reduction of serum CRP among subjects with high supplemental doses or higher baseline CRP. Garlic significantly lowered CRP by 0.82 mg/L ($p < 0.001$) among studies with a daily garlic dose ≥1,200 mg/day and by 2.44 mg/L ($p = 0.002$) among studies with baseline CRP ≥2 mg/L, meaning trials with higher dose garlic or higher baseline CRP showed a significant decrease in mean effect sizes of serum CRP, compared with shorter periods or subjects with lower serum CRP. The latter finding is clinically significant, since it has been shown that in patients with intermediate cardiovascular disease risk, hsCRP levels ≥2 mg/L may reclassify patients into the high risk category. The mechanism by which garlic compounds exert anti-inflammatory effects is not completely known, but may be related to their ability to attenuate the production of inflammatory cytokines via the NF-κβ pathway.

This meta-analysis has several limitations including the small sample sizes of the included studies, the inclusion of studies with combined therapy (where garlic was administered with conventional medicines or other supplements) and heterogeneity of studies. Further large and well-designed randomised controlled studies are warranted to further investigate these findings.

Effects of *Curcumin longa* on the metabolic parameters of fatty liver disease

Wei Z, Liu N, Tantai X *et al.* The effects of curcumin on the metabolic parameters of non-alcoholic fatty liver disease: a meta-analysis of randomized controlled trials. *Hepatol Int* 2018;1–12.

Non-alcoholic fatty liver disease (NAFLD) is a chronic disease that affects approximately 30% of the adult population in Western countries. The disease results from accumulation of fat around liver cells and is associated with diabetes, dyslipidaemia, obesity, cardiovascular disease and metabolic syndrome. The first-line therapy for NAFLD is lifestyle intervention, with weight loss being the key intervention. However, less than 10% of patients with NAFLD achieve the weight loss necessary to induce regression of fibrosis. Curcumin is a natural polyphenol that has antioxidant, anti-inflammatory, antimicrobial and anti-carcinogenic effects. Several small clinical trials have demonstrated positive outcomes and a potential benefit for people with NAFLD; however, the precise mechanisms of action have not yet been established. The aim of the current meta-analysis was to assess efficacy and safety of curcumin in patients with NAFLD.

Databases including PubMed, EMBASE and the Cochrane Library were searched up to March 2018. The review included studies that were randomised, placebo-controlled, with patients aged over 18 years, and NAFLD (grades 1 to 3) assessed via liver ultrasound. Studies that compared curcumin to placebo were included. The Cochrane risk bias tool was used to evaluate study quality. Efficacy was calculated using changes in biomarkers including serum lipid biomarkers (LDL-C, total cholesterol, HDL-C, and triglycerides) and metabolic biomarkers (HbA1c, insulin and HOMA-IR), liver function biomarkers (ALT and AST) and weight. The meta-analysis was completed using a random effects model.

In total, four RCTs were included with a total of 229 NAFLD patients (115 curcumin and 114 placebo). The number of participants ranged from 20 to 87 and the dosing of curcumin ranged from 500–1,000 mg/day curcumin and 3,000 mg/day turmeric). Duration of supplementation ranged from eight weeks to six months. All studies were parallel group and had high quality according to the Cochrane risk bias tool.

When the data was pooled, curcumin was found more likely to lower LDL-C, triglycerides, fasting blood glucose, HOM-IR, weight and AST levels compared with placebo, and the difference was statistically significant. One RCT reported adverse events in the curcumin group.

The authors propose that curcumin may have multiple mechanisms of action that make it beneficial for treatment of NAFLD, including its ability to decrease expression of HMG-CoA reductase, and to decrease lipogenesis and adipogenesis. Furthermore, curcumin may improve insulin sensitivity, and decrease production of inflammatory cytokines, thereby reducing hepatic apoptosis. Another potential mechanism is via curcumin's role on gut microbiota. The oral bioavailability of curcumin is very low, and curcumin reaches the gut almost unaltered, where it thereby exerts prebiotic effects and could potentially improve metabolic parameters of NAFLD.

This meta-analysis provides an overview into the use of curcumin for NAFLD. While the authors recognise that curcumin may have potential benefits on metabolic parameters of NAFLD, it is noted that there are several limitations to this study. Limitations include small sample size, population characteristics, and curcumin dose and duration of supplementation in the studies included. More RCTs with larger sample sizes are required to confirm findings of this meta-analysis.

CPE points

The *AJHNM*-based CPE questionnaire system is a voluntary system designed to assist members in the accumulation of NHAA CPE points. Questions are divided into the appropriate subject categories (herbal medicine and medical science) and each question refers to an article in this issue of the *Australian Journal of Herbal and Naturopathic Medicine*. Points accumulated through completion of these questions should be recorded in the NHAA CPE diary. Each completed question is worth one mark in the relevant category. Your completed CPE diary should be returned with your membership renewal at the end of the calendar year. For further information, please see the NHAA CPE Members' Manual on the NHAA website www.nhaa.org.au.

CPD — MedJourn

With reference to the study investigating the role of the Mediterranean diet (MD) and stroke risk, which of the following statements is incorrect?:

- Risk of stroke was significantly reduced with greater adherence to the MD across the whole study population.
- Moderate adherence to MD was associated with a significant reduction in stroke risk in women but not men.
- A significant risk reduction was observed in both high and low cardiovascular risk subgroups.
- Vegetable and moderate alcohol intake were associated with the stroke risk reduction.

With reference to the Cochrane review on omega-3 supplementation during pregnancy, which of the following statements is correct?:

- Omega-3 supplementation was associated with reduced preterm birth (<37 weeks) and early preterm birth (<34 weeks).
- Increased risk of low birthweight babies was reported for omega-3 supplementation.
- Perinatal outcomes revealed possible reduced risk of perinatal death and reduction in neonatal care admissions.
- Omega-3 supplementation was possibly associated with prolonged gestation (>42 weeks).

Regarding the follow-up study of ART-conceived adolescents and arterial hypertension, which of the follow statements is incorrect?:

- 24-hr systolic BP and diastolic BP was markedly higher in the ART group than control.
- Eight of the ART adolescents met the criteria for diagnosis of arterial hypertension.
- Premature vascular ageing persisted in the ART-conceived adolescents at the five-year follow-up.
- Endothelial dysfunction, decreased vascular stiffness, and structural heart disease may be related to the vascular ageing.

MedPlant

Regarding the study investigating ginger and vitamin B6 for treatment of the first trimester nausea and vomiting of pregnancy (NVP), which of the following statements is correct?:

- After four days of supplementation, ginger was no more effective than placebo for reducing severity of nausea.
- After one day, B6 was more effective than placebo for reducing severity of nausea and vomiting.
- After four days, ginger was more effective than placebo for reducing retching and distress of vomiting.
- After four days, ginger was more effective for reducing severity of nausea and amount of vomiting.

Regarding the study comparing the efficacy of saffron with duloxetine for the treatment of fibromyalgia, which of the following is true?:

- At the end of the trial, HRSD scores had only decreased in the duloxetine group.
- Saffron was more effective than placebo for reducing FIQ and BPI scores.
- Saffron was equivalent to duloxetine for improved mental and physical symptoms associated with fibromyalgia.
- There were statistically significant differences in adverse effects detected in the two treatment arms.

With reference to the study investigating *Rhodiola rosea* supplementation on mental performance, physical capacity and oxidative stress biomarkers in healthy men, which is correct?:

- Rhodiola ingestion significantly increased plasma total antioxidant capacity.
- Rhodiola ingestion did not improve some psychomotor test scores.
- The current study showed significant changes in salivary cortisol in healthy individuals are exercise.
- The results of the current study demonstrate that rhodiola ingestion significantly improves physical performance in young men.

With regard to the meta-analysis studying the effect of garlic supplementation on serum C-reactive protein, which of the following is true?:

- Subgroup analysis demonstrated that garlic did not significantly lower CRP in studies using ≥1,200 mg/day.
- Eleven studies were included in the meta-analysis, with study periods ranging from 3 to 41 weeks.
- The median garlic dose used in studies was 1,200 mg/day.
- Compared with the controls, garlic intake significantly reduced serum concentrations of CRP by 0.8 mg/L.

With regard to the meta-analysis studying the effects of curcumin on the metabolic parameters of fatty liver disease, which is correct?:

- Curcumin was more likely to lower LDL-C, triglycerides, fasting blood glucose, HOM-IR, weight and AST levels compared with placebo.
- Five RCTs were included in the meta-analysis.
- Dosing of curcumin ranged from 200 mg/day to 3,000 mg/day.
- A random effects model was not used to complete the meta-analysis.

In the study investigating the efficacy of saffron for macular degeneration, which is not correct?:

- Ninety-seven participants completed the trial.
- Mean BCVA improved 0.69 letters (p=0.001) and mean-pooled mfERG latency reduced 0.17 ms (p=0.04) on saffron compared to placebo.
- Mean BCVA improved 0.73 letters (p=0.006) and mean pooled mfERG response density improved 2.8% (p=0.038).
- There were significant differences in adverse effects occurrence between treatment arms.

In the meta-analysis reviewing efficacy and safety of green tea for NAFLD, which is correct?:

- Six studies with 256 participants were included in the qualitative study.
- Green tea produced a significant effect on high-density lipoprotein cholesterol concentrations.
- NAFLD diagnosis in the studies was not confirmed by ultrasonography.
- Green tea produced significant effects on alanine and aspartate blood concentrations.

MedJourn and MedPlant References

Broadhead GK *et al.* Saffron therapy for the treatment of mild/moderate age-related macular degeneration: a randomised clinical trial. Graefes Arch Clin Exp Ophthalmol 2018;1–10.

Dehghan M, Mente A, Rangarajan S *et al.* Association of dairy intake with cardiovascular disease and mortality in 21 countries from five continents (PURE): a prospective cohort study. Lancet 2018;392:2288–97

Iacovou M, Craig SS, Yelland GW, Barrett JS, Gibson PR, Muir JG. Randomised clinical trial: reducing the intake of dietary FODMAPs of breastfeeding mothers is associated with a greater improvement of the symptoms of infantile colic than for a typical diet. Ailment Pharmacol Ther 2018;48:1061–1073

Jówko E e*t al.* Effects of Rhodiola rosea supplementation on mental performance, physical capacity, and oxidative stress biomarkers in healthy men. J Sport Health Sci 2016.

Mansour-Ghanaei F *et al.* Green tea as a safe alternative approach for non-alcoholic fatty liver treatment: A systematic review and meta-analysis of clinical trials. Phytother Res 2018;32(10):1876–1884.

Meister TA, Rimoldi SF, Soria R *et al.* Association of assisted reproductive technologies with arterial hypertension during adolescence. J Am Coll Cardiol 2018;72(11):1267–74

Middleton P, Gomersall JC, Gould JF, Shepherd E, Olsen SF, Makrides M. Omega-3 fatty acid addition during pregnancy. Cochrane Database Syst Rev 2018; Issue 11:CD003402. Available from: doi:10.1002/14651858.CD003402.pub.3

Rutten-Jacobs LAC, Larsson SC, Malik R *et al.* Genetic risk, incident stroke, and the benefits of adhering to a healthy lifestyle: cohort study of 306 473 UK Biobank participants. BMJ 2018;363:k4168. Available from: doi:10.1136/bmj.k4168

Shakiba M *et al.* Saffron (Crocus sativus) versus duloxetine for treatment of patients with fibromyalgia: A randomized double-blind clinical trial. Avicenna J Phytomed 2018;8(6):513.

Sharifzadeh F *et al.* A comparison between the effects of ginger, pyridoxine (vitamin B6) and placebo for the treatment of the first trimester nausea and vomiting of pregnancy (NVP). J Matern Fetal Neonatal Med 2017;1–6.

Sun H, Paizao L, Oliva JT *et al.* Brain age from the electroencephalogram of sleep. Neurobiol Aging 2019. Available online: doi:10.1016/j.neurobiolaging.2018.10.016

Taghizadeh M, Hamedifard Z, Jafarnejad S. Effect of garlic supplementation on serum C-reactive protein level: A systematic review and meta-analysis of randomized controlled trials. Phytother Res 2018.

Wei Z *et al*. The effects of curcumin on the metabolic parameters of non-alcoholic fatty liver disease: a meta-analysis of randomized controlled trials. Hepatol Int 2018;1–12.

SAVE THE DATE

BioCeuticals

7th Research Symposium
3-5 May 2019

HILTON SYDNEY

MOOD, GENES & COGNITION

JOIN US AT THE 7TH BIOCEUTICALS RESEARCH SYMPOSIUM, A THREE-DAY MASTERCLASS HIGHLIGHTING THE FUTURE OF PERSONALISED MEDICINE FOCUSING ON MOOD, GENES AND COGNITION:

Save $100 WITH OUR Early Bird RATE ENDING 10 FEBRUARY 2019

CONFIRMED INTERNATIONAL SPEAKERS

Dr Dale E Bredesen, (USA) Professor of Neurology

Dr Dale E Bredesen is internationally recognised as an expert in the mechanisms of neurodegenerative diseases such as Alzheimer's disease. Dr Bredesen's research has led to new insight that explains the erosion of memory seen in Alzheimer's, and has opened the door to a new therapeutic approach. He has found evidence that Alzheimer's disease stems from an imbalance in nerve cell signalling. Dr Bredesen will cover the mechanisms underlying the neurodegenerative process, and the translation of this knowledge into effective therapeutics for Alzheimer's disease and other neurodegenerative conditions.

Dr Jay Lombard, (USA) Internationally Acclaimed Neurologist

Dr Jay Lombard is an internationally acclaimed neurologist, author, and keynote speaker who creates solutions for brain health and intractable neurological disorders. Dr Lombard integrates biological, psychological, and existential components in his holistic treatment approach.
He is the co-founder and creator of Genomind, a precision medicine company utilising genetic testing to improve neuropsychiatric conditions, including Alzheimer's, autism, and depression.
Founder of TedMed, Jay Walker, describes Dr Lombard as "part Freud, part Sherlock Holmes."
Dr Lombard's discoveries have been regarded by key opinion leaders as fundamentally shifting the paradigm of psychiatric medicine.

Ms Amanda Archibald, (USA) Registered Dietitian, Nutritionist and Public Health Advocate

Ms Amanda Archibald is a registered dietitian, nutritionist and public health advocate who has pioneered the combination of the science of nutrigenomics with the culinary arts. Amanda will draw particular attention to the mechanisms of food-gene relationships related to the core issues known to affect long-term health – inflammation, oxidative stress, blood sugar and fats, and gut health – while emphatically encouraging the discovery of taste and culinary treasure in the process. She will present an empowering new system for choosing, preparing and cooking ingredients for optimal long-term health based on a language recognised by DNA.

Dr Brandon Brock, (USA) Board Certified Chiropractic Neurologist, Family Nurse Practitioner and Nutritionist

Dr Brandon Brock has a passion for providing easy to comprehend skills that can be utilised in a clinical setting. He received the most outstanding functional neurology teacher of the year award from the ACA Council of Neurology for five years and twice from IAFNR (International Association of Functional Neurology and Rehabilitation). Dr Brock will present his unique and integrated understanding of functional neurology blending nutrition, pharmacology, immunology and endocrinology to provide a comprehensive and multi-perspective approach to clinical presentations.

FIND OUT MORE Take advantage of limited time **Early Bird** rates! Go to:
bioceuticals.com.au/education/events