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Editorial

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Next year will see 100 editions and a quarter of a century of publication of the *Australian Journal of Herbal Medicine*. This in itself is not exceptional for an association that will be turning 100 years old in the next decade, but it does provide a fascinating history of the changes that have occurred in the profession over the last 25 years. The most significant changes have been in government regulation and education.

In 1988 the Commonwealth Department of Community Health gave notice that it intended to introduce new legislation to nationally regulate all therapeutic goods, including herbal medicines. At the time it was considered this might result in drastic consequences for herbalists with each medicine dispensed being subject to an annual registration fee of $350, herbalists making their own medicines being subject to an annual license fee of $6,250 and practitioner manufacturing principles having to conform to the Australian Code of Good Manufacturing Practice. Submissions were sent to Canberra arguing that herbalists should be exempt from the legislation as they had previously been in the Therapeutic Goods and Cosmetics Act of 1972. In March 1989 the Department succumbed and advised that practitioners had been made exempt and the 1972 Act would stand.

The practitioner was still able to make their own medicine but would they still have access to all the herbs? In 1992 the Commonwealth Drugs and Poisons Scheduling Standing Committee (now National Drugs and Poisons Schedule Committee) scheduled a number of herbs containing pyrrolizidine alkaloids, restricting the use of such herbs as *Borago off, Pulmonaria* spp*, Senecio* spp*, Tussilago farfara* and *Symphytum off* (which, based on lack of scientific evidence, was later rescheduled to allow its use topically). Many more herbs are listed on the different schedules including *Gelsemium, Convallaria, Ammi visnaga, Rauwolfia, Lobelia, Arnica* and of course *Digitalis* and *Ephedra.*

In 1997, just 15 years ago, the line between practitioner and manufacturer was still blurred. The Traditional Medicines Evaluation Committee (later to become the Complementary Medicines Evaluation Committee) was formed to deal with traditional medicines. This resulted in an outburst from the Australian Medical Association declaring herbs as drugs are potentially dangerous and placing greater restrictions on access to herbs and herbal material by herbalists.

With medicines at least partially now under control, the security of the future of the profession was the big topic, as it remains today. Government registration has been on the agenda since 1925 but in 1989 it was recognised that it would depend on more than just government registration to secure its future; the profession would need to continiually assess and re-assess its standards, aims, goals and policies. This has progressed significantly over the last 25 years and continues to do so.

The educational standards in Australia for qualified herbal and naturopathic practitioners have gone from the weekend workshop to certificate, diploma and degree level, with many sets of competencies in between. The Australian National Training Authority has a Health Training Package (HTP) for an Advanced Diploma of Western Herbal Medicine. The NHAA has a comprehensive set of curriculum training guidelines incorporating the HTP and in many areas exceeding those standards laid down in the HTP by including traditional knowledge and concepts such as history and philosophy, which are integral to herbal medicine education.

All this recent work is well documented, while much of the earlier history of herbalists and herbal medicine in Australia has been written and archived for posterity. In 1989 the first female president of the NHAA, Robyn Kirby, was instrumental in setting up a *History of Herbalism in Australia* exhibition in the historical Rocks area of Sydney. During its 3 weeks of display some 600 people from 18 different countries visited the exhibition. The majority of the material has since been stored in the NSW State Library for safe keeping. Sue Evans continued this work in her 2009 publication *Challenge, tension and possibility: an exploration into contemporary western herbal medicine in Australia.*

This is my 70th and final edition of the journal as editor, ending a journey of 17 years that has seen much growth in the quality, content and professionalism of the journal. I look forward to watching the progression not only of the journal but of the association and the profession at large. With such significant changes happening globally we are ensured of interesting times ahead!

Commentary

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This paper provides a reply to the Wong (AJHM 24:3;97-9) critique of the Rapaport et al (2011) study which found no significant difference between St John's wort, citalopram and placebo in treating minor depression. Additionally we address the reasons why St John's wort and antidepressant depression studies are increasingly not demonstrating efficacy over placebo, and provide potential solutions.

Are St John's wort and SSRI antidepressants really ineffective in depression?

We were pleased to see a review by Wong in *Aust J Herbal Med* 24:3;97 (2012) appraising the quality of the most recent St John's wort (SJW) study by Rapaport et al (2011). The author raises some interesting points which deserve further exploration. Thus we provide a reply to the Wong paper in order to 1) respond to the critique of the Rapaport study, 2) address the reasons why SJW and antidepressant depression studies are increasingly not demonstrating efficacy over placebo, and 3) summarise the results of our new data exploring response patterns of SJW compared with placebo and antidepressants.

First it should be clarified that the search methods outlined by Wong was for 'major depression' and clearly the study reviewed concerned 'minor depression'. Further Wong chose to review the Rapaport et al (2011) paper stating that the Sarris, Fava, Schweitzer and Mischoulon (2012) was a reanalysis of SJW data and not an 'original' paper. This is incorrect as the data presented in this paper were data that had not been previously presented before, i.e. continuation data.

The critiqued Rapaport study revealed that SJW was no more effective than placebo (and the antidepressant citalopram) in treating minor depression in a sample of 73 participants. This is potentially due to an overinflated placebo response and also the likelihood of 'minor' depression not being effectively treated by pharmacological interventions (Fournier 2010). Regardless, the assertion by Wong that 'the study was not conducted with a superiority of research due to several limitations in the research methods' is highly questionable. The study design was methodologically sound and the SJW preparation used had been thoroughly scrutinised and vetted by NCCAM prior to awarding of funding. Regarding the sample size, the study was powered to assess the difference between both active treatments and placebo. The main flaw of the report, we recognise, was the failure to detail the standardisation of the SJW extract used. Several recent studies comparing SJW against placebo or selective serotonin reuptake inhibitors (SSRIs) have yielded equivocal results, with neither SJW nor the active drug separating from placebo.

So does this mean that SJW and antidepressants are not effective in reducing depression, or is there another explanation? It should be noted that in antidepressant randomised controlled trials (RCTs) of clinical depression, the placebo response rate in depressive disorders has been gradually increasing by approximately 7% per decade over the past four decades (Walsh 2002). In recent RCTs the average placebo response rate in major depressive disorder (MDD) trials may in fact be as high as 35-45%, with the average drug-placebo difference being only a modest 11-20% (Khan 2002, Yang 2005). This pattern is also apparent with SJW, with a meta-analysis by Werneke, Horn and Taylor (2004) showing, compared with previous meta-analyses, a reduced relative risk for response to SJW in treating MDD of 1.73 (CI 95% = 1.40, 2.14).

So why is a high placebo response rate occurring (and thus obfuscating the true effect of medications) and what are the potential solutions? Possible contributing factors to this problem raised by Fava and colleagues (2003) include study design flaws, diagnostic misclassification and inclusion or exclusion criteria issues, outcome measures with poor sensitivity, measurement and data entry errors, participants' depression oscillating and usually getting better due to 'regression to mean', and high attrition rates reducing statistical power. Another simple explanation concerns a potentially modifiable issue: due to increased competition to recruit participants and the sometimes inflated financial compensation to both researchers and patients, the study's 'strict' inclusion criteria may not be adhered to. Participants may be recruited with 'minor depression', potentially occurring

due to psychosocial factors, and these participants may not be the ideal candidates for a biological intervention.

Proposed solutions have focused on selecting patients with particular characteristics such as increased illness severity or non-transient endogenous depression, use of specific sensitive outcome measures and modifying trial length and design, for example with placebo run-in periods.

Whilst a common anecdotal belief concerning antidepressant response time purports that 'medications take several weeks or months to work', the data do not support this. In fact, among trials that ultimately detect a difference between the active medication and placebo, a statistically significant difference is usually apparent as early as week 2, and almost always by week 4 of treatment (Posternak 2005; Walsh 2002). Interestingly Posternak and Zimmerman (2005) have proposed nearly identical response patterns for both placebo and antidepressants. In reviewing 76 double blind RCTs they found that drug-placebo differences were most pronounced during the first two weeks of treatment and diminished in a stepwise fashion thereafter. Critically more than 80% of the improvement on placebo was noted to occur, on average, in the first half of 6 week trials. Thus if patients experience an antidepressant effect from medication, this occurs shortly after initiation of the treatment, and in studies using placebo this perceived antidepressant response may also occur in the first few weeks.

While the pattern of antidepressant and placebo response has been studied, this has not yet occurred with SJW. In view of this we examined post hoc the conditional probability (the probability of an event occurring assuming another event has already occurred) of response to SJW compared with placebo and antidepressants via an analysis of a pooled sample of MDD patients from two 8 week RCTs comparing SSRIs versus SJW (The Hypericum Depression Trial Study Group study 2002 and the Fava et al study 2005). From a pooled total of 483 patients included in the intent-to-treat analyses, for the 149 patients who were classified as responders (≥50% reduction on Hamilton Depression Rating Scale) at week 8, the conditional probabilities of early partial response revealed that in participants who ultimately respond to SJW, an initial partial response occurs early (Sarris et al in submission). This pattern is mirrored for placebo and antidepressant medication.

Another recent reanalysis of the Hypericum Depression Trial Study group study by Chen et al (2011) found that patient belief with regard to what treatment they had received appeared to have a significant impact on overall response, independent of actual treatment received. Further comparative study of SJW and placebo response patterns should clarify the most effective way to use SJW for mood disorders.

The take home messages from our commentary is that future depression studies need to have careful inclusion and exclusion criteria which are adhered to, and that clinicians prescribing SJW are advised to be aware that if no response occurs within one month of treatment it is unlikely the individual will respond to SJW. In this case reconsideration of the treatment strategy is needed. This may involve clinicians taking more assertive measures by switching interventions, augmenting treatment or referring appropriately.

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Phytoestrogens and breast cancer: friends or foes?

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Phytoestrogens

Phytoestrogens, which are widely distributed in plants, are structurally similar to mammalian estrogens and can thus bind weakly to estrogen receptors. The three major classes of phytoestrogens are the isoflavones, lignans and coumestans. The best known phytoestrogens derived from the diet are genistein, daidzein and glycitein which are the isoflavones found in soy beans, particularly in ferments of soy. They almost exclusively occur as glycosidic conjugates and in unconjugated or conjugated forms in most soy protein products in high concentrations. Smaller amounts have been found in other beans and in some vegetables and fruits (Kurzer 1997).

The interest in phytoestrogens derives from the observation that hormonally dependent cancers are not highly prevalent in Asian countries and amongst vegetarians when compared with data collected in Western populations (Adlercreutz 1999). These diets have consistently shown low plasma concentrations of insulin and IGF1, two potent mitogens, and high concentrations of IGF1 binding proteins (IGFBP). This combination of low IGF1 and high IGFBP is known to reduce breast cancer risk in premenopausal women (Giovannucci 1999). These findings have suggested that phytoestrogens may have a preventive effect against various cancers.

The three most estrogenic phytoestrogens are genistein, coumestrol (a coumestan) and equol. These phytochemicals belong to a larger class of polyphenols characterised by non-steroidal structures similar to mammalian estrogens, such as estradiol, and have estrogenic properties. Given their low molecular weight and stable structure, phytoestrogens are able to interact with estrogenic enzymes and receptors (Adlercreutz 2003). This interaction allows phytoestrogens to bind and alter the structure of estrogen receptors and alter transcription (Santii 1998). Most of all phytoestrogens inhibit the enzyme needed for hormone conversion, which may reduce cancer by lowering the biological activity of sex hormones in specific tissues (Adlercreutz 2003). Evidence that phytoestrogens can mimic endogenous estrogens has raised concerns about their effects on cell growth and proliferation (Bath 2002).

To resolve the dilemma regarding the potential benefits or harmful effects of phytoestrogens in breast cancer development, numerous studies have attempted to characterise the estrogenic and growth stimulatory actions of phytoestrogens. Most of these studies have been carried out in breast cancer cell breast lines and the result of these studies points to the unique quality of phytoestrogens to bind to estrogen receptors ER-alpha and ER-beta and modulate the activity of ER signalling cascades and transcription factors, thereby exerting an inhibitory effect on cell proliferation and survival. Many phytoestrogens display a somewhat higher affinity for ER-β compared with ER-α (Cowly 2006).

The isoflavones in particular have shown the ability to reduce breast cancer risk by affecting the endogenous sex hormone concentration, influencing cancer growth through effects on estrogen receptors, inhibition of tyrosine and inhibition of angiogenesis (Adlercreutz 2004). By reducing the concentration of circulating free 'active' hormones, the isoflavones play a significant role in inhibiting the progression of breast cancer.

Highly reactive oxygen species have been shown to play a role in the development of cancer and several studies have shown that phytoestrogens can act as antioxidants, although the concentrations at which antioxidant activity is observed are unlikely to be reached through dietary means (Harper 1999). There is growing evidence that phytoestrogens could have a protective effect on the initiation or progression of breast cancer by inhibiting the local production of estrogens from circulating precursors in breast tissue. Once ingested, phytoestrogens interact with many of the same enzymes as endogenous estrogens and have been shown to interfere with the process of estrogen metabolism (Bardin 2004). Most importantly one of the most potent effects of phytoestrogens is their ability to inhibit the sulphotransferases. Circulating steroid sulphates are thought to be the major source of estradiol in postmenopausal breast tumours and sulphation is a key step in the activation of some dietary pro-carcinogens (Kirck 2001).

A large number of research studies on the effects of phytoestrogens on breast cancer tend to conclude that they inhibit cell signalling pathways. For example genistein is an inhibitor of protein tyrosine kinase (Wei 1995). At high doses genistein has been found to inhibit AP-1 transcription factor activity and induce apoptosis in breast cancer cell lines. Genistein pre-treatment inactivates NK-kB and may inhibit growth and increase apoptosis induced by chemotherapeutics such as cisplatin, docetaxel and doxorubicin (Hieh 1998). Apigenin and quercetin are inhibitors of the phosphatidyl inositol kinase (PI3K) pathway (Jeong 1999). Studies show that both compounds inhibit E2-induced DNA synthesis and proliferation of ER-positive and ER-negative breast cancer cells (Collins-Burow 2000). Resveratrol has been reported to inhibit SRC tyrosine kinase and block Stat 3 activation in malignant cells (Miodini 1999) and may modulate breast

tumour growth. Both RAS/RAF/MEK/ERK (a signalling pathway associated with cell proliferation, differentiation and apoptosis) and PI3K/AKT/mTOR (an intracellular signalling pathway important in apoptosis) can be activated by growth factors and phytoestrogens may modulate the control of breast tumour growth. In addition recent studies have shown that resveratrol modulates the PI3K pathway through an ERα-dependent mechanism (Brooks 2005). Although some kinetic studies show that phytoestrogens may bind competitively with steroid substrates to inhibit steroidogenic enzymes, other evidence shows they can alter enzyme expression (Whitehead 2006). Indeed recent studies have shown that certain phytoestrogens and low dose mixtures of phytoestrogens are potent inhibitors of aromatase expression (Whitehead 2006).

Breast Cancer

Breast cancer is the most common type of cancer in the general population and the second leading cause of cancer death in Australian women. About 5% of newly diagnosed cases of breast cancer are metastatic and 30% of treated patients have a systemic recurrence. Once metastatic disease develops, the possibility of a cure is very limited with the five year survival rate at about 20% and the median survival duration varying from 12 to 24 months (Adlercreutz 2003). Estrogens may bind to two types of receptors in target cells: estrogen receptor-alpha (ER-alpha) and ER-beta, both of which can transactivate gene expression in target cells. Breast cancer cells express very high amounts of ER-alpha and far less ER-beta.

Breast cancer is not a single disease, but a collection of diseases that have distinct histopathology features, genetic and genomic variability as well as diverse prognostic outcomes. Although no individual model would be expected to completely encompass this complex disease, there is consensus amongst the researchers on the commonality of certain specific conditions that increase a woman's chance of developing breast cancer, whether it is an inflammatory breast cancer or a tubular carcinoma (Mumber 2007):

- Age: Studies show that the risk of breast cancer increases with age. This disease is uncommon in women under the age of 35. Most breast cancers occur in women over the age of 50 and the risk becomes higher above the age of 65.
- Race: Breast cancer occurs more commonly in Caucasian women as compared with African, American and Asian women.
- Personal history: Women who have suffered with cancer in one breast have a higher risk of developing cancer in the other breast compared with women who have never had cancer.
- Family history: The risk of developing breast cancer is higher if the women in any family (mother, sister and daughter) have also contracted breast cancer.
- Certain breast changes: Having a diagnosis of atypical hyperplasia or lobular carcinoma in situ (LCIS) may increase a woman's risk at a young age.
- Genetic alteration: Changes in certain genes (BRA1, BRA2 and others) increase the risk of breast cancer. However 25% of BRAC-positive patients never develop breast cancer. In families where many women have had the disease, gene testing can sometimes show the presence of specific genetic changes that increase the risk of breast cancer.

Evidence suggests that another highly significant factor associated with an increased risk for breast cancer is the length of exposure of the patient to estrogen, whether it is made by the body, taken as a drug or delivered by a patch. As estrogens are known to be potent mitogens in mammary epithelial cells, regulating estrogen metabolism is of prime importance in treatment of breast cancer. The incidence rate of breast cancer continues to increase with age despite the loss of ovarian hormones in postmenopausal women (Geller 2005). This apparent paradox has been resolved by the fact that extragonadal sites, including breast, brain, muscle, skin, bone and adipose tissue, can synthesise potent androgens and estrogens from relatively inactive circulating steroid precursors derived from the adrenal cortex and to a much lesser extent the ovaries. Indeed after menopause nearly 100% estrogens are formed in peripheral tissues and exert their effects locally in a paracrine or intracrine manner.

In postmenopausal women the concentration of 17-beta-estradiol (E2) present in breast tumours is at least 20-fold higher than that in circulation, but in premenopausal women with carcinoma this ratio was only 5-fold. This suggests that particularly in postmenopausal breast cancer, local estrogen biosynthesis is predominant (Adlercreutz 2004).

Estrogens are believed to contribute to tumour growth by promoting the proliferation of cells with existing mutations and/or by increasing the opportunity for mutations (DeLemos 2001). There are several enzymes and receptors involved in the estrogen pathway that have been suggested to play a role in the development of breast cancer. These are:

- 17-beta-hydroxysteroid dehydrogenase 1 (HSD17B1), the enzyme responsible for the conversion of estrone (E1) to estradiol (E2) which is the most potent estrogen. In human breast cancer, HSD17B1 is expressed in proliferative disease. These enzymes catalyse the interconversion of relatively inactive 17β-keto steroids (e.g. androstenedione and estrone) and active 17β-hydroxysteroids, such as testosterone and estradiol (Collins-Burow 2000).
- The aromatase enzyme, CYP19, a key enzyme in the conversion of androgens to estrogens. Over 60% of breast carcinomas express this enzyme (Harris RM 2004) with higher levels of mRNA expression and activity compared with non-malignant tissue (Sanderson 2004).
- Cytochrome P450 1B1 (CYP1B1) catalyses the

conversion of estrone and estradiol to potentially carcinogenic catechol estrogen 4-hydroxyestrogen (4-OH). This enzyme is expressed in the mammary glands, ovary and uterus. Over-expression of this enzyme has been associated with an increased risk of breast cancer (Kirk 2005).

- 3β-hydroxysteroid dehydrogenase: In relation to breast cancer this enzyme has received little attention. There are two isoforms, 1 and 2, the latter being mainly expressed in the adrenal glands and gonads and type 1 being expressed in the placenta and other tissues including skin and breast where it is considered mainly to convert DHEA to androstenedione (Gallo 2006).
- Catechol-O-methyltransferase (COMT) enzyme which is principally responsible for both the inactivation and detoxification of carcinogenic catechol estrogens. This enzyme is ubiquitous and is found in many tissues including the uterus, liver, kidney, breast, lymphocytes and erythrocytes (Lavigne 1997).
- Hyperhomocysteinemia can create a pathogenic effect largely through metabolic accumulation of intracellular S-adenosyl-L-homocysteine. The inhibition of the methylation metabolism of catechol estrogens of which this is a marker, would facilitate the development of estrogen induced hormonal cancer (Wu 2002).

The four main classes of compounds recognised as phytoestrogens, the isoflavones, coumestans, lignans and stillbenes (Caltagirone 2000), exert the unique quality of being estrogen agonist and antagonist (Barnes 2004). As agonists, phytoestrogens exert a protective effect against cardiovascular disease, menopausal symptoms (including osteoporosis) and cancer.

Conclusion

Of all the isoflavones, genistein is still attracting the most attention because of its estrogenic and antiestrogenic effects which, at high concentration, have shown inhibition on cancer cell lines through the modulation of the transforming growth factor (TGF) (Kim 1998). Genistein's greater affinity as a ligand for ER-beta than ER-alpha, suggest that it has potentially significant therapeutic actions. Interestingly this receptor shows a different anatomical distribution from ER-a, being expressed more prominently in tissues such as breast, prostate and urinary tract (Kuiper 1997). Apigenin and quercetin inhibit melanoma growth and metastatic potential.

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Iron inhibition by plant polyphenols: an adjunct to treatment in hereditary haemochromatosis

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Hereditary haemochromatosis (HH) is a common genetic disorder characterised by excessive iron accumulation in the tissues. Orthodox treatment involves frequent blood removal by venesection along with pharmaceutical management with proton pump inhibitors (PPIs) and iron chelating agents. Plant polyphenols (in particular those of *Camellia sinensis*) have long been regarded as potent inhibitors of iron absorption from the gastrointestinal tract (GIT) and numerous epidemiological studies have associated increased tea consumption in populations with reduced iron status. Several clinical studies have been performed in recent decades on the effect of polyphenols in inhibiting dietary iron absorption in humans. While the evidence base is limited, a review of the current literature suggests that consumption of tea polyphenols with meals can significantly reduce iron absorption and may be beneficial as an adjunct to treatment in patients with HH.

Introduction

Hereditary haemochromatosis is a genetic multi-organ disorder associated with a polymorphism in the haemochromatosis gene (HFE) (Pietrangelo 2010). Individuals with HH lack the capability to limit iron absorption into the bloodstream and thus the disorder is characterised by excessive iron accumulation in the tissues, resulting in the development of diabetes, arthropathy, cardiomyopathy, cirrhosis and hepatocellular carcinoma (Pietrangelo 2010). Disease progression is insidious and affected individuals are often asymptomatic before diagnosis is confirmed with elevated serum ferritin and gene testing for HFE (Frenkel 2011).

Primary management of HH relies on weekly blood removal (venesection) until transferrin saturation falls below 50%, following which treatment is indicated less frequently (Crawford 2005). Secondary approaches are aimed at reducing iron absorption from the gastrointestinal tract (GIT), primarily through the avoidance of vitamin C which has been shown to enhance the uptake of dietary iron by up to 350% (Thankachan 2008) and the use of proton pump inhibitors (PPIs). PPIs are effective in reducing iron absorption from the duodenum (McColl 2009) and have to some extent superseded the emphasis on dietary approaches to managing HH. This is reflected in current recommendations for patients with HH which suggest dietary modification is not necessary for managing the condition (Barton 1998). In addition to vitamin C there are several food constituents known to enhance iron absorption, including alcohol (Olynyk 2005), fructose (O'Dell 1993) and muscle tissue, or meat (Hurrell 2010). Factors limiting iron absorption include calcium, magnesium, manganese and zinc (Osiecki 2010), polyphenol containing beverages including tea, coffee and wine (Hurrell 2010) and soluble fibres such as pectin (Monnier 1980).

Plant polyphenols

Perhaps the most well studied food constituent compounds with regard to their effect on iron absorption are the polyphenols, notably those of tea (*Camellia sinensis*). Polyphenolic constituent compounds such as chlorogenic acid, gallic acid, monomeric flavonoids and tannins are known to form insoluble compounds with iron and render it unavailable for absorption (Thankachan 2008). Epidemiological studies suggest a linear relationship between tea consumption and iron deficiency among populations with marginal iron intake (Temme 2002, Mennen 2007, Hogenkamp 2008). A number of clinical studies have also been performed with regard to polyphenol containing beverages and their effect on iron absorption (Samman 2008, Thankachan 2008, Hurrell 1999, Kaltwasser 1998, Cook 1995) however the clinical significance of research to managing iron overload syndromes has scarcely been addressed to date (Kaltwasser 1998). The following article aims to review current literature regarding polyphenol consumption and iron absorption and its relevance as an intervention in the management of HH.

Methodology

The author searched online databases Google Scholar, PubMed, MedLINE, EBSCO and ProQuest from 1992 onwards to retrieve suitable articles for review. Search terms included *hemochromatosis*, *haemochromatosis*, *hereditary hemochromatosis*, *diet*, *iron*, *absorption*, *iron-overload*, *tea*, *polyphenol*, *tannin*, *gallic acid*, *gallate*, *ferritin*, *wine* and *coffee* in various combinations. Using evidence based practice guidelines for best clinical relevance (Straus 2005) articles chosen for inclusion were limited to full text, peer reviewed articles in the format of controlled trials. Animal trials, in vitro studies and case analyses were not chosen for inclusion. Following a search a total of 7 articles were identified

that met criteria for inclusion. After closer investigation, two cross sectional studies were excluded and retained for wider reading, and the remaining five articles were entered for review.

Discussion

Thankachan et al (2008) used a randomised controlled design to observe the effects of both tea and ascorbic acid (AA) on iron absorption in a group of iron deficient women. Subjects were randomised to receive a test meal labelled with radioactive iron in combination with 1 cup of black tea (78 mg polyphenols) or AA in water solution (at a 2:1 molar ratio). Each subject then consumed a second test meal with 300 mL water (control) and subjects were then crossed over to receive the opposing intervention with a third test meal. Red cell radioactivity was measured prior to each meal and again 14 days following consumption; and was used to estimate whole body iron accumulation from each test meal. Blood hemoglobin (Hb), serum ferritin and serum transferrin were also measured in all subjects before and following each intervention.

Results indicated that the addition of tea to the meal significantly inhibited iron absorption in both iron deficient and iron replete subjects compared with water; however inhibition was slightly less in the iron deficient group. This was acquiescent with the authors' hypothesis that iron deficiency is a stronger predictor of iron absorption than meal composition alone (Thankachan 2008). Ascorbic acid was shown to significantly increase iron absorption in both deficient and replete subjects, however absorption was again increased among the iron deficient group. The authors also identified a dose dependent relationship between polyphenol consumption and iron inhibition among healthy controls where consumption of 78 mg or 156 mg polyphenols resulted in a 50% or 70% reduction in iron absorption respectively (Thankachan 2008).

Authors Samman et al (2001) investigated the addition of green tea or rosemary (*Rosmarinus officinalis*) extract to meals and their effect on iron absorption. Twenty-seven female subjects were randomised to receive either green tea extract (37.3 mg polyphenols) or rosemary extract (32.7 mg polyphenols) with a meal for 4 days. Each subject then consumed 4 meals, two with added extract (A) and two controls with no extract (C) in the sequence ACCA. Iron absorption from the meals was measured using whole body radioactive counting before consumption of the test meals and 2 weeks after consumption; while blood Hb and serum ferritin were measured at both enrolment and completion. Results indicated that while serum ferritin and Hb were unchanged following the intervention, both the tea and rosemary extracts significantly reduced the amount of iron absorbed from the test meal by 28% and 21% respectively (Samman 2001).

Hurrell and colleagues (1999) examined the efficacy of a range of polyphenol containing beverages (tea, herbal tea, coffee and cocoa) on iron absorption in 77 healthy subjects. The trial consisted of 8 separate absorption studies involving groups of 9 or 10 subjects in which each individual consumed 4 test meals in combination with 3 different beverages and a water control. The authors utilised measures of red cell radioactivity to assess iron uptake, both before consumption and 14 days after consumption of each test meal. Polyphenol content of the beverages ranged from 396 mg (black tea) to 177 mg (peppermint) to 52 mg (camomile).

Following interpretation of the results the authors identified a logarithmic relationship between iron absorption and polyphenol consumption with the highest polyphenol containing beverage (black tea) reducing absorption by 90%; while beverages with low polyphenol content (herbal teas) hindered absorption by just 30% (Hurrell 1999). Interestingly the authors found that black tea diluted to 5% of original strength still reduced iron absorption by 70%; concluding that black tea polyphenols were significantly inhibitory even at low concentrations and therefore may be a useful strategy in reducing iron absorption in patients with overload disorders (Hurrell 1999).

The effect of polyphenols in various wines was examined by Cook and colleagues (1995) using a non randomised experimental design. The researchers conducted 4 controlled absorption studies in a similar fashion to Hurrell et al (1999), each with 7 or 8 subjects consuming 4 radio labelled test meals accompanied by 3 wines and a water control. The highest content of polyphenols was found in the red pinot noir wine (2.98 g/L) followed by the red aramon (1.95 g/L) and the white wine (0.19 g/L). Again red cell radioactivity was measured at baseline and 14 days following consumption of each test meal as a measure of iron absorption. Results followed a similar trend to previous experiments where polyphenol content mirrored inhibition of iron absorption; with maximal inhibition achieved with a 358 mg polyphenol dose. However inhibition of iron absorption was not seen to be statistically significant in any of the wines studied, despite their relatively high polyphenol content.

Complexities in the study design reveal a number of confounding factors that may have been responsible for non significant results. Alcohol itself is a known potentiator of iron absorption (Olynyk 2005) though to what extent this relationship exists is unknown. The authors accounted for this relationship by comparing a low alcohol form of each wine with its full alcohol counterpart, and expectedly the low alcohol wines showed a greater inhibitory effect on iron absorption. However the authors failed to account for the effect of the iron content of the wines themselves, later quantified as almost 10% of daily required intake (Cook 1995). If not for the multiple confounders, the data suggests there exists a potential for low alcohol red wines as a component of dietary intervention for those individuals with iron overload who continue to consume alcohol.

Kaltwasser and colleagues (1998) conducted a more recent non randomised controlled trial involving 18 subjects, all of whom were diagnosed with hereditary haemochromatosis. The study observed the effect of tea drinking on iron accumulation over a period of 52 weeks. An initial absorption study was performed in each subject who first consumed an iron radio labelled test meal and was then assessed for whole body radioactivity 7 days and 14 days following consumption. Subjects then consumed a second test meal with tea, and iron absorption was again measured after 7 and 14 days. Among all individuals intestinal absorption of iron was inhibited by 70% when the test meal was consumed with tea (Kaltwasser 1998).

Subjects were then assigned to either the intervention group or the control. All subjects consumed their regular diet for the next year; with the intervention group instructed to consume black tea with meals 3 times daily, and the control group permitted to drink only water or non polyphenol containing beverages with meals for the subsequent period. All subjects also abstained from venesection treatments until completion of the study. Blood measurements for Hb, packed cell volume (PCV), mean cell volume (MCV), mean cell Hb, serum iron, total iron binding capacity (TIBC), TIBC saturation and serum ferritin were recorded every 4 weeks for all subjects, and iron storage levels and total body iron were calculated from venesection at completion of the experiment.

At completion results indicated a reduction in both serum ferritin and TIBC saturation in the treatment group compared with the control group, as well as a reduction in iron storage levels and total iron removal required at final venesection (Kaltwasser 1998). The study design allowed for variation in polyphenol consumption by the treatment group who were not assigned a specific brand of tea, brewing method or polpyhenol concentration; however overall all blood markers were significantly improved in the treatment group compared with the control. The authors noted that while all results from the experiment were promising, of particular significance to individuals with HH was the reduction in blood required to be drawn at post experimental venesection (Kaltwasser 1998).

Conclusion

Small study populations, short durations and variability in experimental composition retract somewhat from the potency of the reviewed literature, however despite methodological shortcomings there is a uniformity of results in favour of the treatment. Whilst the clinical relevance of absorption studies conducted in healthy or iron deficient subjects is also questionable when considering patients with inherent disorders of absorption, of particular interest is the work by Kaltwasser and colleagues which demonstrated an inverse relationship between polyphenol consumption and the requirement for venesection in patients with established HH. The available research indicates that plant polyphenols, in particular those of tea (*Camellia sinensis*), are potent inhibitors of iron absorption from the GIT, and suggests that in combination with the avoidance of known potentiators of iron uptake (meat, alcohol, fructose and vitamin C) consumption of tea polyphenols may be beneficial in individuals with HH and aid in the management of their condition.

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The antiviral activity of *Gynostemma pentaphyllum* against yellow fever virus

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Objectives: Yellow fever is a disease of significant public health concern with no known drug for treatment. There is therefore the need for a suitable antiviral drug against the disease. This has given rise to the present research into medicinal plants for suitable alternative antiviral drugs since most antiviral drugs known have side effects on the host cells.

Methods: The antiviral activity of the crude extracts of *Gynostemma pentaphyllum* on yellow fever virus was evaluated. The antiviral properties were determined against yellow fever virus using three methods: protection of chicken egg embryo against viral infectivity by the extracts, protection of mice against viral infectivity by the extracts and percentage inhibition of viral induced hemagglutination by the extracts. Phytochemical analysis of the extracts was also carried out.

Results: The phytochemical analysis of the extracts revealed the presence of saponins, alkaloids, glycosides, tannins, flavonoids, carbohydrates, reducing sugar, resins, acidic compounds, fats and oil and proteins. The egg embryo and mice protection studies against yellow fever viral infectivity showed that the extracts were able to give up to 100% protection to the embryonated eggs and mice and hence prevented egg/mice mortality. The extracts gave plausible percentage inhibitions of yellow fever virus in embryonated eggs up to 90%. It was observed that the antiviral effect decreased with decrease in concentration.

Conclusion: The research has shown that the plant *Gynostemma pentaphyllum* possesses potent antiviral potential and could serve as a possible source of lead antiviral drugs against yellow fever since the disease has no known drug for treatment.

Key words: antiviral, medicinal plant, *Gynostemma pentaphyllum*, yellow fever, embryonated eggs, albino mice, phytochemical analysis

Introduction

Yellow fever is a pansystemic viral sepsis with viremia, fever, prostration, hepatic, renal and myocardial injury, hemorrhage, shock and high lethality. It stands apart from other viral hemorrhagic fevers in its severity of hepatic injury and the universal appearance of jaundice (Monath, 2001, 2008). It is caused by the yellow fever virus (YFV) that is transmitted to humans through the bite of the *Aedes* or *Haemagogus* mosquitoes (Lindenbach 2007). YFV, belonging to the *Flavivirus* genus, is a single stranded RNA genome virus that possesses a spherical nucleocapsid surrounded by a lipid envelop in which the envelope (E) protein and membrane (M) protein are embedded. Binding of YFV to the cell surface is believed to be mediated by the E protein (Lindenbach 2007). The disease is endemic in tropical regions of Africa and South America; nearly 90% of yellow fever cases and deaths occur in Africa (Tolle 2009).

Yellow fever (YF) is a disease of significant public health importance with an estimated 200 000 cases and 30 000 deaths annually (PAHO 2004). Prevention of the disease has been by the usage of a safe and efficacious life attenuated vaccine (the 17D vaccine) but in a few vaccinees (1:200 000 to 1:300 000) the virus can cause adverse effects such as YEL-AVD (yellow fever vaccine-associated viscerotropic disease) and YEL-AND (yellow fever vaccine-associated neurotropic disease) leading to meningoencephalitis or an acute viral hemorrhagic syndrome with multiple organ system failure. The fatality rate in cases suffering from disease due to 17D vaccination is 60% (Monath 2005, Barrett 2009). There is no currently approved antiviral drug against YF and since the 1980s the number of cases of yellow fever has been increasing, making it a reemerging disease (Barrett 2007).

Effective antiviral therapeutic agents are sought to combat viral diseases in such a manner that will be toxic to the virus with minimal or no toxicity to the host cells. Unfortunately most antiviral agents have long term side effects and viruses also have the ability to adapt, mutate or negate the effect of certain antiviral agents. Currently medicinal plants are being increasingly projected as suitable alternative sources of antiviral agents because of their multiple targets, minor side effects, low potentials to cause resistance and low costs (Esimone 2010).Within the last decade the search for antiviral agents from medicinal plants against viral pandemics, which have long been a threat to human health, has been dramatically intensified (Esimone 2007, Saddi 2007, Rocio 2009, Liu 2007).

This need for new sources of lead drugs for the development of potent antiviral agents has led to the present research. The plant used in this study, *Gynostemma pentaphyllum*, has been claimed in complementary medicine to possess antiviral activities. It has also been reported to have various activities such as a cholesterol reducing effect (Huang 2005) and hypoglycemic effects (Samer 2006). Locally the traditional medicine practitioners use it for the treatment of bacterial and viral infections. These claims led to the screening of this medicinal plant for its antiviral activity against yellow fever virus which is a major public health concern.

Materials and methods

Collection and extraction of plant materials

The leaves of *Gynostemma pentaphyllum* (GP) plant were collected from Nibo in Awka south LGA, Anambra State, Nigeria. The leaves were identified by Prof CC Okeke of the Department of Botany, Nnamdi Azikiwe University, Awka. They were oven dried at 50^0C for 24 h and ground to powder using a mechanical grinder.

Forty gram (40 g) portion of the plant powder was macerated in 400 mL of distilled water in a conical flask and left at room temperature for 24 hours. For the methanolic and petroleum ether extract, the 40 g portion of plant powder was macerated in 200 mL of either methanol or petroleum ether and left at room temperature for 48 hours. These were filtered using Whatman No 1 filter paper. The filtrates were concentrated to dryness in the oven at 50^0C.

Phytochemical analysis of plant extracts

The extracts were first reconstituted in the respective solvents used for their extraction and then tested by standard phytochemical methods (Harborne 1998) for presence of alkaloids, flavonoids, tannins, saponins, glycosides, protein, carbohydrate, terpenoids, resins, fats and oil, acidity, steroids and reducing sugar.

Collection of embryonated chicken eggs and Swiss albino mice

Pre-incubated 9 day old embryonated chicken eggs were collected from Arroma farms Nig Ltd, Awka, Anambra State. Adult Swiss albino mice (males and females) weighing between 12 g and 18 g were collected from the National Veterinary Research Institute, Vom, Nigeria. The albino mice were fed with chicken mash and tap water. They were reared until the females became pregnant and gave birth to their litters. These suckling mice 3-7 days old were used for the antiviral assay.

Collection and preparation of the viral inoculum

Yellow fever virus already passaged in mice was obtained from the virology laboratory, University College Hospital (UCH) Ibadan and transported back to Awka in an ice pack. The virus was harvested from the brain cells of the mice using a 5 mL syringe with 21 gauge needle. The virus was filtered using Millipore micro filter of 0.45µm pore size and stored in cryo vials at –20°C until used. For the egg assay, one cryo vial of the virus stock was repassaged twice in nine day old embryonated chicken eggs, harvested and stored in cryo vials at –20°C until used.

Determination of the 50% embryo infectious dose (EID_{50}) and 50% embryo lethal dose (ELD_{50})

Stepwise 10 fold (1/10) dilutions of the virus suspensions were made up to 10^{-5} in sterile test tubes using phosphate buffered saline (PBS). Five embryonated eggs were inoculated with 0.1 mL of each dilution using separate syringes and needles. The eggs were incubated at 37°C for 4 days. They were turned at least four times a day and candled each day to see if any of them had died. The dead eggs were removed from the incubator, chilled and harvested and used for hemagglutination assay. At the end of the 4 days the remaining eggs were chilled overnight and harvested and also quantified by hemagglutination assay. The result of the hemagglutination assay was used to determine the infectivity titre while the number of dead eggs and living ones were used to determine the lethal dose. These calculations were done using the mathematical technique of Reed and Muench (1938).

Determination of the 50% mice lethal dose

Stepwise ten-fold (1/10) dilutions of the virus suspension were made up to 10^{-5} in sterile test tubes using phosphate buffered saline (PBS). Five suckling mice less than one week old were inoculated intracerebrally with 0.01 mL of each dilution using a separate needle and syringe for each. A total of twenty five (25) suckling mice were used for the titration. The mice were observed seven days for symptoms of encephalitis and death. At the end of the seven days the number dead were used to calculate the lethal dose using Reed and Muench technique (1938).

Assay of antiviral activity against yellow fever

The assay of antiviral activity against yellow fever was determined by inoculating nine day old embryonated egg and albino mice.

Egg inoculation: The principle and procedure applied in preparation of the inoculum and egg inoculation were as described by Hawkes (1976) and Senne (1998). The nine day old embryonated chicken eggs to be used for the test were checked for viability by candling and inoculated through the blunt ends using 2 mL syringe and 21 guage needle. The eggs were inoculated with 100 EID_{50} of the virus and extracts as follows: five eggs were inoculated for each concentration of extracts (200 mg/mL, 20 mg/mL and 2 mg/mL), five eggs for each inoculation regimen (1 h pre inoculation (P1), at inoculation (0 h) and 1 h post inoculation (P0) and five eggs for each extract (3 extracts).

About 0.1 mL of the passaged virus and 0.1 mL of sterile PBS were inoculated into 5 eggs. This served as positive control while 2 mL of PBS alone inoculated into

5 eggs served as negative control. For the toxicity control 0.2 mL of the different extracts was each inoculated into 5 eggs. The eggs were incubated at a temperature of 37^{O}C for four days. They were turned at least four times daily and also candled daily to check for egg mortality. The number of dead eggs and those alive were used to calculate the percentage protection given to the embryonated eggs by the extracts. At the end of four days the eggs were chilled overnight at 4^{O}C and the allantoic fluid harvested and used for hemagglutination test. The result of the hemagglutination test was used to calculate the percentage inhibition of the extracts.

Mice inoculation: Ten microlitres of different concentrations of methanolic extract of the plant were separately inoculated into five suckling mice less than one week old intraperitoneally. Using 1 mL syringe with 30 guage needle the mice were inoculated as follows: five mice per concentration (20 mg/mL, 2 mg/mL and 0.2 mg/mL), five mice per inoculation regimen (1 h pre infection, at infection and post infection). For the pre infection 0.01 mL of each extract was inoculated intraperitoneally into the mice and the mice left for 1 h before introducing 0.01 mL of 100MLD$_{50}$ of the passaged virus. For at infection 0.01 mL of 100MLD$_{50}$ of the virus and 0.01 mL of the extract were inoculated at the same time. For the post infection 0.01 mL of 100MLD$_{50}$ of the virus was inoculated into the mice 1 h before the introduction of the extract.

Positive control was 0.01 mL of virus and 0.01 mL of PBS inoculated into five suckling mice while negative control was 0.02 mL of PBS inoculated into the mice.

The mice were observed for symptoms of encephalitis (paralysis) and death for seven days. The number of mice alive was used to calculate the percentage protection given to the mice by the extracts.

Results

The phytochemical analysis of the crude extracts of the plant *Gynostemma pentaphyllum* (Table 1) showed the presence of saponins, alkaloids, glycosides, tannins, carbohydrates, flavonoids, resins, acidic compounds, proteins, reducing sugar and fats and oil.

Table 2 indicates the percentage protection of mice against yellow fever virus using methanolic extract of *Gynostemma pentaphyllum*. All the concentrations of the extracts inoculated at both pre infection and at infection gave plausible protection of 100% to the mice. The 0.2 mg/mL inoculated one hour after the virus gave the least protection of 60% to the suckling mice. In the positive control all the mice died whereas for the negative and toxicity control none of the mice died.

The aqueous extract of *Gynostemma pentaphyllum* at the concentration of 200 mg/mL gave 100% protection to the embryonated eggs for the three inoculation modes (pre infection, at infection and post infection). At 20 mg/mL it gave 100%, 60% and 80% protection for pre infection, at infection and post infection respectively. The 2 mg/mL

Table 1: Phytochemical constituents of *Gynostemma pentaphyllum* extracts

	Type of extract		
Constituents	**Ether**	**Aqueous**	**Methanol**
Saponins	+	+++	++
Alkaloids	++++	-+++	+
Glycosides	-	++	+++
Tannins	-	+++	+
Carbohydrates	-	++	+++
Reducing Sugar	-	-	++
Flavonoids	++++	-	+++
Resins	++	-	+
Steroids	-	-	-
Terpenoids	-	-	-
Fats and Oils	+	-	-
Acidic Compounds	-	+++	-
Proteins	-	+	++++

Key:
(-) = not present
(+) = present in small concentration
(++) = present in moderately high concentration
(+++) = present in very high concentration
(++++) = Abundantly present

concentration gave 20%, 80% and 20% protection for pre infection, at infection and post infection respectively. The positive control (virus alone) showed the death of all eggs inoculated and therefore gave 0% protection to the embryonated eggs. For the toxicity control all eggs inoculated survived showing that the extracts were not toxic to the cells (Figure 1).

The methanolic extract of *G. pentaphyllum* at 200 mg/mL gave 80%, 100% and 80% protection for the

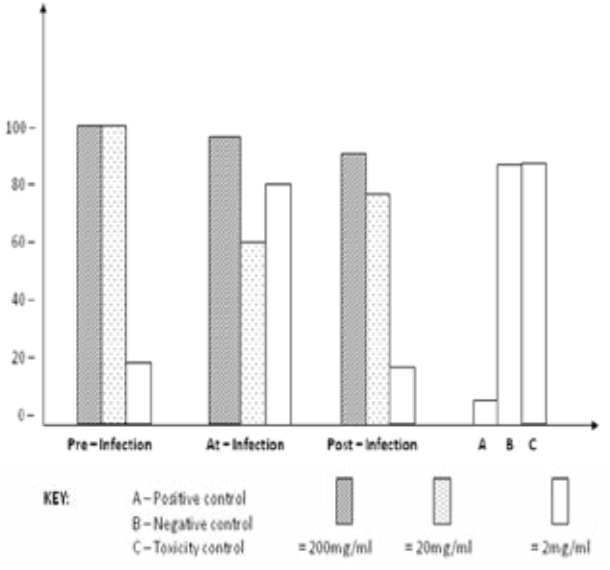

FIG. 1: Protection of embryonated eggs against yellow fever virus by aqueous extract of *Gynostemma pentaphyllum*

pre infection, at infection and post infection inoculation modes respectively. At 20 mg/mL it gave 100% protection for the three inoculation modes and at 2 mg/mL it gave 20%, 100% and 40% protection for the pre infection, at infection and post infection respectively (Figure 2).

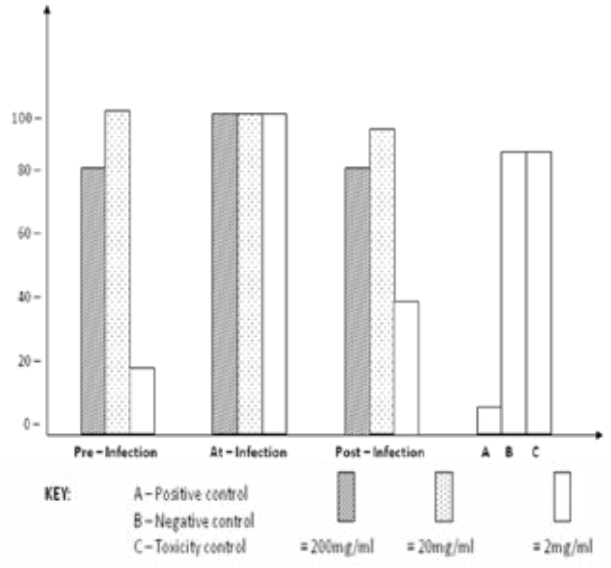

FIG. 2: Protection of embryonated eggs against yellow fever virus by methanolic extract of *Gynostemma pentaphyllum*

The ether extract of *G. pentaphyllum* at the concentration of 200 mg/mL gave 60%, 60% and 100% protection to embryonated eggs for the pre infection, at infection and post infection inoculation modes respectively. At 20 mg/mL it gave 100%, 80% and 100% protection for the pre infection, at infection and post infection respectively. The 2 mg/mL concentration of extract gave 100%, 60% and 80% protection for the pre infection, at infection and post infection respectively (Figure 3).

On the percentage inhibition of viral induced hemagglutination by the aqueous extract of *Gynostemma pentaphyllum* the 200 mg/mL (pre infection) gave the highest inhibition (85%) while the 2 mg/mL (pre infection) gave the lowest inhibition of 35%. The highest egg mortality was given by the 2 mg/mL (pre infection) and 2 mg/mL (post infection). Concentrations of 200 mg/mL (pre infection), 20 mg/mL (pre infection), 200 mg/mL (at infection) and 200 mg/mL (post infection) showed no egg mortality (Table 3).

The percentage inhibition of viral induced hemagglutination by the methanolic extract of *Gynostemma pentaphyllum* showed that the 20 mg/mL (at infection) and 20 mg/mL post infection gave the highest inhibition of 90% while the 2 mg/mL (pre

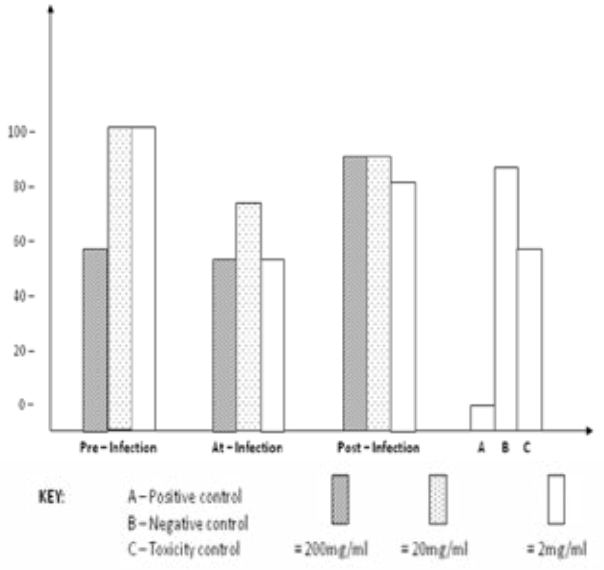

FIG. 3: Protection of embryonated eggs against yellow fever virus by ether extract of *Gynostemma pentaphyllum*

Table 2: Mice protection against yellow fever by methanolic extract of *Gynostemma pentaphyllum*

Inoculation regimen controls	Extract concentration (mg/mL)	Mice mortality (D/N)	No living	% Protection of mice by extracts
Pre - Infection	20mg	0/5	5/5	100
	2mg	0/5	5/5	100
	0.2mg	0/5	5/5	100
At - Infection	20mg	0/5	5/5	100
	2mg	0/5	5/5	100
	0.2mg	0/5	5/5	100
Post Infection	20mg	1/5	4/5	80
	2mg	1/5	4/5	80
	0.2mg	2/5	3/5	60
Positive Control	-	5/5	0/5	0
Negative Control	-	0/5	5/5	100
Toxicity Control	20gm	0/5	5/5	100

Key: D = No of dead mice N = No of mice inoculated MHA = Median haemagglutination titre

infection) gave the lowest inhibition. The highest egg mortality was given by 2 mg/mL (pre infection) while all concentrations inoculated at infection, 20 mg/mL (pre infection) and 20 mg/mL (post infection) showed no egg mortality (Table 4).

The percentage inhibition of viral induced hemagglutination by the ether extract of *Gynostemma pentaphyllum* showed that 200 mg/mL (post infection) gave the highest inhibition of 90% while 2 mg/mL (at infection) gave the least inhibition of 55%. The highest egg mortality was given by the 200 mg/mL (pre infection), 200 mg/mL (at infection) and the 2 mg/mL (at infection). The positive control showed all the eggs dead while the negative control showed that none of the eggs died. The toxicity control at 200 mg/mL showed that two of the eggs died indicating that there was minimal toxicity of the ether extract of GP on the embryonated eggs at 200 mg/mL. This may also be the reason for death of some eggs at the same concentration for both pre infection and at infection regimen (Table 5).

Discussion

The phytochemical screening of the extracts of *Gynostemma pentaphyllum* showed the presence of saponins, alkaloids, glycosides, tannins, flavonoids, carbohydrates, reducing sugar, resins and proteins. The presence of saponins, glycosides and flavonoids in the extracts of *Gynostemma* is consistent with other findings about the leaves of the plant (Xin 2004, Cui 1999) The leaves of *Gynostemma pentaphyllum* have been shown to contain more than 90 saponins and more than 100 dammarane type glycosides have been isolated and

Table 3: Inhibition of yellow fever virus replication in chick embryo by aqueous extract of *Gynostemma pentaphyllum*

Inoculation regimen controls	Extract concentration (mg/mL)	Egg mortality (D/N)	MHA litre (Log 2 reciprocal) allantoic	% inhibition
Pre infection	200	0/5	1.8	85
	20	0/5	2.2	82
	2	4/5	7.8	35
At infection	200	0/5	2.6	78
	20	2/5	4.2	65
	2	1/5	5.0	58
Post infection	200	0/5	2.2	82
	20	1/5	2.8	77
	2	4/5	7.4	38
Positive control	-	5/5	12	
Negative control	-	0/5	0	
Toxicity control	20	0/5	0	

Key: D = No of dead eggs N = No of eggs inoculated MHA = Median haemagglutination titre

Table 4: Inhibition of Yellow fever virus replication in chick embryo by methanol extract of *Gynostemma pentaphyllum*

Inoculation regimen controls	Extract concentration (mg/mL)	Egg mortality (D/N)	MHA litre (Log 2 reciprocal) allantoic	% inhibition
Pre infection	200	1/5	4.0	67
	20	0/5	3.6	70
	2	4/5	6.8	43
At infection	200	0/5	1.4	88
	20	0/5	1.2	90
	2	0/5	2.0	83
Post infection	200	1/5	2.0	83
	20	0/5	1.2	90
	2	3/5	5.8	52
Positive control	-	5/5	12	
Negative control	-	0/5	0	
Toxicity control	200	0/5	0	

Key: D = No of dead eggs N = No of eggs inoculated MHA = Median haemagglutination titre

identified from it (Zhang 1993, Cui 1999).

The extracts have shown varying degrees of antiviral activities against the yellow fever virus assayed. The antiviral activities may be attributed to the rich phytochemicals contained in the extracts since various studies have shown that phytochemicals such as tannins found in almost all plant parts cure or prevent a variety of viral infections (Serafini 1994, Nonaka 1990).

Flavonoids on the other hand have been shown to exhibit inhibitory effects against viruses including HIV and respiratory syncytial virus (Li 2000). Plant polysaccharides have also been shown to exhibit potent antiviral activities especially against enveloped viruses (Hosoya 1991, Premanathan 1990). In fact Abram et al (1993) attributed the medicinal properties of *Gynostemma pentaphyllum* to be mainly due to the presence of saponins in the plant.

The antiviral screening of the extracts against yellow fever virus was demonstrated using three different methods: (a) protection of mice against viral infectivity by the extracts, (b) protection of chicken egg embryo against viral infectivity by the extracts, and (c) percentage inhibition of viral induced heamagglutination by the extracts.

The mice inoculation assay for the determination of the extracts' protection of mice against viral infectivity showed that the extracts were able to prevent the symptoms of encephalitis and death in many of the mice and some of the extracts gave up to 100% protection to the mice. This result corroborates other research findings that showed the antiviral activities of this plant using animal models against such viruses as Epstein-Barr virus (EBV), Herpes Simplex virus (HSV-1) and HIV–AIDS virus (Lipipum 2003, Abram 1993). Nevertheless this is the first report of the antiviral activity of this plant against yellow fever virus.

The result of the percentage protection of the embryonated eggs against viral infectivity also proved that the extracts were able to give up to 100% protection to the embryonated eggs and hence prevented mortality due to viral infection.

The result of the percentage inhibition of the replication of yellow fever virus by the extracts in embryonated chicken eggs showed that the extracts gave potent activity against the virus. Some of the extracts gave plausible percentage inhibitions of up to 90%. The result of the embryo mortality clearly showed that the extracts were not toxic to the chicken embryonated eggs since all embryos used for toxicity control survived by the fifth day of the experiment. The extracts with antiviral effect showed activity in two subsequent dilutions of the maximum non toxic concentration. This is in line with the suggestion made by Vanden et al (1993) that the antiviral activity of crude plant extracts should be detectable in at least two subsequent dilutions of the maximum non toxic concentration to ensure that the activity is not directly correlated with the toxicity of the extracts.

It is interesting to note that the extracts had activity against the yellow fever virus at different times of inoculation (one hour pre infection, 0 h at infection and one hour post infection). The extracts that inhibited viral replication at 1 h pre infection might have acted on the viral entry step of the replication cycle and prevented the virus from attachment and further replication inside the host. Those extracts that inhibited the yellow fever virus at zero hour might have acted on the virus before attachment by mechanism of binding on the active site of the host cell blocking the virus from attaching to the host receptors or by binding on the active site of the virus. According to Vanden et al (1986) polyphenols act principally by binding to the virus and/or the protein of the host cell membrane thus arresting adsorption of the

Table 5: Inhibition of Yellow fever virus replication in chick embryo by ether extract of *Gynostemma pentaphyllum*

Inoculation regimen controls	Extract concentration (mg/mL)	Egg mortality (D/N)	MHA litre (Log 2 reciprocal) allantoic	% inhibition
Pre infection	200	2/5	3.4	72
	20	0/5	3.0	75
	2	0/5	1.4	88
At infection	200	0/5	1.4	68
	20	2/5	3.8	68
	2	2/5	5.4	55
Post infection	200	0/5	1.2	90
	20	0/5	2.2	82
	2	1/5	2.8	70
Positive control	-	5/5	12	
Negative control	-	0/5	0	
Toxicity control	200	2/5	0	

Key: D = No of dead eggs N = No of eggs inoculated MHA = Median haemagglutination titre

virus. The extracts that inhibited 1 h post infection must have inhibited a post entry step of the viral replication. The extracts gave up to 100% protection with the three modes of inoculation. Viral infectivity was also inhibited up to 90% by the extracts of the plant inoculated in three different modes. This shows that the three modes gave good inhibition of viral growth and protection on the embryonated eggs and mice and that no mode of inoculation worked better than the other. This suggests that the extracts could be used as both preventive and curative therapy. It was observed that the antiviral activity decreased with a decrease in concentration as the 200 mg/mL concentration showed the highest activity while 2 mg/mL showed the least activity.

Conclusion

The extracts of the plant *Gynostemma pentaphyllum* used in this study have shown credible antiviral activities against the yellow fever virus and could be recommended as a potential source for a yellow fever remedy.

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Valeriana officinalis

Jeremy Brown, Australasian College of Natural Therapies, Sydney

Botanic name: *Valeriana officinalis*

Common name: Valerian

Botanical description

Reaching a height of 1.5 metres, valerian is a perennial herb with creeping, aromatically pungent rhizomes, and hollow stems (Weiss 1988, Heinrich 2004). Oblong and ovate shaped, the fruits have 4 ridges and are single seeded. Its leaves are compounded and its flowers are white/pinkish and arranged in flat topped terminal clusters (Wyk 2009, WHO 1999). Due to the isovaleric acid released on the decomposition of the valepotriates, a distinctive pungent odor is produced (Mills 2000, Culpeper 1826).

Part/s used

The plant materials of interest are the dried roots, rhizomes and stolons (British Herbal Pharmacopeia 1976).

Relevant constituents

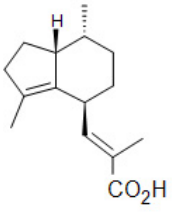

Figure 1: Valerenic acid (non-volatile cyclopentane sesquiterpene)

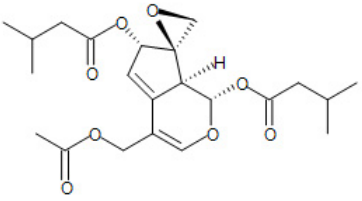

Figure 2: Valtrate (iridoid)

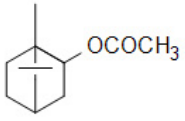

Figure 3: Bornyl acetate (essential oil)

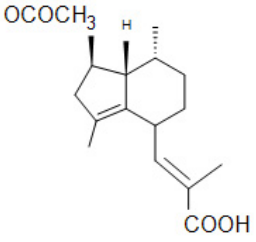

Figure 4: Acetoxyvalerenic acid (sesquiterpene)

(Barnes 2007, Hechtman 2012)

Preparation method/s

Valerian is native to Europe and Asia and was naturalised in North America but has been observed as being a difficult herb to cultivate in Australia (Wyk 2009, Hall 1988). Preferring a moist lime rich soil with roots in the shade, its flowering parts favour gentle morning sun. In cultivation, the flowers are removed to encourage rhizome growth; the rhizomes and roots are then harvested in the second year once the leaves have died off (Bown 2008, Hall 1988, American Herbal Pharmacopoeia (AHP) 1999). The essential oil content can vary with harvest times and older literature reports the ideal time is the autumn. Other factors influencing plant chemistry are growing conditions, age of root, drying techniques and method of analysis (AHP 1999). The roots and rhizomes must be dried carefully at a temperature not above 40°C according to ESCOP (1996). Interestingly, depending on this development it has been observed that valerian may show individually different results, even though commercially it is more cost effective to harvest the roots the same year the plants are sown rather than in the second year (AHP 1999, Hall 1988). According to Adams (2006), the liquid extract is made at a ratio of alcohol to water of 45:55.

Historical information

Valerian is considered one of the most important herbal sedatives and is also considered the most widely recognised herbal tranquilliser in Australia (Wyk 2009, Hechtman 2012). Valerian has a history that is over 2000 years old with Dioscorides (AD 40-80) writing on several species of it, with others also reporting on its sedative effects, including Galen (ca AD 131-208) (AHP 1999). Valerian has a rich folklore tradition of use in many countries, although its origin is considered to be placed in Europe, Asia and then naturalised in North America. It is rumoured to have been used by the Pied Piper of Hamelin in his quest to remove vermin as well as being associated with strange behaviour in felines (Hall 1988, Sarris 2010, Weiss 1988). The name itself, valerian, was first used in domestic books of home remedies around the 11th century. It was described for use to treat epilepsy in the late 16th century by Fabius Columba (AHP 1999, Griggs 1997). According to its energetics, valerian was considered by Disocorides to possess 'warming properties' and it was widely used by Eclectic physicians who commented that valerian was 'one of the best calmatives for the collective condition termed nervousness' (Sarris 2010, Hechtman 2012). It was included in *King's American Dispensatory* and quoted as an aromatic stimulant as well as having some other unique indications. One of them includes having 'bitter' properties (Bown 2008, Culpeper 1826, AHP 1999). Finally it was listed in both the *United States Pharmacopoeia* (USP) from 1820-1936 and the *British Herbal Pharmacopeia* (BHP) in 1867 and then in the *European Scientific Cooperative of Phytotherapy* in 1996. Additionally it is now included in *Commission*

E (Thomsen 2009, ESCOP 1996). Valerian has been used as a flavouring agent in some foods and liqueurs as well as being used as bait to trap wild cats and rodents (Bown 2008). Valerian now continues to be both a safe and effective herbal remedy for many uses which will be discussed in the next section.

Medicinal actions (contemporary usage)

Medicinal actions include sedative (AHP 1999), hypnotic (BHP 1976), anxiolytic (Mills 2000), spasmolytic (Bone 2003), hypotensive (Barnes 2007, AHP 1999), carminative (BHP 1976), mild anodyne (Barnes 2007), nervine tonic (Thomsen 2009), cerebral stimulant (Felter 1922). Other uses include anti-arrhythmic (the valepotriates thought to be responsible are unlikely to be present in commercial products).

Homeopathic uses include neuralgic pains, rapid pulse and blood congestion in the head (Lockie 2000).

Medicinal indications (contemporary usage)

Medicinal indications for the use of valerian include insomnia (Bent 2006), restlessness and anxiety (Thomsen 2009), dysmenorrhea (Mirabi 2011), nervous headache (Sarris 2010), nervous tension (Mills 2000), hypochondriasis (BHP 1976), symptoms of menopause (Wyk 2009), nervous palpitations (Weiss 1988), nervous asthma (AHP 1999), benzodiazepine withdrawal (Head 2009b), restless leg syndrome (Cuellar 2009), rheumatic pains (BHP 1976), ADHD (AHP 1999), migraine (Thomsen 2009), chorea (Bone 2003), intestinal colic (BHP 1976), cholecystitis (Hechtman 2012), epilepsy (Griggs 1997), obsessive compulsive disorder (Pakseresht 2011), anxiety and alterations in hyperthyroidism (Hechtman 2012).

Externally it is indicated for treatment of eczema (Bown 2008), ulcers (Bown 2008), minor injuries (Bown 2008) and may be used as a bath additive for its sedative properties and effects on fibromyalgia (Mills 2000, Braun 2010). The specific indication for valerian is conditions presenting with nervous excitability (BHP 1976).

Pharmacokinetics

Authors Barnes et al (2007) state that there is limited data on the pharmacokinetics of *Valeriana officinalis*. Furthermore the AHP (1999) has reported that the only available data is regarding its valepotriates, although they have shown that when administered to rats (in vivo) the valepotriates were absorbed poorly with only 0.19% absorption efficiency. They also observed that the greatest quantity was from the stomach lining and intestines.

According to Barnes et al (2007) the mean elimination half-life for valerenic acid in valerian is 1.1 hours. The pharmacokinetics for valerian were explored with six healthy volunteers in a single dose study receiving a 70% ethanol extract of valerian root (5:1) equating to 600 mg in the morning. Maximum serum concentrations of valerenic acid occurred between one and two hours after administration. For one subject however, maximum blood serum concentration peaked at both one and five hours after administration. It appears that the direct antispasmodic activity of valerian is attributed not to its work on the ganglion receptors but to the smooth muscle receptors. Valerian oil exhibited antispasmodic activity on isolated guinea pig uterine muscle, however it proved inactive when tested in vivo (Anderson 2005). Authors Donovan et al (2004) and Barnes et al (2007) also found that typical doses of valerian in healthy volunteers were unlikely to produce significant effects on CYP3A4 activity (the protein that catalyses many reactions involved in drug metabolism) and no effect on CYP2D6 (liver microsomal enzymes) pathways of metabolism. Although both a small and preliminary investigation, it does indicate that valerian is unlikely to participate in interactions with drugs that are dependent on those pathways of metabolism. Further research is needed however, in regards to the pharmacokinetics of valerian, including those of different constituents and preparations.

Pharmacodynamics

Much of the research into the pharmacological action of *Valeriana officinalis* has been focused on its sedative and spasmolytic properties (AHP 1999). The essential oils appear to have the sedative effect; while the valepotriates seem to have a regulatory effect on the autonomic nervous system (Head 2009a). This effect appears to be due to the fact that valerian interacts with neurotransmitters such as gamma-aminobutyric-acid (GABA). As well as producing a release of GABA that is dose dependant, it also inhibits the enzyme induced breakdown of GABA in the brain, with an affiliated sedation. Additionally Head and Gregory (2009a) and Heinrich et al (2004) state that it binds to benzodiazepine receptors and this also confirms other research reported by Braun & Cohen (2010) that found it comparable to oxazepam in a double blind trail. There appears to be an interaction between hydroethanolic extracts of valerian root and the GABA benzodiazepine-chloride receptor channel complex in in vitro experiments (ESCOP 1996). There was an affinity for GABA receptors (Wyk 2009) although at this stage the constituents responsible for the activity were not identified. In short the neuropharmacological activity of valerian appears to be complex, and only partially understood.

Relevance of pharmacodynamics research to contemporary usage

There is a wide variety of opinions about whether the research information and the contemporary usage of valerian are consistent. For example in 2010 a meta-analysis of eight randomised control trials (RCTs) was selected to investigate the use of valerian in the treatment of insomnia. The authors Fernandez-Sab-Martin et al

concluded that although its effectiveness for 'subjective' improvement of insomnia was verified, they recommend that to improve insomnia, future investigations should be oriented towards other 'more promising treatments'. It was also said that valerian has not been demonstrated to be effective through 'quantitative or objective measurements'. Furthermore Anderson et al (2005) stated that 'the evidence for the efficacy of valerian to improve sleep remains weak'. However many authoritative herbal texts, both old and new, affirm its use (AHP 1999, BHP 1976, Felter 1922). In light of this the evidence base on valerian will need to be improved.

A further systematic review and meta-analysis was published in the *American Journal of Medicine*. Authors Bent et al (2006) stated that after an 'extensive literature search' they found 16 randomised controlled trials. Of these only 6 studies were methodologically robust enough to include. The authors concluded that there were 6 studies with a dichotomous outcome of quality of sleep that showed statistically significant benefit, although there was evidence present of publication bias in this summary measure. It should also be noted that 9 of the studies did not have positive outcomes with regard to sleep quality, but the authors did determine that 'the available evidence suggests that valerian might improve sleep quality without producing side effects.

Future studies should assess a range of doses of standardised preparations of valerian and include standard measures of sleep quality and safety'. There was also agreement with Bent et al (2006) from Fernandez-Sab-Martin et al (2010) that there was a 'statistically significant improvement in the subjective variable of sleep quality'. Bent et al (2006) argues that there was an 80% greater chance of sleep improvement when compared with placebo. Furthermore the AHP (1999) argues that their research suggests the contemporary valerian usage is consistent with the research information. In the cases where the research and valerian usage is not consistent one may hypothesise that there may be bias in the research publication (Bent 2006). Of course this opinion is argued by others, such as Fernandez-Sab-Martin et al (2010) who claim there is no publication bias in the very same research.

As we have seen, valerian has over two centuries of use. The two different epistemological approaches (traditional evidence vs. statistical and scientific evidence) are not likely to always be consistent and sometimes, but not always, yield different results. However it seems that both approaches typically refer to the efficacy of valerian in sleep disturbances.

Cautions

Valerian appears to be quite a safe herb. Authors Fernandez-Sab-Martin et al (2010) stated that 'the safety factor valerian offers makes it highly desirable compared to pharmacological alternatives for insomnia'. While in theory morning somnolence is a possible side effect of therapy as with numerous pharmaceutical sedatives, evidence suggests that this is not associated with valerian (Braun 2010). However for concurrent use of valerian with pharmaceutical drugs, authors Barnes et al (2007) state that there is only limited data available for potential interactions with other medicines. They went on to further say that there may be interactions with barbiturates and that concurrent sedative use is not recommended. According to Braun & Cohen (2010) the use of valerian and alcohol appears to be acceptable. As for the toxicity of valerian, one case study reported a dose taken at 20-fold the recommended therapeutic dose and appeared to not be life threatening. Furthermore physical addiction appears unlikely and illustrates valerian's therapeutic attractiveness when compared with its pharmaceutical counterparts (Braun 2010).

In older literature it was reported that atropine decreases the hypotensive activity of valerian by 50%, although the cause has not been established (AHP 1999). In addition hepatotoxicity has been associated in the past, admittedly isolated (Heinrich 2004). One study did report vivid dreams, headache and gastrointestinal symptoms occurring, although this is considered rare (Braun 2010). Use with pregnancy appears unrestricted although safety in pregnancy has not yet been established (Braun 2010). It should be noted that in some rare cases valerian may cause nervousness and heart palpitations in some sensitive individuals (AHP 1999).

Contraindications

It is not recommended to administer valerian to children under 3 years of age (Thomsen 2009).

Dosage

The dosage range for valerian varies considerably in the literature, particularly in older texts (Barnes 2007).

- 3-9 g dried root/rhizome per day (Bone 2003)
- 2-6 mL 1:2 liquid extract per day or 5-15 mL 1:5 tincture per day (Mills 2000)
- 1-3 g as an infusion or decoction up to three times a day (Barnes 2007)
- Essential oil 0.05-0.25 mL (2-6 drops) up to two times daily (AHP 1999)
- As a bath additive 100 mg (AHP 1999)

Clinical trials provide the following recommendations:

- Insomnia: doses vary from 400 mg per day (3:1) to 1215 mg per day (5 to 6:1) (Barnes 2007)
- Restless leg syndrome: 800 mg dried root/rhizome per day (Cuellar 2009)
- Dysmenorrhea: 255 mg (Mirabi 2011)
- Nervous tension (anxiety): 600 mg per day (Braun 2010)
- Fibromyalgia: 100 mg as a bath additive (AHP 1999, Braun 2010)
- Obsessive compulsive disorder: 765 mg (Pakseresht 2011)
- Anticonvulsant for epilepsy: an upper limit of 10 g per day (Eadie 2004)

- Benzodiazepine withdrawal with sleep disturbance: 100 mg three times daily (Head 2009a, Braun 2010).

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Essential oils and steam distillation

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What is essential oil?

When I think about this it seems such an enigma. Wikipedia describes it as 'a concentrated hydrophobic liquid containing volatile aroma compounds from plants', a pragmatic answer: a concentrated substance that is repelled from a mass of water (hydrophobic) that has smell or odour (aroma compounds). Apparently they are 'essential' as well!

To feel a plant, reach out and pick a leaf or flower, crush it in the palm of your hands and inhale — this is the silent language of aroma and sensation which cannot be translated into academic words that make sense. Aroma is certainly a powerful conduit of subtle change, response and reaction. Essential oils are aroma.

To be able to steam distil, gathered fresh plants from the garden or wildcrafted is a profound experience. Even though the process is quite simple, it becomes part of a magical alchemy of transformation. The energy of steam changes the chemistry of a plant so that its constituents are released into the steam. That in itself is enough, however that liquid then releases millions of tiny bubbles of aromatic essential oil. They float to the surface of the water and gathers there as the volatile aromatics – the essential 'soul' of the plant.

The process of steam distillation

Steam distillation is the method mostly used to produce essential oils. The plant material is subjected to steam under pressure. It does not come into contact with the boiling water. Water boils at 100°C while steam has a higher temperature than boiling water; a steam burn is worse than a boiling water burn.

Distilling for essential oil by steam distillation

Following on from the previous article on hydrosols and hydro-distillation (AJHM 24:3;101), rosemary (*Rosmarinus officinalis*) is an excellent plant to use to illustrate the difference between the two distillation methods and the differences in the hydrosols that are produced. A copper alembic column still is used for steam distillation. The only difference between this and the one used for hydro-distillation, the traditional alembic pot still, is that the former has a column that sits on the pot with a sieve plate at the bottom. The herb is placed in the column and the column is placed on top of the pot of boiling water. The sieve plate keeps the herb material above the water so that only the steam can come into contact with it.

The column still is set up in a sheltered spot in the garden. It has already been cleaned by distilling rye flour slurry. To do this 450 g of rye flour is mixed in in 4 L of water and poured into the pot. The whole still is assembled and the rye flour mix is heated until it boils, becomes frothy and seeps out through the joins. Once it comes out of the condenser bucket as a distillate the heat is turned off. When the still is cool enough to handle, it is dismantled and scoured with Eco Cream Cleanser which it contains a citrus oil that cleans the copper. It is rinsed and dried thoroughly, leaving it in the sun to finish the drying process. Before starting a new distillation, I ensure the still is totally clean by distilling hot water and checking that the distillate is tasteless, odourless and clear.

Traditional alembic column and parts

The pot is 2/3 filled with water and placed on a gas ring to bring the water to the boil. It is important to have everything at hand for a distillation. The water pump is kept on a steady flow of cold water running through the condenser bucket and the sterile beakers and oil separators are ready to collect the distillate. A bowl of rye flour paste is ready to seal the joins in the still so it doesn't lose precious steam.

And of course the herb has been harvested and prepared. The leaves and flowers have been stripped from the stems of the rosemary and sit fragrantly waiting.

Five good handfuls of rosemary are placed into the column. When the water in the pot is boiling, the onion dome is carefully removed and the column is placed on the pot making sure it is square. The onion dome is placed on the column, checking that it is level. Because the rosemary is cold there is time before the steam works its way through. Once it is heating it is important to work quickly so as not to lose any steam or precious essential oil. The joins are quickly but carefully sealed with the rye flower paste between the column and the pot and between the column and the onion dome. As the copper heats it bakes the paste dry and seals the joins.

By the time this is done the onion dome will be hot which means the steam has spiralled up the column. The heat bursts open the cells of the plant and the volatile oils

are released and carried in the steam. The steam spirals in the onion dome and any particles of dust or physical matter fall back. The steam flows down the bird's beak, through the connecting pipe and into the condenser coil.

Just as in the hydro-distillation it is vital to have a steady stream of cool water running around the condenser coil throughout the whole distillation. It is even more important when distilling with steam as it is a hotter, faster distillation. The water needs to be boiling vigorously to create a good body of steam under pressure to burst the cellulose of the plants and release the essentail oil.

The distillate will flow faster than a hydro-distillation. The essential oil will be released in the first 200 mL of distillate. A 10 L still should collect 500 mL of hydrosol. The oil has been expelled from the mass of water and floats to the top. The hydrosol is not as milky as it is in a hydro-distillation and of course there is significantly more oil released. The aroma is stronger, sharper and more intense. At this point the pH level is checked.

Depending on the purpose, distilling may be continued for more hydrosol. There will be very little oil in the next 500 mL but often the hydrosol is still of a good aroma, flavour and pH level. As soon as the pH increases the distillation is stopped.

This is the best part! The two oil separators, one 500 mL and the other 60 mL, are clamped on to a retort stand. The larger separator is used when distilling botanicals that will yield between 3 and 10 mL of oil from 500 mL, such as rosemary, eucalyptus, lavender, clary sage, manuka, thyme or peppermint. The smaller one is used to collect precious drops of plants such as rose geranium, balm, rose, lemon verbena, chamomile or yarrow.

The hydrosol is poured into the large oil separator. As this is done the oil mixes with the hydrosol again. This is really something to watch as the millions of miniscule bubbles stream to the surface, tiny drops of essential oil released from the water like a sponge being squeezed. The oil collects at the top, often a golden colour, and the hydrosol settles and clears.

A clean beaker is placed at the bottom of the oil separator. The tap is carefully turned on to release the hydrosol, leaving the oil to collect at the bottom of the separator. Once the hydrosol has drained off completely, the tap is turned off. The reward is 3 mL of precious essential oil! It will need to be distilled another 3 times to get the desired 10 mL, but with this will come 2 litres of fragrant rosemary hydrosol.

At this stage there will still be some moisture in the oil. If left, bacterial growth and degradation of the oil could result. The test tube with the oil is put into the freezer where it dries the oil out and freezes any moisture. The oil is then poured off into an amber dripulator bottle and becomes part of the precious collection of my own distilled essential oils.

By this time next year I hope to have 25 of my own distilled oils. Already in stock is 50 mL eucalyptus, 50 mL peppermint, 10 mL rosemary, 3 mL lemon verbena, 10 mL lime, 10 mL manuka, 10 mL ginger and 10 mL thyme. Spring is the time for harvesting German chamomile, orange, grapefruit and lime flowers, spring manuka and kanuka, tarata, rose geranium, bay leaves and balm. In summer it will be lavender, clary sage, lemon verbena, basil, peppermint, *Helicrysum* (immortelle or everlasting oil), yarrow, angelica and some trimming of the citrus as the fruits form tiny balls for petitgrain.

Every distillation is different depending on the season, soil and climate. The table below shows an approximate guide to volumes and quantities expected; for example the harvest of three mature flowering tops of *Lavendula augustifolia* weighs approximately 1 kg and will yield approximately 25-30 mL of oil. Most other plants will yield only a quarter to half that amount.

Jill Mulvaney set up and ran a natural skincare business for many years which found her importing raw materials, manufacturing and teaching. Jill and her partner are both avid distillers of hydrosol, essential oils and spirits. They run workshops and demonstrations throughout NZ and sell alembic stills worldwide. They share the knowledge of this ancient process, using natural organic seasonal botanicals and beautiful handcrafted copper. www.alembics.co.nz.

Approximate guide to volumes and expected quantities

Size	Plant quantity	Example of *Lavandula augustifolia*	Approx. yield of essential oil	Hydrosol
5 L column	250 g or 2-3 handfuls	1 mature plant flowering tops	1-2 mL	250-400 mL
10 L column	500 g or 5-6 handfuls	1.5-2 mature plants	3-5 mL	500-1.5 L
20 L column	1.5 – 2 kg	4-6 mature plants	5-15 mL	3-5 L
40 L column	5 kg	8-10 mature plants	20-50 mL	5-10 L
150 L column	20-30 kg	60-90 mature plants	200-500 mL	20-50 L

Comparison of hydro-distillation and steam distillation

Hydro-distillation	Steam distillation
Plant is subjected to boiling water	Plant is subjected to pressure and steam
Distillation is slower and cooler	Distillation is fast and hot
Hydrosol is often milky	Hydrosol is mostly clear
The distillate shows little essential oil floating on the surface, most remains in suspension	Essential oil is evident on top of the hydrosol and is removed
Aroma is complex as are the flavours	When the oil has been removed the aroma of the hydrosol is light and delicate, the flavour less intense
Nothing has been separated from the distillate, it remains complete	Has a dual result with both essential oil and the hydrosol, however the hydrosol has lost the element of the essential oil

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Constant severe nausea accompanied by weight loss in a 24 year old male

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Blastocystis is a single cell parasite that infects the gastrointestinal tract of humans (where it is referred to as *Blastocystis hominis*) and animals. Previously considered to be a harmless yeast present in normal intestinal flora, *B. hominis* has more recently and controversially been described as a pathogenic protozoan which is frequently accompanied by intestinal symptoms (Selcuk 2007).

Presenting complaint

Aaron, a 24 year old part time university student and retail assistant, presented with severe ongoing nausea which had occurred daily for the last 18 months and weight loss of approximately 30 kg due to feeling unable to eat without experiencing increased nausea. He was fatigued throughout most of the day.

Aaron's nausea was worse in the morning, immediately before and after meals, with large meals, and with physical exertion of any kind. He reported that his digestive problems had initially begun the morning after a night of excessive alcohol and food intake. Aaron believed it was possible that one of his drinks might have been 'spiked' as he had felt unusually unwell while drinking.

Frustrated with a diet of mainly bread, crackers, pasta and water, and exhausted by his ongoing symptoms, Aaron expressed concern that he may eventually become so ill that he would have to quit both uni and his job.

Medical history

Aaron's childhood history was unremarkable with no major illnesses or injuries. He was not aware of any allergies and could not recall any previous digestive issues apart from the occasional childhood 'bug'.

Family and social history

There was no family history of any serious medical conditions or digestive disorders and none of his friends became ill after the same bout of drinking. He had travelled overseas to China 24 months prior to becoming ill. Aaron admitted consuming excessive amounts of alcohol on approximately a monthly basis prior to the development of his current condition.

Pathology and investigation

Shortly after first experiencing symptoms a fecal sample was found to contain *Blastocystis hominis*. Aaron was prescribed the antibiotic Flagyl (metronadiazole). He was unsure if he completed the course as he experienced a worsening of symptoms while taking the antibiotic. A follow up stool examination was not performed.

Three months after onset Aaron's doctor referred him for an endoscopy, ultrasound of the abdomen, comprehensive blood tests including liver function and a food movement test. All tests were NAD with the eventual diagnosis being 'possible functional dyspepsia'. Aaron had also performed a finger prick 'self test' for gluten intolerance which had returned a negative result.

Over the following months Aaron's doctor prescribed Nexium (proton pump inhibitor) which worsened the nausea. Several other PPIs were trialled with the same effect. Motilium (domperidone) made no change to symptoms. Two weeks prior to his visit Aaron had commenced taking the tricyclic antidepressant Endep 25 (amytriptyline) at night which had lessened his morning nausea but made him feel dizzy and 'hung over'.

Observations and physical examination

Bowel function was regular producing a stool of formed to hard consistency every second day. Aaron mentioned experiencing diarrhea and cramping around the time he initially developed nausea, but now only experienced these symptoms occasionally.

Ongoing digestive problems included constant severe nausea, occasional belching, some flatulence, no sensation of reflux or regurgitation, fullness after eating even a small amount of food and difficulty swallowing large mouthfuls of food or large tablets.

His current weight was 62 kg (previously 92 kg) and he was 178 cm tall. Dark circles were observed under the eyes and the fingernails had deep vertical ridges. Zinc test recorded little or no taste sensation. Urinary indican test returned a high level result but due to the lack of protein in Aaron's diet the results were considered potentially inaccurate.

Treatment

The initial treatment plan focused on providing symptomatic relief, soothing and regulating digestive function and modifying bowel flora. Aaron was very reluctant to change his current diet at this stage so was instead asked to keep a comprehensive diary of all food consumed, any symptoms experienced and to maintain his water intake. Prescribing options were tailored to suit Aaron's very tight budget and the difficulty he had in swallowing tablets:

Probiotic and vegetarian enzyme combination: one capsule opened and taken with a little manuka honey three times daily 15-30 mins before food.

Ulmus rubra powder (which he had on hand already): one teaspoon combined with a little water or mashed banana three times daily after or between meals.

Zinc supplementation was not started at this stage due to the possibility of inducing nausea.

Herbal formula

Herb	Conc.	Total
Berberis vulgaris	1:2	20 mL
Matricaria recutita	1:2	20 mL
Echinacea root blend	1:2	25 mL
Hydrastic canadensis	1:3	15 mL
Althea officinalis	1:5	20 mL
TOTAL		100 mL

Dose 5 mL twice daily for the first week, thereafter 7.5 mL twice daily.

All products to be taken at least 2 hours away from prescription medication to reduce the possibility of herb/medication interaction, specifically altered drug absorption or clearance.

I recommended repeat fecal testing for *Blastocystis hominis* and a 'parasite and ova screen'. Blood testing for liver function and a full blood count were also requested. Aaron was confident that his GP would be able to authorise the tests, removing the necessity for referral to a private pathology service.

Follow up

At his second consultation 2 weeks later Aaron reported a significant lessening in the frequency and severity of nausea. Consequently he had been able to eat more and increase his activity levels. He had experienced some bloating initially after taking the digestive enzyme/probiotic, but this had resolved after a couple of days. Aaron had approached his doctor about reducing the dose of Endep with the intention of discontinuation and planned to do so over the next 2 weeks.

Fecal tests revealed the presence of *B. hominis*. Fecal testing for other parasites was not performed. Blood tests were all within normal range with the exception of a mild elevation in alanine transaminase (ALT) and aspartate transaminase (AST). Elevated AST and ALT levels can be indicative of liver inflammation or hepatocellular injury: ALT 45 U/L (5 -40), AST 49 U/L (10-40).

Treatment

With a repeat positive diagnosis of *B. hominis* and mildly irregular liver chemistry, treatment was aimed at reducing gut parasite levels, balancing gut flora, supporting liver function and stimulating digestion.

Aaron was advised to continue *Ulmus rubra* powder as desired for symptomatic relief. Dietary recommendations included reducing wheat based products, reintroducing vegetables and lean protein and implementing five small regular meals daily. Aaron had been given a pure rice protein supplement by a friend and intended to use it to supplement his dietary protein intake if needed.

Herbal treatment

Herbal capsule containing extracts equivalent to dry:

Herb	Total
Juglans nigra fruit hull	400 mg
Artemisia annua herb	400 mg
Tabebuia avellanedae inner stem	200 mg
Berberis vulgaris stem bark	360 mg
Allium sativum bulb	720 mg
Citrus paradisi seed	250 mg
Thymus vulgaris oil	2 mg
Rosmarinus officinalis oil	1 mg
Origanum vulgare oil	10 mg

Capsule to be opened and mixed with manuka honey three times daily.

Herbal digestive and liver support formula tablet containing extracts equivalent to dry:

Herb	Total
Silybum marianum fruit	2.1 g
Taraxacum officinale root	500 mg
Citrus reticulata fruit peel	500 mg
Gentiana lutea root	100 mg
Zingiber officinale rhizome	100 mg
Citrus reticulata essential oil	12.5 mg
Matricaria recutita flower essential oil	5 mg

Dose one tablet to be sucked for 1 minute then chewed before each main meal.

All products to be taken at least 2 hours away from prescription medication.

Follow up

Over the next month Aaron reduced and then ceased Endep, with the support of his GP. This worsened his digestive but then showed steady improvement. Three months after his initial consultation he was free of digestive problems with the exception of occasional morning nausea. Bowel function improved to one bowel motion every day. Aaron's energy and physical activity levels improved as did the variety of his diet and food intake. I continued to work with Aaron to rebuild confidence in his ability to make sound dietary choices. Further prescriptions included a probiotic formula to restore beneficial gut flora and mineral supplementation. Aaron continues to take the herbal digestive and liver support tablet once daily.

Discussion

Although *Blastocystis hominis* is often found in asymptomatic individuals, its presence in this case was significant due to the patient's previous good health and the absence of other irregular test results. It is difficult to determine if Aaron's mildly elevated AST and ALT levels were of any clinical significance in relation to his symptoms as these chemical changes have many potential causes including prescription medication reactions and alcohol intake.

Aaron may have contracted the parasite on or prior to his night of overindulgence, but it is possible in my opinion that his extreme food and alcohol intake on that occasion may have resulted in sufficient gastrointestinal irritation to prevent his usual gut based immune defences from dealing with the parasite in an appropriate way.

Aaron's wholehearted commitment to and compliance with treatment, including the less palatable forms of herbal treatment necessitated by his swallowing difficulties, resulted in a swift recovery. Aaron is on track to graduate from university at the end of this year, still works part time and has rejoined his rugby team. He has sworn off binge drinking and is attempting to convince his mates to do the same!

Iron inhibition by plant polyphenols: an adjunct to treatment in hereditary haemochromatosis

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Reviews of articles on medicinal herbs

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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.

Licorice looks promising for treatment of periodontal diseases

Messier C, Epifano F, Genovese S, Grenier D. 2012. Licorice and its potential beneficial effects in common oro-dental diseases. *Oral Dis* 18;32–9.

Glycyrrhiza glabra and *Glycyrrhiza uralensis* are the most common sources of licorice used in herbal medicine. Licorice is produced from unpeeled roots and stolons of those species. This article reviews the effects of licorice extracts and some of secondary *Glycyrrhiza* metabolites on the oral microenvironment.

Generally licorice is recognised as safe when used in foods and over the counter medications, however consumption of large amounts of licorice may cause hypertension through inhibition of the renal enzyme 11 beta-hydroxysteroid dehydrogenase which converts cortisol into cortisone. The inhibition causes an increase of cortisol in the collecting ducts of kidneys which in turn causes excretion of potassium and retention of sodium.

Development of dental caries is a chronic disease of progressive destruction of the hard tooth structures by acidogenic/aciduric bacteria embedded in the dental plaque. The primary agents of caries are mutans streptococci: *Streptococcus mutans* and *Streptococcus sorbinus*. In addition to producing organic acids *S. mutants* produces extracellular polysaccharides which contribute to formation of biofilm, trapping those acids at the tooth surface. The acids cause dissolution of calcium and phosphate from enamel structure. While glycyrrhizin did not affect the *S. mutants* growth, studies have shown that glycyrrhizin inhibited glucosyltransferase activity which is involved in biofilm formation.

Glycyrrhizol A, glycyrrhizol B and two other compounds isolated from *G. uralensis* have been reported to exhibit antibacterial properties. Sugar free licorice lollipops enriched in glycyrrhizol A used twice daily caused marked reduction in salivary *S. mutants*.

Periodontal diseases are associated with different types of pathogens. Gram negative anaerobic bacteria accumulate in sub gingival areas producing toxins and causing inflammatory response from the host. A crude extract from *G. uralensis* was reported to inhibit the growth of *P. gingivalis*, a key agent of chronic periodontitis. A licorice constituent 18 beta-glycyrrhetinic acid was shown to markedly reduce alveolar bone loss in mice infected with a virulent strain of *P. gingivitis*. Alveolar bone destruction is mediated by activated osteoclasts. It was shown that isoflavonoid glabridin could inhibit osteoclast maturation.

Denture stomatitis affecting denture wearers is an inflammation of palatal mucosa characterised by creamy white pseudomembranes. Immune status of the host and virulence of *C. ablicans* are key factors in initiation of oral candidiasis. The ability of *C. ablicans* to form biofilms makes this pathogen resistant to antifungal medications. Studies investigating the fungicidal effect of two licorice compounds, licochalcone A and glabridin found that licorice phenol licochalcone A inhibited biofilm formation by 30-80%.

Animal studies have shown that flavonoid liquiritigenin had immunomodulating activity protecting mice from disseminated candidiasis.

Some studies investigated the effect of licorice on controlling pain and reducing healing time in aphthous ulcers also known as canker sores. The studies produced conflicting results indicating that more research was required.

Considering possible adverse effects of prolonged intake of licorice, a localised application may present an attractive option for acting on pathogens and improving host inflammatory response in periodontal diseases.

Green tea mediated suppression of IgE production

Wu S, Silverberg J, Joks R, et al. 2012. Green tea (*Camelia sinensis*) mediated suppression of IgE production by peripheral blood mononuclear cells of allergic asthmatic humans. *Scand J Immunol* 76:3;306–10.

Allergic asthma, a condition characterised by airway hyper-responsiveness and bronchial inflammation, affects approximately 25% of the world population. Green tea (*Camelia sinensis*) is a traditional beverage and remedy known for beneficial properties such as antioxidant and anticancer activity. It also contains bioactive ingredients including polyphenols, catechins and caffeine.

This in vitro study aimed to evaluated how green tea extract (GTE) and the purified catechin epigallocatechin gallate (EGCG) act on the induction of IgE immune responses.

Peripheral blood (40 mL) was drawn from three allergic asthmatic patients and immunoglobulin (Ig)

levels (IgM, IgG, IgA, IgE) were detected in serum. None of the subjects had received allergen immunotherapy within the previous 6 months.

The blood samples were cultured and exposed to several different extract doses. When 1, 10 or 100 ng / mL of GTE was added to cultures, IgE production was suppressed in a dose dependent manner (89.3 ± 5.7%, 56.9 ± 8.9%, 0.2 ± 4.1% respectively), compared with control (general linear models, P = 0.07, <0.0001, and <0.0001, respectively). When 5 or 50 ng / mL of EGCG was added to cultures, IgE production was also suppressed in a dose dependent manner (87.0 ± 7.0% and 72.6 ± 14.4% respectively), compared with none (P = 0.02 and <0.0001 respectively). The addition of cat pelt antigen (1 AU/ mL) and GTE (1–100 ng / mL) or EGCG (0.5–50 ng / mL) also resulted in suppression of IgE production (up to 31% and 98% respectively).

The researchers concluded that this demonstrated GTE, or its catechin EGCG, suppressed in vitro allergen and non-allergen specific IgE production in allergic asthmatics, further suggesting that GTE or EGCG has immunoregulatory effects on human IgE responses.

These results suggest a potential therapeutic option using components of green tea to treat asthma and other diseases of altered IgE regulation. Further investigation is warranted to assess clinical application.

Olive leaf as a hypoglycemic agent

Wainstein J, Ganz T, Boaz M et al. 2012. Olive leaf extract as a hypoglycemic agent in both human diabetic subjects and in rats. *J Med Food* 15:7;605–10.

Olive tree (*Olea europaea* L.) leaves have been used as a traditional remedy in European and Mediterranean countries. They contain bioactive compounds that have been attributed with a number of effects, including antioxidant, antihypertensive, antiatherogenic, anti-inflammatory, hypoglycemic and hypocholesterolemic. The effects of olive leaf extract (OLE) are attributed to antioxidant and phenolic components such as oleuropein, which has previously been reported to have anti-hyperglycemic action in animal studies.

Diabetes is a common condition that is creating an increasing burden of disease in developed and developing countries. Olive leaf tea and chewing olive leaves are traditional folk remedies for the treatment of diabetes.

This two arm randomised placebo controlled trial investigated whether OLE impacted on glycemic control in diabetic humans and animal models.

In the first arm, 79 adults with type 2 diabetes mellitus (T2DM) were randomised to treatment with 500 mg olive leaf extract tablet or matching placebo once daily for a period of 14 weeks. The eligible patients had been diagnosed with T2DM at least 1 year before the study's onset, were 18–79 years of age, had a body mass index < 40 kg/m^2, Hba1c < 10%, and were on oral and/or diet therapy for T2DM. Participants were instructed to consume a diet consistent with American Dietetic Association recommendations and an exercise training program was prescribed. The OLE tablet or matching placebo was taken orally once daily throughout the study period, before breakfast. All subjects maintained their usual diabetes therapy which consisted of the oral hypoglycemic agents sulfonylurea and/or metformin. None were treated with insulin.

Those treated with OLE for 14 weeks had significantly lower HbA1c levels than placebo (P= 0.037). Compared with placebo, OLE treatment was also associated with a significant decrease in fasting insulin levels (P= 0.01). Postprandial insulin and glucose levels did not differ significantly.

Concurrently, in a series of animal models, normal, streptozotocin (STZ) diabetic and sand rats were used in the inverted sac model to determine the mechanism through which OLE affected starch digestion and absorption.

In the in vitro animal model OLE inhibited both digestion and absorption of starch in a concentration dependent manner. A distinct dose response was observed at concentrations of 10, 20 and 40 mg. In vivo OLE was added to starch and given by intubation to healthy rats. Significantly lower blood glucose levels at 30, 60 and 120 min after the intubation were observed. When repeated in STZ diabetic rats, the addition of OLE to the starch administered by intubation resulted in significantly lower blood glucose tolerance.

In vitro and ex vivo experiments in animal models imply the inhibition of starch absorption as a possible mechanism through which OLE reduces blood glucose tolerance curves. However animals were administered much higher doses than the human subjects participating in the clinical trial arm.

The researchers concluded that the reduction of HbA1c in humans receiving 500 mg/day doses of OLE suggests that clinical efficacy requires a relatively low dosage. Furthermore the high dose delivered to animals was not associated with adverse events, indicating that lower doses of OLE can be considered safe for human use.

The study results suggest that treatment with OLE is associated with a beneficial hypoglycemic effect in patients with diabetes and low risk of adverse effects, even at a higher dose.

Ginsenoside: novel agent for breast cancer?

Wang W, Zhang X, Qin J-J, Voruganti S, Nag SA, Wang M-H et al. 2012. Natural product Ginsenoside 25-OCH$_3$-PPD inhibits breast cancer growth and metastasis through down-regulating MDM2. *PLoS ONE* 7:7;e41586.

Breast cancer, a leading cause of death amongst women, has prompted researchers to explore novel therapeutic agents which are both effective and safe with prolonged administration. Ginsenosides, the saponin constituents of ginseng, have clinically demonstrated

anti-tumour effects.

A recently isolated component of *Panax notoginseng*, 20(S)-25-methoxyl-dammarane-3β, 12β, 20-triol (25-OCH_3-PPD) was studied to evaluate its effect on tumour growth as well as its mechanism of action on primary and metastatic breast cancers in mice.

Female nude mice bearing human breast cancer xenografts of MCF7 (wild type p53) or MDA-MB-468 (mutant p53) cells, were randomly divided into treatment groups and control group (10-15 mice/group). The intervention 25-OCH_3-PPD was administered by i.p. injection at doses of 5 or 20 mg/kg/day, 5 days per week for 6 weeks (MCF7) or 4 weeks (MDA-MB-468) respectively. The control group received the vehicle only.

Results show that at 5 and 20 mg/kg the intervention inhibited MCF7 xenograft tumour growth by around 60% and 90% respectively ($p<0.01$) and MDA-MB-468 tumour growth by 50% and 87% respectively ($p<0.01$). Body weights between the intervention and control groups were not significantly different. Altogether the data demonstrates that 25-OCH_3-PPD has significant anti-breast cancer activity in vivo and no indication of toxicity at the doses given over the treatment period.

The researchers then assessed the underlying mechanisms of action of 25-OCH_3-PPD in vitro and in vivo with particular focus on MDM2 expression, again in MCF7 and MDA-MB-468 cells. MDM2 is an oncogene which plays an important role in cancer development, progression and metastasis. It is amplified and over-expressed in various human cancers and high levels are suggestive of a poor prognosis.

Western blot analysis of tissue homogenates showed that MDM2 protein levels were decreased by 25-OCH_3-PPD in a dose and time dependent manner, suggesting that MDM2 is a target of the intervention. Further analyses revealed that MDM2 transcription was reduced in a dose dependent manner ($p<0.01$) when exposed to various concentrations of 25-OCH_3-PPD for 24 hours. Compared with vehicle control, there was a demonstrated destabilisation and turnover of MDM2 protein in both cell types after exposure to 25 μM of the ginsenoside for 24 hours.

In previous studies it has been observed that 25-OCH_3-PPD can decrease cell survival, inhibit cell proliferation, induce cell apoptosis and initiate cell cycle arrest in breast cancer cells. By deliberately over expressing MDM2, the researchers noted that antitumour activities of the intervention were reduced, further suggesting that MDM2 is indeed a target of therapy.

Finally the antimetastatic ability of the ginsenoside was demonstrated via inhibition of cell migration, especially at 10 μM. On days 8 and 15, 25-OCH_3-PPD showed significant inhibition of lung metastases compared with vehicle control.

Favourable safety profiles at effective doses and significant antimetastasis activity of this ginsenoside warrant further investigation of this novel MDM2 inhibitor as a potential human breast cancer agent.

Bilberry may ameliorate stress induced depression

Kumar B, Arora V, Kuhad A, Chopra K. 2012. *Vaccinium myrtillis* ameliorates unpredictable chronic mild stress induced depression: possible involvement of nitric oxide pathway, *Phytother Res* 26;448–97.

Vaccinum myrtillus (bilberry) has long been known as a potent inhibitor of reactive oxygen/nitrogen species. Unpredictable chronic mild stress (UCMS) induced oxidative damage has been postulated to be involved in the etiopathogenesis of depression. This study explores the nitric oxide (NO) mechanism in the bilberry effect against UCMS induced depression. NO is a transmitter molecule synthesised from its precursor L-arginine by nitric oxide synthase (NOS). Several studies have shown that NOS inhibitors demonstrate preclinical antidepressant like properties.

Male LACA mice (Laboratory Animal Centre A-strain) were randomised into nine groups. Each group received different combinations of the following drugs: bilberry extract, fluoxetine (Prozac), L-arginine and L-NAME which is an NOS inhibitor. Each group excluding the control group was subjected to stress for 21 days. On day 21 the animals went through forced swim test (FST), locomotor activity test, elevated plus maze (cognition) test and chemical and neurological evaluations.

Exposure to stress significantly increased the immobility period in FST. Daily bilberry extract administration (125, 250 and 500 mg/kg) dose dependently reversed the immobility period increase. The efficacy of fluoxetine was similar to that of 500 mg/kg bilberry extract. Administration of L-arginine increased the immobility time suggesting NO involvement. L-NAME potentiated the antidepressant action of the sub-effective dose of bilberry extract (250 mg/kg).

Daily stress caused significant decrease in locomotor activity of mice. The high dose bilberry extract (500 mg/kg) almost completely restored ambulatory movements. Again the effect was similar to fluoxetine. Interestingly the combination of bilberry extract with nitric oxide modulators did not show any significant change in movements.

The glutathione levels and enzymatic activities of superoxide dismutase (SOD) and catalase were significantly decreased in stressed mice. Bilberry extract dose dependently reversed that effect. The efficacy of 500 mg/kg dose was comparable to that of fluoxetine (10 mg/kg). Pre-treatment with L-NAME enhanced the effect of 250 mg/kg bilberry dose on antioxidant profile and glutathione levels.

The level of thiobarbituric acid which is formed as a by-product in lipid peroxidation was noticeably increased in chronically stressed mice. Daily treatment

with bilberry extract produced a dose dependent reduction in thiobarbituric acid reactive substances. Co-administration of L-arginine with bilberry extract reversed positive bilberry effect on the level of reactive substances. Pre-treatment with L-NAME increased bilberry effect in reducing lipid peroxidation.

Nitric oxide plays an important role in the central nervous system. Studies have shown that manipulation of NO pathways represents a novel therapeutic approach to the management of mental depression. This study leads to the conclusion that bilberry extract may produce an antidepressant-like effect by inhibiting the NO synthase enzyme. A sub-effective dose of bilberry may potentiate the effect of antidepressants belonging to the nonspecific nitric oxide synthase inhibitor group.

SJW and psoriasis

Najafizadeh P, Hashemian F, Mansouri P et al. 2012. The evaluation of the clinical effect of topical St Johns wort (*Hypericum perforatum* L.) in plaque type psoriasis vulgaris: A pilot study. *Australas J Dermatol* 53:2;131–5.

Psoriasis is a common condition characterised by hyperproliferation and abnormal differentiation of keratinocytes. Psoriasis often involves multiple body systems and recurs chronically. Plaque psoriasis is typified by red erythematous skin plaques, usually covered with silver flaking scales, that can be itchy or painful or both.

Conventional treatment for patients with mild to moderate psoriasis generally involves topical therapy, with systemic therapies and phototherapy used in moderate to severe cases.

St John's wort (*Hypericum perforatum*) is often used in herbal medicine for relief of stress and anxiety. One of the herb's constituents, hyperforin, has been reported to have wound healing, anti-inflammatory and anti-bacterial effects. Another marker constituent, hypericin, has been shown in animal studies to down regulate CD8 mediated cytotoxicity and inhibit tumour necrosis factor alpha (TNF-α) induced apoptosis in vitro and therefore modulate the immune system.

This trial aimed to evaluate the effect of a topical St John's wort ointment compared with placebo on plaque type psoriasis. The ointment formulated for the study was prepared from an ethanolic St John's wort extract (5% wt/wt), vaseline (84% wt/wt), propylene glycol (10% wt/wt) and avicel (1% wt/wt). The vehicle was the same in the placebo and the active ointments contained the same percentages of propylene glycol, vaseline and avicel (the ointment form was selected because of its stability). The pH of the formulation was measured using a digital pH meter during the stability studies at 0, 30, 60 and 90 days.

Ten patients aged between 20 and 55 years with mild plaque psoriasis were selected (mean PASI [psoriasis area severity index] scores 8.7 ± 3.8). The inclusion criteria were clinical diagnosis of symmetrical plaque type psoriasis. Overall 20 plaques were treated, two plaques in each patient.

Each patient had a 4 week washout of any topical and systemic therapy. They each received both the St John's wort ointment and a placebo, one applied to the right and the other to the left side of the patient's body randomly. The patients were asked to apply the formulated ointment and vehicle twice a day for 1 month. Photographs were taken at the beginning and at the end of the study.

The plaques were located on both sun exposed and unexposed sites although most of the plaques were located on unexposed sites.

A statistically significant difference was not found between the vehicle treated and drug treated sides before treatment ($P = 0.17$, $P = 0.76$, $P = 0.26$), but after treatment all three factors, erythema, scaling and thickness, were significantly lower where the active ointment had been applied ($P = 0.01$, $P = 0.004$, $P = 0.04$ respectively).

Compared with the plaques to which placebo ointment was applied, the active treatment resulted in significant suppression of erythema and plaque thickness, with the improved effect on scaling most obvious.

The main limitations of this study were the small sample size, the fact that the investigators were not blinded and the subjectivity of the outcome measures. However the findings are promising and larger studies are warranted to further investigate this treatment for plaque type psoriasis.

New international research confirms dose dependant efficacy of *Vitex agnus-castus* extract Ze 440 (Premular®) in relieving symptoms of PMS

Michelle Boyd, herbalist, and head of practitioner education at Flordis, focuses on a most effective herbal remedy for the treatment of premenstrual syndrome (PMS).

2011 systematic review of *Vitex agnus-castus* (VAC) evaluated the efficacy of this herb in alleviating symptoms of PMS and highlighted the only (better than placebo) benefits were derived from the use of European dosage forms.[1] Further, of the studies identified in this review, only one extract of VAC has received recognition for 'well established use' classification from the Committee on Herbal Medicinal products of the European Medicine Agency (EMA), namely Ze 440.[2,3]

Most recently (2012), new international research, published in the International Journal of Phytotherapy and Phytopharmacology, *Phytomedicine*, confirms that this unique VAC extract (Ze 440), is the most effective dosage and preferred option to relieve the symptoms of PMS in women.[4] This multicentre, double-blind, placebo-controlled, parallel-group study included 162 females aged 18 to 45 years with PMS. Participants were randomised to receive either placebo or one of three different doses of Ze 440 over three menstrual cycles. The patients' symptom severity was assessed for the main symptoms of PMS identified as irritability, mood alteration, anger, headache, bloating and breast fullness. Study results showed the improvement for the group on the normal dosage for Premular of one tablet per day was superior in the reduction of all six individual symptom scores, compared to the placebo and smaller dosage groups. Interestingly, a higher dose demonstrated no significant difference in reducing symptom severity compared to the normal Premular dose. Importantly, after treatment with the normal Premular dose, nearly half (49%) of all patients had no symptoms and 31% had mild symptoms. The tolerability rating for Premular was also noted as very good by over two-thirds of patients (69%) and good by almost one-third (31%), with no serious adverse events occurring in the study.[4]

Prior chronic use of oral contraceptives was allowed in this study, with the dose remaining unchanged throughout the study period. Oral contraceptives are known to affect premenopausal symptoms; however the researchers noted no statistically significant effect of oral contraceptives on either total or individual symptom scores.[4]

On the basis of these findings, the study authors conclude that, for patients suffering from PMS, Premular (Ze 440) once-daily should be the preferred option.[4]

The clinical evidence from this study supports the dose recommendation provided by both the European Scientific Cooperative of Phytotherapy (E/S/C/O/P 2003) and the Committee on Herbal Medicinal products of the EMA's 'well established use' classification for Ze 440 (Premular).[2,3]

This study also adds to an already established body of clinical evidence to support Premular's efficacy and safety. In previous clinical trials, Premular has also shown to be significantly ($p<0.001$) better than placebo in reducing PMS symptoms such as irritability, anger and headache.[6,7]

Premular is one of a pedigree of European herbal medicines, developed and currently produced by Swiss company Zeller. Zeller has over 130 years of experience in the development and manufacture of herbal medicines.

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Reviews of medical journal articles

Melissa Gearing, Sarah Kottmann, Susan Jarmo

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 herbal medicine. A dominant theme is often present throughout the journals which will be reflected in the reviews.

Anesthesia and cognitive deficits in children

Ing C, DiMaggio C, Whitehouse A, Hegarty M, Brady J, Ungern-Sternberg B et al. 2012. Long-term differences in language and cognitive function after childhood exposure to anesthesia. *Pediatrics* 130:3;476–85.

The neurotoxic effects of anesthesia exposure in developing brains are well established in animal models, with neurodegenerative changes found to be dose dependant and increased with multiple agents. In the animal model, long term neurocognitive changes, including differences in learning, memory, motor activity, attention and behaviour during adulthood, have also been identified.

A window of vulnerability in rodents appears to occur during peak synaptogenesis between postnatal day 7 and 30. In the human brain this happens over a wider period of time, occurring in the primary sensorimotor cortex near the time of birth, temporal cortex at 9 months and prefrontal cortex at 3 years.

In studies demonstrating an association of anesthesia with disability, only children with multiple anesthetic exposures have been associated with deficits, but an effect with a single exposure has not been identified. This study aimed to determine if (1) exposure to anesthesia for surgery or a diagnostic test during the first 3 years of life is associated with differences in any of a range of directly assessed neuropsychological outcomes; and (2) if the differences persist with only a single exposure.

Data was obtained from the Western Australian Birth Cohort (Raine) Study, an established birth cohort consisting of 2868 children born from 1989 to 1992, originally created to evaluate the long term effects of prenatal ultrasound. After birth, all children were assessed at 1, 2, 3, 5, 8, 10, 13 and 16 years of age. Parents kept detailed diaries of their child's medical history and during follow ups parents filled out questionnaires describing illnesses and medical problems.

Only children who completed follow up from age 1 to 3 were included. Of the 1781 children, 1523 were unexposed while 206 had a single exposure and 52 had multiple exposures. The exposed group had significantly worse scores in tests of receptive, expressive and total language and cognition, specifically abstract reasoning. Evidence for a significantly increased risk of disability in exposed children was determined from this. There was no different between exposed and unexposed in behaviour and motor function domains.

In this birth cohort, children exposed to anesthesia before age 3 had an increased long term risk of clinical deficits in receptive and expressive language and abstract reasoning. This increased risk was found even in children with a single exposure to anesthesia. Results indicate that the association between anesthesia and neurodevelopmetal outcome may be confined to specific domains. Part of the association of neurocognitive deficit with anesthesia may be due to the innate differences between children requiring surgery and diagnostic procedures and those not requiring them. However the fact that the vast majority underwent minor procedures suggests significant comorbidity is unlikely to confound results.

Breastfed babies have a lower risk of major depression in adulthood

Peus V, Redelin E, Scharnholz B, Paul T, Gass P, Deuschle P et al.. 2012. Breast-feeding in infancy and major depression in adulthood: a retrospective analysis. *Psychother Psychosom* 81;189-90.

We all know and have heard the benefits of breastfeeding your baby in many ways beyond basic nutrition. Breast milk is packed with disease fighting substances that protect your baby from many illnesses such as stomach viruses, respiratory illness, ear infections and meningitis. It has also been shown to help prevent allergic reactions to foods and prevent adulthood diseases such as hypertension and diabetes.

For this reason a recent study in Germany looked at the effect of breastfeeding in the prevention of major depression in adulthood. The study consisted of those who were breastfed for at least 2 weeks and those who were bottle fed. Both males and females were included in the study; 52 were patients being treated for major depression and were compared with 106 people who had never had depression.

It was found that 72% of the group who had never had depression were breastfed compared with 42% of the patients suffering from depression. Researchers looked at possible factors such as age, gender and maternal education level but factors remained the same. It was concluded that non-breastfed individuals had a greater risk of depression in adulthood. It was also interesting to note that the length of time being breastfed did not affect the outcome. The researcher hypothesised that breastfed

babies may receive a boost of oxytocin which is regarded as being stress protective, and that certain constituents of breast milk may be beneficial in preventing depression.

Breast milk has also been found to lower the risk of other conditions such as hypertension, which has been shown to be associated with depression. However it could not be exclusively concluded that breastfeeding is in itself a contributing factor as other components such as the mother infant interaction and relationship must be considered. This is just one more example of the importance of breastfeeding in infancy.

Cancer outpatients at risk of malnutrition

Bozzetti F, Mariani L, Lo Vullo S, Amerio ML, Biffi R et al. 2012. The nutritional risk in oncology: a study of 1,453 cancer outpatients. *Support Care Cancer* 20;1919–28.

The practice of nutritional screening of cancer outpatients is rarely performed despite guidelines by the American Society of Parenteral and Enteral Nutrition. The European Society for Clinical Nutrition and Metabolism defines nutritional screening as a 'rapid and simple process conducted by admitting staff or community healthcare teams'. Since cancer patients represent the most common candidates for aggressive therapies, they might present a state of malnutrition which can reduce compliance to the oncologic therapies and can be worsened by such treatments.

This study aimed to define the pattern of scores of nutritional risk in 1453 outpatients with cancer who presented for diagnosis, therapy or follow up at multiple centres. Those affected by endocrine diseases or showing severe organ function impairment were excluded. The endpoints of the study were to (1) define prevalence and rate of malnutrition and of nutritional risk in cancer outpatients and (2) the need for a nutritional intervention and to investigate the association of some patient related, tumor related and therapy related variables with the nutritional risk.

Demographic and clinical data were collected for each patient including age, sex, tumor stage and site, nutritional data including the percentage of the weight loss at different interval times before and during the illness, and the body mass index (BMI). Systemic and digestive symptoms such as fatigue, anorexia, nausea/vomiting, early satiety, dysgeusia/dysosmia, odynophagia/dysphagia and diarrhea/constipation were classified through a 4 point score (no, mild, moderate and severe).

A nutritional risk score (NRS) was assessed using the Nutritional Risk Screening 2002 (NRS-2002) which has been clinically validated. At the initial screening the patient was assessed and if they presented as having a BMI of < 20.5, lost weight in the last three months, reduced dietary intake in the last week or were severely ill, they moved to the final screening where a quantification of the previous parameters was completed and summed with the severity of disease. The final scoring range was from 0 to 6 (0 being no risk).

Results showed 32% of patients were defined at nutritional risk, the authors commenting that 'the most striking finding of this study was that one third of our patients were considered with a high NRS'. Even if oncologists feel uncomfortable or not confident providing nutritional counselling, such a remarkable prevalence of outpatients with high nutritional risk should alert them to face the issue. It would be in the best interest of all involved due to the deleterious effects of malnutrition on compliance with oncologic therapies, the response to treatment and the growing experience, that early nutritional intervention when tumor burden is still limited is able to achieve a clinical benefit.

Risk of rheumatoid arthritis with elevated rheumatoid factor

Nielsen S, Bojensen S, Schnohr P, Nordestgaard B. 2012. Elevated rheumatoid factor and long term risk of rheumatoid arthritis: a prospective cohort study. *Brit Med J* 345;e5244.

Rheumatoid arthritis is an autoimmune disease affecting 0.5-2% of the population. Although modern treatment for rheumatoid arthritis can induce remission in many patients, diagnosis in the early disease stages is important for preventing irreversible damage to the synovial lining and cartilage of diseased joints and for preventing progression into later disease stages. At present there is no good clinical indicator for long term development of rheumatoid arthritis.

Rheumatoid factor is an autoantibody targeting the Fc region of IgG antibodies. Testing for rheumatoid factor is the most widely used blood test in the classification of rheumatoid arthritis. It is often stated that rheumatoid levels increase with age, but convincing data for this statement is difficult to find. About 80% of all patients with rheumatoid arthritis will eventually be seropositive for rheumatoid factor, while only 40% are positive at clinical onset of the disease. However it is unknown whether an elevated level of rheumatoid factor in individuals in the general population without rheumatoid arthritis is associated with later development of rheumatoid arthritis.

This study tested the hypothesis that elevated levels of rheumatoid factor are associated with long term development of rheumatoid arthritis. Baseline plasma levels of IgM rheumatoid factor were measured in 9712 people without rheumatoid arthritis from the general population. They were followed up for 28 years, during which time 183 developed rheumatoid arthritis. Turbidity was used to measure concentrations of rheumatoid factor of IgM type in plasma. Plasma samples were drawn in 1981-83 and frozen at –20°C until measurement in 2009-10. Investigators were blinded to rheumatoid arthritis diagnosis and vise versa.

The principle findings in this study are that those with

elevated levels of rheumatoid factor had higher long term risk of developing rheumatoid arthritis. The authors comment that these findings are novel and do not serve as evidence that rheumatoid factor plays a causal role in the pathogenesis of rheumatoid arthritis. An interesting finding was that of a particularly high absolute risk of developing rheumatoid arthritis for women with elevated rheumatoid factor who smoked. The authors concluded that individuals in the general population without rheumatoid arthritis but with an elevated plasma level of rheumatoid factor have up to 26-fold greater long term risk of developing rheumatoid arthritis and up to 32% ten year absolute risk of rheumatoid arthritis.

Anticancer activity of propolis

Sawicka D, Car H, Halina, M et al. 2012. The anticancer activity of propolis. *Folia Histochem Cytobiol* 50:1;25–37.

Propolis is a resinous substance produced by bees. The main components of propolis are fatty, aliphatic and aromatic acids, flavonoids, alcohols, terpenes, sugars and esters. It has been ascribed a number of therapeutic properties and this review aimed to summarise the mechanism of action for the active compounds of propolis in the apoptotic process and their influence on the proliferation of cancer cells.

The study of active compounds in propolis resulted in the extraction of CAPE and chrysin, which are believed to be mainly responsible for the antitumor therapeutic activities of propolis. The cancer inhibitory effects of CAPE and chrysin have been confirmed in a variety of culture cell lines.

CAPE exhibits strong antitumor effects in oral cancer cells: fibroblasts from oral submucous fibrosis (OSF), neck metastasis of gingiva carcinoma (GNM) and tongue squamous cell carcinoma. Previous studies have identified that CAPE inhibits colorectal cancer cells proliferation and induces cell cycle arrest by down regulation of b-catenin protein expression and activation of the cyclin-dependent kinase inhibitors which prevent pRb phosphorylation.

Chrysin (5,7-dihydroxyflavone) is a natural flavonoid found in plant extracts (e.g. *Passiflora caerulea, Populus tremula*), honey and propolis. It has antioxidant and anti-inflammatory effects and has been shown to influence the apoptotic process.

In vitro studies show different sensitivities of tumour cells to extracts of propolis in the context of apoptosis. Results from several studies indicate different degrees of sensitivity to water-soluble propolis extracts among cancer cells and normal fibroblasts. Ethanolic extracts of propolis have also demonstrated action on apoptosis of breast cancer cells in vitro. However the mechanism of propolis-induced apoptosis appears to be independent of the kind of cancer cells studied, but dependent on the concentration of propolis extract. The literature indicates that propolis induces apoptosis through the release of cytochrome c from mitochondria to the cytosol.

The results of the in vitro studies considered for review suggest that propolis, CAPE and chrysin have cytotoxic properties against cancer cells through the induction of apoptosis or cell division and cell growth arrest. The studies presented in this review suggest that propolis and its compounds, CAPE and chrysin, may inhibit cell cycle proliferation or induce apoptosis in tumor cells. The results of these studies also suggest the inhibition of NF-kB activation, the suppression of anti-apoptotic proteins, such as IAP, c-FLIP, Akt kinase, and the initiation of extrinsic pathway of apoptosis by induction of TRAIL and Fas receptor stimulation in cancer cells.

The researchers concluded that while many studies have shown the inhibitory effects of propolis and its compounds on growth and cancer cell proliferation, further work is warranted to investigate the efficiency and mechanism of their beneficial effects. More information would also be required to understand how that translates into clinical practice.

Evaluation of vegetarian diet

We in the complementary health field are very much aware of the merits of healthy diets and their effects on people. However diet was largely overlooked by the medical profession until quite recently, so it is not unusual that the doctor looking after your health has only four hours of nutrition training. It was therefore refreshing to find that the June 4 edition of the *Medical Journal of Australia* committed the whole edition of the journal to explore the nutritional adequacy of vegetarian diets. The publication collected the results of some recent research that address the questions regarding the nutritional characteristics of vegetarian diets.

Questions about meat free diet and its likely problems include the adequate amount of protein, iron, zinc, vitamin B12 and omega-3 polyunsaturated fatty acids. Rosemary Stanton mentions that the body's ability to absorb these nutrients in the context of various diets has not been addressed well in the past. This research now provides evidence based answers to these questions.

Reid et al explored the nutritional needs of different population groups and found that a well-planned vegetarian diet can meet almost all of the nutritional needs of children and adults of all ages. In their research they designed nutrient rich meal plans in order to meet nutrient requirements without excess calories. While they found that in general the nutrient levels were adequate, the increased iron requirement of pregnant women was not met. They pointed out this increased requirement is difficult to meet even with a non vegetarian diet and supplementation is often necessary. The omega-3 polyunsaturated fatty acids also fall short of the minimum requirements, so supplementation was suggested.

Others explored the protein content of vegetarian diets and found that a vegetarian diet can easily meet the

human protein requirements. They suggest the inclusion of a variety of plant sources including legumes, soy products, grains, nuts and seeds. This is even easier if a lacto-ovo vegetarian diet is adopted as both dairy and eggs contain complete proteins. It was also found that the consumption of plant proteins rather than animal proteins by vegetarians contributes to their reduced risk of chronic diseases like diabetes and cardiovascular disease. Marsh et al (2012) also pointed out that most Australians eat significantly more protein than is required.

Omega-3 polyunsaturated fatty acid levels of vegetarians were measured through blood tests and found that vegetarians have much lower EPA and DHA levels, which may be a result of a poor conversion of precursors from the diet. The supplementation of EFA or increased intake of seeds (chia, sunflower, flax), nuts (walnuts), oils (flaxseed, soybean, wheat germ), eggs and dairy can help to provide enough precursors to count for the low conversion. This recommendation is valid even though vegetarians with low blood levels of EPA and DHA do not show clinical signs of DHA deficiency.

Iron content of vegetarian diets was found to be well balanced and vegetarians do not appear to face greater risk of iron deficiency than non vegetarians, except perhaps pregnant women, however their increased needs are often not met by non vegetarian diets either. Authors conceded that bioavailability non-haem (plant origin) iron is affected by absorption as well as by the person's iron stores. Iron absorption, especially non-haem iron is inhibited by polyphenol containing beverages such as tea, coffee, cocoa and red wines. Calcium was also found to be an inhibitor of both haem and non-haem iron. Iron absorption was enhanced by vitamin C and other antioxidants. The current recommendation for vegetarians is 1.8 times a normal RDI to count for the reduced bioavailability of non-haem iron. Long term adaptive mechanisms of the human body do help to achieve the right balance by changing the level of absorption and excretion to maintain healthy iron levels.

Zinc availability has similar mechanisms to iron. Zinc absorption is inhibited by phytic acid found in legumes, unrefined cereals, seeds and nuts. This can be counteracted by soaking and cooking. Iron supplements were also found to be competing with zinc absorption. Protein enhances zinc absorption as zinc attaches itself to protein, different types of proteins do have different levels of influence on the absorption, for example casein in milk has an inhibitory effect on zinc absorption, but soy protein does not. Zinc is also subject to the homeostatic mechanism that has a long term control of iron stores through a balance of absorption and excretion.

Vitamin B12 is an essential vitamin that is found almost exclusively in animal origin foods. In a cross sectional analysis of 689 subjects, half of the vegans and 7% of the vegetarians were deficient in Vitamin B12. The contributing factors include both inadequate intake and absorption or impaired utilisation. Lacto-ovo vegetarians have a reliable intake of vitamin B12 through adequate consumption of dairy and eggs. There is no plant based food with enough vitamin B12 to provide adequate dietary intake. Supplementation or consumption of fortified foods is recommended. Supplementation is a must during pregnancy and breast feeding.

Comment:

Although a well balanced vegetarian diet is sufficient to provide a variety of nutrients necessary for being healthy, there can be issues around bioavailability and absorption. Bioavailability can be enhanced by proper preparation methods and the addition of other food items such as fruit juices. Insufficient digestive acids in the digestive system can be enhanced with bitters such as dandelion or globe artichoke. Including medicinal herbs in the diet directly can keep the diet more manageable and make it more interesting, e.g.cooking with ginger, garlic, chilies, turmeric, fenugreek, rosemary, sage and cinnamon.

Licorice in non alcoholic fatty liver disease

Hajiaghamohammadi AA, Ziaee A, Samini R. 2012. The efficacy of licorice root extract in decreasing transaminase activities in non-alcoholic fatty liver disease: a randomized controlled clinical trial. *Phytother Res* 26:9;1381-9.

Non alcoholic fatty liver disease (NAFLD) linked to metabolic syndrome has become a common liver disorder in developing countries. A clinical study, the first of its kind, was undertaken to investigate the effects of licorice on NAFLD. In this double blind randomised clinical trial 66 patient were divided into two groups. All the patients had elevated liver enzymes and increased lipid accumulation in the liver. Those at risk of licorice side effects such as hypertension where excluded from the study.

The case group was treated with a capsule containing 2 g of licorice root extract for 2 months while the control group received a placebo. Weight, BMI and liver transaminase levels were measured before and after treatment for each patient in the study.

Results showed that in the licorice group there was a reduction in alanine aminotransferase (ALT) from 64.09 to 51.27 IU/mL and aspartate aminotransferase (AST) decreased from 58.18 to 49.45 IU/mL ($p < 0.001$).

The control group had much smaller drops that were not statistically significant. BMI had no statistically significant change in either group.

In this study it can be seen that licorice demonstrated a significant change in liver enzymes and protection against continued liver damage at low oral doses providing further evidence for the hepatoprotective effect of the herb.

Book reviews

Practical Herbs

by Henriette Kress

Yrtit ja yrtititerapia Henriette Kress, Helsinki Finland 2011

ISBN 978 9 526 75750 6

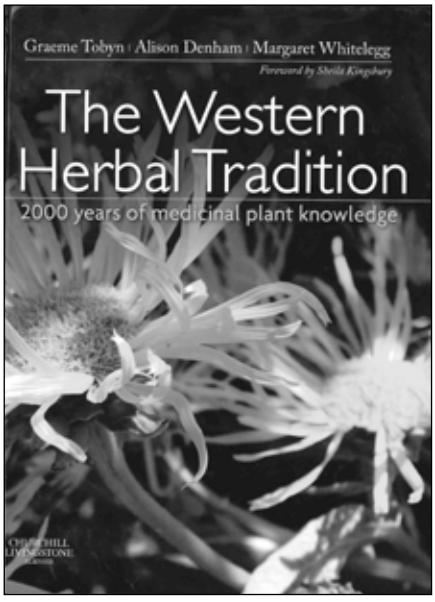
Reviewed by Liz Hammer

Practical Herbs is a small attractive book about wild crafting, manufacturing and using herbs for simple acute ailments. The author resides and practises in Finland; however as she points out the herbs discussed can be found in most temperate environments, including Australia.

The book has 150 pages with colour photos and has two main sections. The first provides basic information on picking and preparing herbs for use in tinctures, salves, syrups and teas, and goes on to provide fairly clear and simple instructions on how to make these preparations. The second section consists of 23 traditional monographs (unreferenced) with all the usual information, ending with suggested preparations for simple acute ailments. These monographs include old favourites such as dandelion, horsetail and *Calendula*, and the less familiar maral root (*Rhaponticum carthamoides*) and speedwell (*Veronica* spp). Be advised effects and uses are clearly gauged at the amateur.

This is not a professional text but will appeal to students of herbal medicine and any herbalist feeling nostalgic about what inspired them to practice herbal medicine. Who can resist such gems as tea of beggarticks (*Bidens* spp) for dry mucous membranes, cinquefoil (*Potentilla* spp) paste for healthy cuticles or a yummy blackcurrant sugar? There is also an intriguing section on constructing an aromatic rose leaf string of beads. I already have plans to raid my rose garden this summer!

Available for loan from the NHAA library.



The Western Herbal Tradition

2000 years of medicinal plant knowledge

By Graeme Tobyn, Alison Denham, Margaret Whitelegg

Elsevier Limited 2011

ISBN 978 0 443 10344 5

Reviewed by Sue Giles

This book is rich in the historical aspects of herbalism. It provides discussion of the many influences that lead to the evolution of our profession and the way we practise today. It traces the historical development and cultural influences of the application of herbal therapy from Greco, Roman and Arabic, through the ages to 18th and 20th century herbalists and 21st century approaches.

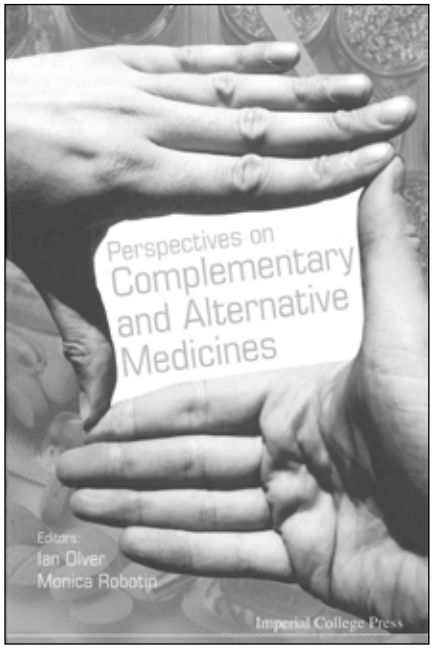
The book reviews several of the old herbals and their authors including *Macer's Herbal, Hildegard & the Myddfai Physicians*, and the more expected inclusions of Fuchs & Gerard. The American, British and European herbalists are all well represented along with mention of many more modern day herbalists such as Hoffman, Mills and Bone who are currently in print.

There is a total of 27 lengthy herbal monographs included, listed alphabetically by botanical name. Some of these are of herbs not commonly used or available (e.g. *Tussilago*, coltsfoot) to us in Australia, but they do

include some lovely accessible herbs we are in danger of neglecting in favour of their more exotic cousins. I particularly enjoyed the inclusion of *Rosa damascena* (damask rose), because this is a herb we seldom see included and yet it is a very useful and commonly overlooked herb. There are also monographs for some of the herbs in my dispensary, *Inula helenium, Arctium lappa* and *Paeonia officinalis*. Each monograph begins with discussion of the plant from a modern perspective and then progresses into a historical review of collated data and applications. Safety aspects are covered well with not only potential drug interactions being discussed, but potential allergenic issues and other areas relevant to a particular plant. There is good discussion of the pyrrolidizine alkaloid content of *Tussilago*. Recommendations for use and application are well covered and chemical constituents are not neglected.

I thoroughly enjoyed my time with this book. It is well indexed and well referenced. I particularly enjoyed the historical aspects of the plant uses, demonstrating evolution to the way we use the plants to day. I only wish it included more monographs. This is a book I would enjoy having in my own library.

Available for loan from the NHAA library.



Perspectives on Complementary and Alternative Medicines

By Ian Olver & Monica Robotin

Imperial College Press London 2012
ISBN 978 1 848 16556 4

Reviewed by Angela McClelland

Ian Olver and Monica Robotin, the editors of this text, have gathered together a wide range of perspectives on complementary and alternative medicine (CAM) in the context of cancer care and present them to readers to form their own conclusions. The contributors include health educators, oncology clinicians, complementary medicine clinicians, researchers and consumers, all given the freedom to express themselves in their own styles. This has resulted in is a 'lively mix of poignant stories, strong opinions and scientific reviews'. As far as I am aware, it is the first Australian book to be published on this topic. All the similar texts I have seen have come from North America and Europe.

The chapters are arranged in a logical sequence with various definitions of CAM, understanding CAM through classification and examples, and reasons people with a diagnosis of cancer might use CAM. Explanations of and literature reviews of different types of CAM followed as well as safety issues, research issues and how natural materials are used for drug development.

Sociological factors behind why people are choosing to use CAM and what this means for health practitioners, health policy makers, funders and health designers explain why we have seen such a huge increase in CAM use in recent years. There is also an interesting discussion around regulation of complementary medicines and the importance of striving toward practitioner registration.

The chapters I particularly enjoyed were from the clinicians. David Joske's narrative on the creation of the SolarisCare centre at Perth's Sir Charles Gairdner Hospital was particularly informative, giving an account of the processes involved in establishing the centre and the challenges and rewards of a model that combines non-ingestable complementary therapies with conventional care. The centre's data collection has shown that the patients who participate in the complementary therapy sessions have reduced symptom distress and improved quality of life.

Views submitted from the other end of the spectrum were strong and did provide a contrast to the rest of the text. I must admit that I was irritated with the superficial sweeping statements that ignored the current evidence from psycho-oncology, neurology, immunology, nutrition and herbal medicine research. It left me with the impression that the authors really weren't interested enough in the topic to look too deeply in case they found something that would contradict their opinions.

However I did find the great majority of this book to be informative and interesting with well written, considered and thoughtful chapters by people who share the common goal of improving the lives of people with a diagnosis of cancer. I would recommend this book to anyone seeking to have a greater understanding of why people with a diagnosis of cancer choose to use complementary and alternative medicines.

Available for loan from the NHAA library.

AJHM based CPE Questionaire

The AJHM 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 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 financial year. For further information please see the NHAA CPE Member's Manual on the NHAA website www.nhaa.org.au.

Herbal medicine questions - AJHM 24(4)

1. Phytoestrogens and breast cancer

a) Studies show that dietary sources of phytoestrogens provide potent antioxidant activity.
b) Ingested phytoestrogens have been shown to have no effect on the process of estrogen metabolism.
c) Phytoestrogens may have a protective effect on the progression of breast cancer by stimulating estrogen production.
d) Phytoestrogens have been shown to inhibit cell signalling pathways in breast cancer.

2. Ginsenoside and breast cancer

a) MDM2 is an oncogene which is important for cancer development, progression and metastasis.
b) The presence of MDM2 in human cancer is suggestive of a good prognosis.
c) The intervention 25-OCH3-PPD was shown to have an affinity for MDM2, despite having no effect on transcription.
d) Both (a) and (c).

3. Olive leaf as a hypoglycemic agent

Olive leaf extract has been shown to have the following effect in diabetes:

a) Significantly impairs insulin response
b) Significantly decreases fasting insulin
c) Reduces HbA1c after 500 mg 2 x day for 14 weeks
d) Induces liver toxicity at high dose in animal studies
e) All of the above.

4. *Vaccinium* in depression

a) Nitric oxide synthase inhibitors demonstrate an antidepressant effect
b) *Vaccinium myrtillus* does not affect nitric oxide production
c) Bilberry potentiates the effect of NOS inhibitors
d) Unpredictable chronic mild stress (UCMS) has been used in animals to model depression
e) a) and c)

5. Licorice for peridontal disease

a) Licorice may cause hyperkalemia through inhibition of 11 beta-hydroxysteroid dehydrogenase
b) One of the primary agents of caries is *Streptococcus mutans*.
c) Organic solvent extract of *G. glabra* has no effect on *C. ablicans*.
d) Liquiritigenin exhibits immunomodulating activity in mice.
e) a) and d)

Medical science questions - AJHM 24(4)

1. Plant polyphenols and haemochromatosis

a) Plant polyphenols are well known for their positive effect in increasing iron levels in the body
b) Plant polyphenols have very little effect on iron levels in the body
c) Plant polyphenols are known for their positive effect in decreasing iron levels in the body
d) Plant polyphenols only have an effect on iron levels in the body when combined with ascorbic acid

2. Breastfed babies and depression

a) Breastfed babies have a reduced risk of hypertension
b) Breastfed babies may received increased levels of oxytocin
c) Breastfed babies have a reduced risk of suffering from depression despite the duration of breastfeeding
d) (a) and (b)
e) All of the above

3. Medical anaesthesia

Children are at an increased long term risk of clinical deficits in receptive and expressive language and abstract reasoning due to:

a) Multiple exposure to anesthesia
b) Single exposure to anesthesia
c) Minor procedures under anesthesia
d) All of the above

Index to Australian Journal of Herbal Medicine

Volumes 1-24
1989 to 2012

MJA – Medjourn abstracts (when separate publication from AJMH)
MPA – Medplant abstracts (when separate publication from AJMH)

Australian Journal *of* **Herbal** Medicine

national herbalists association of australia

The NHAA invites contributions to the Australian Journal of Herbal Medicine

The Australian Journal of Herbal Medicine publishes material on all aspects of western herbal medicine with emphasis on the philosophy of herbal medicine and the phytochemistry, pharmacology and clinical applications of medicinal plants.

Editorial policy

- Subject material must relate to herbal medicine.
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- Contributions are subject to peer review and editing.
- Contributions to the Australian Journal of Herbal Medicine must not be submitted elsewhere.

Peer review

- All feature articles will be reviewed by two independent peer reviewers.
- Reviewed articles will be returned to the author for modification if required.

Contribution requirements

- Files should be saved as Word for Windows or equivalent and should be sent electronically by email as a complete version or by post with an original printed version and an electronic copy on CD or USB stick. All figures and pictures must be saved as a high resolution .pdf, .jpg or .tif file.
- All statements must be referenced and a full reference list must be included. If the statement is the author's observation or opinion this should be made clear.
- All statements should be of a professional nature and exclude any inflammatory, derogatory, racist or other inappropriate style of writing.
- Papers should be no more than 5000 words including tables and references. The number of references should not exceed 30 (except for review articles).
- An abstract of the article should be included.
- A brief profile of the author should be included.

Referencing (inability to use required referencing may result in delay or rejection of article)

- Text citation should appear as surname of first author and year of publication in parentheses at the end of a statement or paragraph such as (Cowper 2007).
- The reference list should be arranged in alphabetical order using the following format:

  Journals: Author's surname Author's initials, Author's surname Author's initial. Year. Title of article. *Journal name* volume:issue;page numbers. (Cowper A, Pengelly A. 2007. Instructions for Contributors. *Aust J Med Herbalism* 19:1;30-41.) Internet references should be in the format accessed 25 May www.nhaa.org.au.

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