

Australian Journal *of* Herbal Medicine





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- Maintain and promote high educational standards for practitioners of herbal medicine.
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- Advocate ethical and sustainable methods of growing, harvesting and manufacturing herbal medicines.
- Provide peer support for practitioners and students of herbal medicine.

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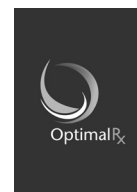
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Editorial

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In seeking speaker proposals for the upcoming *8th International Conference on Herbal Medicine* it was encouraging to see just how much complementary medicine (CM) research is being conducted around Australia. In addition to university academics and undergraduates carrying out vital research, many practitioners are returning to undertake higher research and coursework degrees in specific areas of CM research.

How much has CM research grown in the last ten years? In 2004 Bensoussan noted that despite its rapid growth, the CM industry did not easily see the advantage of investing in research instead of marketing, as companies were not able to protect medicines against negative research findings. He further identified that funding agencies such as the National Health and Medical Research Council (NHMRC) and the Australian Research Council were hesitant to fund research in an area of little understanding compared with conventional medicines. Bensoussan proposed that if 5% of the GST raised from the estimated \$160 million of GST collected each year from sales of CM products was invested in CM research annually over the next five years, this would create an annual budget of approximately \$8 million.

In November 2006 the Commonwealth Government did announce that it would provide \$5 million in funding through the NHMRC to investigate the use and effectiveness of CMs. In 2008 funding of \$1.74 million was awarded to establish three National Institute of Complementary Medicine Collaborative Centres and a further \$5.3 million for 13 projects to be funded by the NHMRC (www.nhmrc.gov.au).

Whilst \$5 million of research funding is a small start, this achievement followed USA's example where \$5 million was invested in 1995, followed in 2006 with the National Centre for Complementary and Alternative Medicine investing \$122 million into CM research and integrated approaches to health care (European Federation for Complementary and Alternative Medicine).

Sarris (2011) noted that whilst research into CM products is on the rise, there is a real need for the study of naturopathic practice, its outcomes and effectiveness as well as the safety of naturopathic and herbal medicines. Sarris proposed an individualised research approach applied to naturalistic practice to collect data from multiple samples (or cases studies), or to be applied

within a controlled design comparing the outcomes of practice to usual care, standard conventional care or other CM modalities. Sarris acknowledges that the method and design of these studies would be difficult. Analysis of results would be questionable due to them being uncontrolled; component/s which were significant could not be separated from placebo; and all variables would be further confounded by the level of the practitioner's skill or other individual characteristics.

Critics of CM claim that unlike studies of drugs derived from plants, many funded studies lack a sound biological underpinning. For example the National Centre for CM in the USA spent \$374 000 to find that inhaling lemon and lavender scents did not promote wound healing. On the other hand, if the treatment was scientifically provable would it continue to be classed as a complementary medicine?

On 1 July 2012 another four health professions joined Australia's National Registration and Accreditation Scheme: Aboriginal and Torres Strait Islander health practice, Chinese medicine, medical radiation practice and occupational therapy (www.ahpra.gov.au). Herbal medicine is unique in the CM industry as it has a solid basis for scientific evidence, safety and efficacy of its practice. It is therefore our hope that with the ongoing rigorous scientific validation of our medicines and practice, and fidelity to our traditions, that we will see our profession take its rightful and recognised place alongside other medical and allied health practitioners within primary healthcare in Australia.

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To the Editor

Regulation of CAM – it's all in the evidence

Simon J Spedding argues in his letter to the editor of the *Medical Journal of Australia* in June 2012, that conventional and complementary medicine should have equal requirements regarding regulation. He points out that while complementary medicine lacks evidence of safety and efficacy, conventional medicine also lacks efficacy in 30-40% of cases.

Regarding safety of conventional medicine, the recent issues with breast implants and hip replacements raise safety concerns. The debate around these issues exposes the outdated views of the medical profession. While conventional medicine was once the main health care provider, now its share has shrunk considerably. These days complementary therapists provide half of the consultations and people spends almost three times more, close to \$3.5 billion on complementary medicine compared with conventional medicine prescriptions at only \$1.3 billion (Spedding 2012).

In this changing situation a more focused evidence based regulation of practitioners is needed. Spedding feels that the current system is inadequate; it is relying on different legislations with inconsistent standards. A number of federal and state regulating bodies are involved in this process, including Medicare, Professional Services Review (PSR), Australian Health Practitioner Regulation Agency (AHPRA), Therapeutic Goods Administration (TGA) and the Australian Competition and Consumer

Commission (ACCC). The process of registration of medical practitioners is strict, while complementary health practitioners are regulated with quite minimal credentials. Medicare restricts the activities of medical practitioners, while private health insurers pay for almost any therapy. Regarding products used, pharmaceutical products are much more regulated than for example slimming products.

To ensure effective, safe and uniform healthcare for all, evidence based regulation of practitioners and products is necessary. This will re-establish the relevance and respectability of both the conventional and complementary health systems to progress to a healthier Australia. I believe that it is in the interest of all serious complementary health professionals that the regulatory regime reflects the higher safety and efficacy standards of both conventional and complementary health products and the standard of practice.

Simon J Spedding is a member of the Advisory Committee on Complementary Medicine, a member of the Royal Australian College of General Practice, and participates in such capacity on the board of the TGA.

Susan Jarmo
MNHAA

Reference

Spedding S. 2012. Regulation of conventional and complementary medicine – it is all in the evidence. *MJA* 196:11;682–3.

Australian Journal of Herbal Medicine

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8th International Conference on Herbal Medicine

Tradition, evidence and integration

The Sebel Albert Park Melbourne **Friday 12 to Sunday 14 April 2013**

Australia's oldest natural therapy association, the National Herbalist Association of Australia, is holding its next International Conference at the lovely Sebel Albert Park in Melbourne in April 2013. This conference attracts some of the world's leading herbalists and naturopaths as well as cutting-edge researchers as presenters.

Presentations will cover a broad range of topics around the theme of *Tradition, evidence and integration* including integrative practice, complex clinical conditions such as cancer and autoimmune disease, epigenetics, spagyrics and how to optimise IT and social media in this profession. There will be research streams in many of the concurrent sessions that similarly cover a broad spectrum of fascinating topics.

Speakers include Simon Mills (UK), Steven Foster (USA), Phil Rasmussen (NZ) Kerry Bone, Voytek Kielczynski, Andrew Pengelly, Karen Bridgman, Justin Sinclair, Hans Wohlmuth, Melanie Koeman, Gill Stannard, Michael Thomsen and many more . .

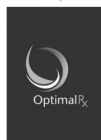
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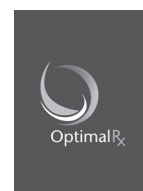
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Phytotherapy for polycystic ovarian syndrome

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Introduction

Polycystic ovarian syndrome (PCOS) is a common gynecological disorder characterised by hypergonadotrophism, hirsutism, obesity, oligomenorrhea and is commonly associated with infertility (D'Hooghe 2002). PCOS is a complex clinical picture and presents a multifaceted etiology related to imbalance of the hypothalamic pituitary adrenal (HPA) axis, thyroid involvement and metabolic syndrome (insulin resistance) (D'Hooghe 2002). There is substantial evidence that PCOS should no longer be considered purely a gynecological disorder, but rather a complex endocrine disorder.

PCOS affects approximately 5-10% of women of reproductive age and is one of the most common causes of anovulatory infertility (Hopkinson 1998). Menstrual disruption typically manifests in PCOS, ranging from oligomenorrhea to amenorrhea.

Etiology

Despite extensive investigations the etiology of PCOS remains poorly understood. The most recent knowledge indicates that abnormal insulin response to glucose stimulus is a key underlying factor in PCOS (Hopkinson 1998, Visnova 2003). Other etiological factors include derangement of the sympathetic nervous control of the ovaries (Lara 1993), estrogen dominance and elevated androgens. Some of the literature suggests a genetic susceptibility to insulin stimulation of androgen secretion, blocking follicular maturation.

Insulin resistance

PCOS and insulin resistance are intimately related endocrine disorders. The most common causes of insulin resistance are obesity, poor diet and stress. Hyperinsulinemia is not a characteristic of hyperandrogenism in general, but is uniquely associated with PCOS (Hopkinson 1998).

In obese women with PCOS, 30-40% have impaired glucose tolerance or diabetes. However women with ovulatory hyperandrogenism can present with normal insulin and glucose tolerance (D'Hooghe 2002, Hopkinson 1998) thus indicating additional etiological factors may be involved.

Elevated androgens

The ovarian and adrenal glands of women with PCOS are usually the sites of production of elevated androgens.

It is postulated that these women have a hyperactive production of CYP17 enzyme, which is responsible for forming androgens in the ovaries and adrenals (from dehydroepiandrosterone sulfate, DHEA-S) (Hopkinson 1998). Elevated total and free testosterone correlate with the typically elevated luteinising hormone (LH) levels. Serum total testosterone is usually up to twice the normal range (20 to 80 ng/dL). High androgen levels in the ovary inhibit follicle stimulating hormone (FSH), thereby inhibiting development and maturation of the follicles (D'Hooghe 2002, Hopkinson 1998).

DHEA is found to be elevated in 50% of women with PCOS (Hopkinson 1998). The elevated DHEA is due to stimulation by adrenocorticotrophic hormone (ACTH), produced by the pituitary in response to stress. The excess DHEA then converts to androgens via adrenal metabolism, which in turn contributes to the typical elevated androgen levels in PCOS.

The skin and adipose tissue add to the complex etiology of PCOS. Women who develop hirsutism have the presence and activity of androgens in the skin which stimulate abnormal patterns of hair growth. Aromatase and 17-beta-hydroxysteroid activities are increased in the fat cells and peripheral aromatisation increases with body weight. The metabolism of estrogens by way of 2-hydroxylation and 17-alpha-oxidation is decreased. Estrogen levels increase as a result of peripheral aromatisation of androstenedione. This cascade results in a chronic hyper-estrogen production (estrogen dominance) (Hopkinson 1998).

Hirsutism occurs in 70% of women with PCOS in the USA, as opposed to only 10-20% of Japanese women diagnosed with PCOS (Visnova 2003). This may be explained by the genetically determined differences in 5-alpha-reductase activity between different cultures, or from a holistic standpoint may reflect differences in endocrine behaviour in accordance with local diet and levels of physical fitness.

Estrogen dominance

The hypothalamic pituitary axis imbalance can contribute significantly to the etiology of PCOS. The result of increased gonadotrophin releasing hormone (GnRH) output causes an elevation in the pulsatile output of LH and results in an elevated LH to FSH ratio (typically 2:1 respectively) (Hopkinson 1998, Stenchever 2001). FSH is not increased as a result of elevated LH

in this case, likely due to the hypothalamus responding via negative feedback to the already chronically elevated estrogen levels.

About 25% of PCOS patients exhibit elevated prolactin (D'Hooghe 2002, Hopkinson 1998) known as hyperprolactinemia. Hyperprolactinemia results from abnormal estrogen negative feedback via the pituitary gland. Elevated prolactin can in turn contribute to elevated estrogen levels.

PCOS holistic diagnostic criteria

Menstrual irregularity

- Eight or fewer menstrual cycles per year
- Unpredictable menstrual cycles
- Amenorrhea for longer than 4 months in the absence of pregnancy or menopause
- Infertility
- History of ovarian cysts
- Irregular bleeding
- Excessive or heavy bleeding

Skin complications

- Adult acne
- Severe adolescent acne
- Cystic acne on face, neck, back shoulders
- Hirsutism with excessive hair on face, body, upper lip, chin, neck, abdomen
- Thinning of the head hair or male pattern balding
- Acanthosis nigricans: discoloration or darkening of skin (may be in patches) around neck, groin, under arms, skin folds or skin tags (see later)

Insulin resistance

- Weight gain, especially around trunk (apple body shape or android body shape, especially after the age of 30 years)
- Dysglycemia
- Difficulty losing weight
- Family history of diabetes or menstrual irregularity

Obesity is found in 50% of patients with PCOS (D'Hooghe 2002, Hopkinson 1998, Stenchever 2001). The body fat is usually located centrally around the trunk. A higher waist to hip ratio indicates an elevated risk of cardiovascular disease and diabetes (D'Hooghe 2002). Insulin resistance and metabolic syndrome are commonly seen in PCOS patients and insulin resistance is now recognised as a risk factor for the development of diabetes mellitus type 2 (Hopkinson 1998). Approximately one-third of obese PCOS patients have impaired glucose tolerance and up to 10% have diabetes mellitus type 2.

Acanthosis nigricans, a condition in which the vulva develops thickened, pigmented velvety lesions, is considered a marker of insulin resistance in women with hirsutism. These lesions can also be found on the nape of the neck, inner thigh and below the breast. Women with severe insulin resistance can develop HAIR-AR syndrome consisting of hyperandrogenism (HA), insulin

resistance (IR) and acanthosis nigricans (AR) (D'Hooghe 2002, Hopkinson 1998). These women will have elevated testosterone (>150 ng/dL) and fasting insulin levels of greater than 25 mIU/dL. Insulin alters steroidogenesis (independent of gonadal production) in PCOS, as insulin and insulin-like growth factor receptors are located within the ovarian tissue (Hopkinson 1998).

Associated with impaired glucose tolerance is the abnormal lipoprotein profile that is commonly seen in patients with PCOS. The typical PCOS lipoprotein profile includes:

- Elevated total cholesterol
- Elevated triglycerides
- Elevated low density lipoproteins (LDL)
- Low high density lipoproteins (HDL)
- Low apoprotein A-12

The culmination of these factors leads to a marked elevation in cardiovascular risk for the PCOS patient. Another metabolic observation that puts these women at higher cardiovascular risk is the incidence of impaired fibrinolysis, shown by elevated circulating levels of plasminogen activator inhibitor. This is associated with atherosclerosis and hypertension.

When these factors are combined, PCOS women are at much higher risk of hypertension, atherosclerosis and exhibit a seven-fold risk of myocardial infarction (Hopkinson 1998).

Recommended naturopathic hormonal evaluation

- Salivary adrenal stress index, including ACTH
- Salivary or serum expanded female hormonal panel, including testosterone and LH to FSH ratio
- Glucose tolerance test
- Thyroid panel
- Blood lipid profile

Typical hormonal disturbances associated with PCOS diagnosis include:

- Elevated LH while FSH is usually low at a ratio of 2:1
- Progesterone can be low
- Sex hormone binding globulin (SHBG) usually low
- Androgens such as testosterone and DHEA-S are usually elevated

Conventional treatment approaches

The conventional treatment for PCOS is dependent on the patient's desired goal of either menstrual regularity in order to achieve pregnancy or menstrual regularity for contraception. Some women seek treatment for the removal of excessive male hair growth patterns such as increased facial hair (common to women with PCOS and elevated androgens).

Women are currently being treated according to their presenting clinical symptoms, including irregular menses, hirsutism and infertility (D'Hooghe 2002, Hopkinson 1998, Stenchever 2001).

Irregular menses

A combined oral contraceptive pill is commonly used to regulate the menses. By both increasing the levels of sex hormone binding globulin (SHBG) and decreasing androgen secretion, this can reduce elevated free testosterone activity. The combined pill worsens insulin resistance and if the patient falls into the categories of being overweight or obese, this therapy is relatively contraindicated (D'Hooghe 2002, Hopkinson 1998).

Hirsutism

Hirsutism is addressed with the administration of the anti-androgens cyproterone acetate or spironolactone. The action of these drugs is to inhibit the binding of dihydrotestosterone (DHT) to the receptors at the hair follicle site (Sweetman 2002).

Infertility

Clomifene citrate is suggested to women with PCOS who are diagnosed with fertility challenges. This drug induces ovulation and does increase risk of multiple pregnancies (Sweetman 2002). It acts by inhibiting the estrogen negative feedback at the hypothalamus, thus enhancing the pituitary's production of FSH.

Other pharmaceutical medications

Other pharmaceutical medications which can be prescribed for PCOS include medroprogesterone acetate, gonadotrophin releasing hormone agonists, glucocorticoids, ketoconazole, flutamide, finasteride and metformin.

Overview of botanical protocol

Strong evidence supports the current hypothesis that the underlying cause of PCOS is due to insulin resistance (a decreased peripheral sensitivity to insulin), hence managing this aspect becomes the most important feature for the phytotherapist. The exact mechanisms for insulin resistance are not yet known within the conventional medical community, however the holistic practitioner finds that insulin resistance has a high correlation to a diet high in refined carbohydrates coupled with a poor adrenal glycemic counterbalance.

As the HPA axis becomes weakened (as a result of chronic stress), insulin sensitivity becomes heightened, adversely affecting the ovaries and thyroid. Elevated insulin and insulin-like growth factor have an effect in stimulating androgen production from the adipose tissue, ovaries and adrenals. Under chronic stress, excess cortisol is produced from the adrenal glands, triggering the release of elevated levels of prolactin and a sympathetic nervous system response (Lara 1993). Prolactin has an inhibitory effect on the production of FSH and elevates the production of LH, worsening the scenario for women with PCOS. It is essential that the adrenals are well supported at a functional level with herbal adrenal tonics such as *Glycyrrhiza glabra* and supported by adaptogens such as *Withania somnifera*.

The first step in restoring ovarian function and a normal menstrual cycle in a PCOS patient is to break the pattern of hyperinsulinemia with a combination of diet and lifestyle strategies. Implementing a low refined carbohydrate diet and exercise is essential for a truly successful protocol.

Primary herbs

Paeonia lactiflora (white peony)

Gymnema sylvestre (gymnema)

Tribulus terrestris (tribulus)

Vitex agnus-castus (chaste tree)

Caulophyllum thalictroides (blue cohosh)

Paeonia lactiflora (white peony)

Paeonia lactiflora has been used for gynecological conditions by both Chinese and Western herbalists, and is used by Western herbalists for PCOS, hyperprolactinemia, endometriosis, ovarian failure and androgen excess. *Paeonia* has been shown to positively influence low progesterone, reduce elevated androgens (testosterone) and acts to modulate estrogen and prolactin (Trickey 1998). In vitro the active constituent paeoniflorin has been shown to affect the ovarian follicle by its action on the aromatase enzyme (Ota 1998). Aromatase is important for follicle maturation, ovulation and corpus luteum function, steroid hormone synthesis and the regulation of the conversion of androgens to estrogens. The biofeedback in the pituitary and hypothalamus relies on aromatase to regulate prolactin and GnRH. The daily dose for *Paeonia* is 4.5 mL to 9 mL of a 1:2 dried plant extract (Bone 2003).

The traditional Chinese/Kanpo formula known as Shakuyaku-Kanzo-To or TJ-68, which is a decoction of *Glycyrrhiza glabra* and *Paeonia lactiflora*, has been the subject of a number of clinical trials, all of which demonstrate activity in the hormonal regulation of androgens. In one trial involving eight women with hyperandrogenism and oligomenorrhea, the formula was given for 2 to 8 weeks. This combination regulated the LH to FSH ratio. Over this period of time, serum testosterone levels decreased to less than 50 ng/dL and this resulted in seven of the eight women ovulating regularly (Yaginuma 1998).

Another trial involved 20 women diagnosed with PCOS. The formula was successful in lowering testosterone in 90% of the women, of which 25% went on to conceive (Takahashi 1988). It is suggested that it acts directly on the ovary, increasing the activity of aromatase, which promotes the synthesis of estradiol from testosterone, thus lowering serum testosterone levels. It also seems to regulate the LH to FSH ratio (Takahashi 1994).

Gymnema sylvestre (gymnema)

Gymnema sylvestre is a traditional Ayurvedic herb used as an antidiabetic, hypoglycemic, lipid lowering agent

and to support weight reduction. *Gymnema* possibly has a trophorestorative action of the beta cells of the pancreas (Bone 1996). The plant part used as medicine is the leaf.

Gymnema is well indicated for PCOS due to its insulin modulating activity and the added benefits of reducing the elevated triglycerides associated with PCOS. Key constituents of *Gymnema* include saponins, especially the gymnemic acids. Gymnemic acid suppresses the sweet taste on the taste buds, so if taken before food masks the sweet sensation. *Gymnema* has demonstrated hypoglycemic activity in experimental models of diabetes and regulated blood sugar in hyperglycemia. The mechanism of action also includes the inhibition of glucose absorption in the intestine.

The daily dose of *Gymnema* is 3.5-11 mL of 1:2 liquid extract (Bone 2003, Merrily 2002). Since conventional medical models are focussing on pharmaceutical agents such as metformin to control PCOS, *Gymnema* may prove to be one of the most significant herbs in the treatment of the underlying factor of insulin resistance.

***Tribulus terrestris* (tribulus)**

Tribulus terrestris, commonly known as puncture vine, is an endemic weed to many regions of the world including the Mediterranean, India, China, South Africa and Australia. The aerial parts, particularly the leaf, are used for medicinal purposes in the Western tradition.

As a result of Bulgarian research, *Tribulus* has become a popular herb for the treatment of female and male endocrine disorders. It acts as a general tonic, aphrodisiac, estrogen modulator and androgen modulator and is used to restore vitality, libido and reduce the physiological effects of stress (Bone 2003, Takahashi 1988).

The Bulgarian research has identified a unique steroidal saponin class known as furostanol saponins, and extracts are standardised to contain at least 45% of these saponins calculated as protodioscin. The leaf is noted to be higher in these unique saponins than the fruit or root. Other active constituents include phytosterols and spirostanol glycosides.

The tonic activities of *Tribulus* have been shown to act by intensifying protein synthesis and enhancing the activity of enzymes associated with energy metabolism. It increased iron absorption from the small intestines and inhibited lipid peroxidation during stress. This leads to more muscle strength and improved endurance and stamina (Bone 2003).

To ensure the desired clinical results it is recommended to use only the Bulgarian grown *Tribulus* standardised to 40% furostanol saponins. It is not interchangeable with the Chinese or Indian *Tribulus*.

The daily dose of *Tribulus* corresponds to extracts containing furostanol saponins as protodioscin at 300 mg to 400 mg per day. In PCOS it is best to use *Tribulus terrestris* on days 5 to 14 of the menstrual cycle to restore menstrual regularity.

***Vitex agnus-castus* (chaste tree)**

Vitex agnus-castus is beneficial for ovulatory factors associated with PCOS; in particular it has been shown to downregulate the production of excess prolactin, a condition known as hyperprolactinemia. *Vitex* is also postulated as having antiandrogenic properties (Mills 2000). Hyperprolactinemia is related to adrenal stress and hyperinsulinemia in PCOS. It is well documented that the active constituents in *Vitex* demonstrate a dopaminergic activity and dopamine inhibits the production of prolactin. The dopaminergic compounds in *Vitex* have been identified as the diterpene, including rotundifuran and 6 β ,7 β -diacetoxy-13-hydroxy-labda-8,14-diene. However recent research is pointing to other phytochemicals which may have this activity. Other constituents of *Vitex* include essential oils, flavonoids (such as casticin) and iridoid glycosides (including aucubin and agnuside) (Bone 2003, Merrily 2002).

Hyperprolactinemia, or the more subtle condition of latent hyperprolactinemia, is one of the most frequent causes for cyclical disorders, including corpus luteal insufficiency. This can lead to premenstrual syndrome (PMS) and progesterone deficiency, secondary amenorrhea and premenstrual mastalgia (De Cherney 2003). In an uncontrolled study, *Vitex* reduced elevated prolactin levels in 80% of 34 women with hyperprolactinemia at a dosage of 30-40 mg per day for one month and improved symptoms of a variety of menstrual disorders including secondary amenorrhea, cystic hyperplasia of the endometrium, deficient corpus luteum function, metrorrhagia, polymenorrhea and oligomenorrhea (Bone 2003).

Vitex reduced the thyroxin releasing hormone (TRH)-induced prolactin release (essentially a pituitary thyroid axis problem), normalised shortened luteal phases, corrected luteal phase progesterone deficiencies and reduced PMS symptoms in women with luteal phase defects due to latent hyperprolactinemia (Bone 2003).

Vitex should be considered a first line botanical therapy for hyperprolactinemia and given for the duration of at least 3 to 6 months. In herbal writings *Vitex* is often attributed to increasing LH, which is not desirable in PCOS. However clinical experience has shown that it is valuable in PCOS, especially when combined with other herbs, probably because of its action in reducing prolactin. The daily dose of *Vitex* is 1-4 mL of a 1:2 dried plant tincture or 500-1000 mg of dried berries daily (Bone 2003). It is best taken as a single dose in the morning (Bone 2003). In PCOS it is best combined with *Tribulus* and *Paeonia*.

***Caulophyllum thalictroides* (blue cohosh)**

Caulophyllum thalictroides is known by the common name of blue cohosh and is native to North America. Within traditional use among the native North Americans it was used for women as a remedy for amenorrhea and

profuse menstruation, both of which are common features of PCOS. It is particularly useful to bring on the menses in PCOS. It acts as a uterine and ovarian tonic and a pelvic anti-inflammatory. The known constituents of *Caulophyllum* root include glycosides, caulosaponin and caulophyllosaponin, which are known to stimulate the uterus. Other identified constituents include N-methylcystine, taspine and thalictroidine (Bone 2003).

The daily dose is 1.5-3 mL of 1:2 dried plant extract (Bone 2003).

Example PCOS formula

Herb	Conc.	Total
<i>Vitex agnus-castus</i>	1:2	12.5 mL
<i>Glycyrrhiza glabra</i>	1:1	12.5 mL
<i>Paeonia lactiflora</i>	1:2	25 mL
<i>Gymnema sylvestre</i>	1:2	25 mL
<i>Schisandra chinensis</i>	1:2	25 mL
TOTAL		100 mL

Dose 15 mL daily or 5 mL three times daily.

In a case of a PCOS patient with amenorrhea, include *Caulophyllum thalictroides* at a dose of 2 mL per day to help induce the menses.

Once a cycle has been initiated, change to *Tribulus* concentrated extract, equivalent to furostanol saponins (as protodioscin) 300-400 mg per day on days 5 to 14 of the cycle to ensure cyclic regularity.

Dietary modification

A review of the extensive literature specific to lifestyle factors in PCOS demonstrates that an essential treatment strategy for ameliorating the symptoms of PCOS and resolving the underlying metabolic derangements is the implementation of a low carbohydrate diet. This will tightly control blood sugar levels and resultant insulin production. High levels of insulin result in high levels of triglycerides and low levels of high density lipoproteins which puts these patients into a high cardiovascular disease risk category.

Modulating the diet not only helps the female endocrine cycle but also serves as preventative medicine against these cardiovascular risk factors. As the insulin levels normalise, this will also improve circulating levels of SHBG therefore limiting the problematic effects of free androgens on the menstrual cycle (Hopkinson 1998, Sweetman 2002).

Women with PCOS are urged to lose 5% to 10% body weight using a moderate protein, low refined carbohydrate diet. When this approach was taken in one clinical trial, 10 of the 11 subjects resumed a normal cycle within 10.5 months (Hopkinson 1998). In a similar study, such weight loss restored ovulation in 60 out of 67 previously anovulatory women (Visnova 2003).

The dietary profile should include approximately

30% good quality fats, 40% protein and 30% complex carbohydrates (D'Hooghe 2002, Hopkinson 1998, Glueck 2003). Literature suggests establishing an energy efficient diet of 1000-1500 kcal per day. It is recommended to avoid alcohol, caffeine, smoking and psychosocial stressors. *Gymnema* is helpful in reducing carbohydrate and sugar cravings, and therefore improving compliance with dietary changes (Bone 2003).

Exercise

Implementing an exercise regimen of approximately 30 minutes per day will assist weight loss and improve the endocrine regulation of stress.

Case history

Overview

Female patient aged 34 presented with irregular menses and was considering attempting to become pregnant. She had been diagnosed with PCOS 2 years ago. Up until 6 months prior to her consultation she had taken the oral contraceptive in combination with Levoxyl, but suffered side effects of heightened emotional lability from these drugs.

Her menstrual cycle varied in length anywhere from 50 to 70 days and she experienced mid abdominal cramping for 24 hours prior to the onset of her menses. The flow was medium to light and lasted for 4 to 5 days, dark red in color, starting with brown spotting for 12 to 18 hours. She had occasional menstrual clots, stringy and lumpy in nature.

Her skin was affected badly by the PCOS and she experienced painful, deep cystic acne on her face, chest and back, which was worse for up to a week before the onset of each period. She had taken two courses of isotretinoin (Accutane) within the past 5 years and regularly used a tetracycline for treatment of her acne. Breast tenderness was an uncomfortable premenstrual feature for her.

She had gained 10.5 kg over the past 3 years, which she had difficulty losing despite exercise on a regular basis. She did however have a high carbohydrate diet and craved sugar intensely.

She was a shift worker in a high stress and responsibility occupation and fatigue was a daily experience.

She was taking prescribed thyroid hormone (thyroxine) for Hashimoto's thyroiditis, diagnosed 4 years prior. At the same time she was diagnosed as having secondary osteoporosis. Recent evaluation showed her spinal density indicated osteopenia, her femoral density indicated osteoporosis and total hip density indicated severe osteopenia.

Additional assessment

Hormonal evaluation showed a typical pattern of a 2:1 LH to FSH ratio, with elevated testosterone and hyperlipidemia.

Treatment protocol

Herb	Conc.	Total
<i>Vitex agnus-castus</i>	1:2	12.5 mL
<i>Glycyrrhiza glabra</i>	1:1	12.5 mL
<i>Paeonia lactiflora</i>	1:2	25 mL
<i>Gymnema sylvestre</i>	1:2	25 mL
<i>Schisandra chinensis</i>	1:2	25 mL
TOTAL		100 mL

Dose 8 mL twice daily.

Additionally:

- *Tribulus* concentrated extract, equivalent to furostanol saponins (as protodioscin) 300-400 mg per day on days 5 to 14 of the cycle to ensure cyclic regularity.
- *Fucus vesiculosus* 1:1 10 mL twice daily.

Rationale

Vitex agnus-castus was indicated for the hormonal imbalance and hyperprolactinemia, often resulting in the symptom of premenstrual breast tenderness. A combination of *Glycyrrhiza glabra* and *Paeonia lactiflora* were included into the formula to utilise the synergy of these plants in TJ-68 to reduce elevated testosterone and induce ovulation. *Gymnema sylvestre* was included in the formula to treat the insulin resistance and hyperlipidemia and assist with reducing associated carbohydrate cravings.

Schisandra chinensis was included in the formula to provide liver support, in particular to improve the liver's ability to conjugate sex hormones and assist in reducing the circulating levels of testosterone and estrogen. *Tribulus* was selected to ensure a healthy follicular phase of the cycle and as an androgen modulator. *Fucus vesiculosus* was indicated for thyroid support as a plant source of iodine and is traditionally recommended by herbalists to assist with weight loss associated with hypothyroidism.

Echinacea spp. root could be a valuable additional inclusion for an autoimmune-mediated hypothyroid condition. In cases such as this, *Echinacea* would serve as an immune modulator.

Conclusion of care

After 5 months on the herbal protocol the patient's cycle had regulated to a 32 day cycle with a consistent 15 day follicular phase and a 17 day luteal phase. Problematic symptoms such as mastalgia, acne and hirsutism diminished significantly during the 5 month program. The lipid profile had improved to within normal range and with the inclusion of the combined regimen of *Gymnema*, dietary modification (low carbohydrate diet) and exercise, she lost a total of 12% body weight in the 5 months.

She went on to begin a full preconception healthcare program and became pregnant in her second month.

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Metabolic and neurological consequences of maternal nutrition: a review

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Introduction

Maternal nutrition, both prior to conception and during pregnancy, is increasingly being recognised as an important determinant of the later life health of the mother's offspring. The food a mother consumes is the primary influence on the prenatal nutritional environment of her fetus. An increasing body of scientific research suggests that biological adaptation may result in a programming of the effects of early nutritional environment through fetal and neonatal imprinting (Kaludjerovic 2010). The exact mechanisms are by no means clear, but it has been suggested that programming may be a result of epigenetic changes.

Epidemiological evidence now links maternal malnutrition to conditions as diverse as cardiovascular disease, diabetes and schizophrenia, amongst others (Kaludjerovic 2010, Langley-Evans 2010). Both over and under feeding have been shown to have an impact (at least in animal studies) and this may influence medical prescribing habits in the future. In the bulk of the Australian community pregnant mothers are more at risk of malnutrition (having insufficient or inappropriate proportions of nutrients in the diet) rather than undernutrition (an overall deficiency of nutrients, including caloric deprivation) (De Souza 2011). The resultant environment then understandably affects the development of the fetus.

This review examines recent developments in this area and teases out certain nutritional factors that may be relevant, with the prospect of developing targeted interventions. The role of herbal medicines is also covered, but with the paucity of evidence surrounding maternal usage of herbs it is difficult to establish any definitive understanding of their activity.

The process of fetal programming

During the prenatal period the embryo or fetus is entirely dependent upon the mother for its nutrition. The developing child is highly sensitive to shifts in the maternal environment (particularly during periods of rapid growth) and adverse circumstances may change the expression of key genes, resulting in perturbation of cellular development and differentiation, and by implication the growth of organs and tissues (Kaludjerovic 2010, Jones 2011). As the fetus grows it continually monitors its surrounds and may adapt its physiological functioning and growth processes in order to best survive.

A simple, yet sometimes controversial example of this is the rebound infantile scurvy that may occur if a mother has taken megadoses of vitamin C during her pregnancy. The adaptation process, whilst facilitating immediate survival benefits, may result in irreversible change to cellular function and structure. Tissue remodelling and altered metabolic functioning are then theorised to be expressed as the development of chronic diseases later in life (Warner 2010, Johnston 1999). Whilst there are a multiplicity of factors that influence maternal environment, including smoking, psychological and physical stress and endocrine disorders, nutrition (both over- and under-) plays a key role, especially as it is so easily modifiable.

Metabolic conditions: diabetes and cardiovascular disease

To date much research into fetal programming and adaptation has focused on the later life development of metabolic conditions, including insulin resistance, type 2 diabetes, metabolic syndrome and cardiovascular disease. As a general rule, a low birth weight may be considered a crude indicator of disrupted fetal growth (Warner 2010). Numerous epidemiological studies, beginning with the work of Barker et al (1989, 1990), have established links between this indicator and resultant increases in cardiovascular mortality and the development of type 2 diabetes. Studies of the Dutch Winter Hunger in 1944 produced some of the most clear initial correlations. Individuals born to mothers who endured famine periconceptually and during pregnancy showed increased risk of cardiovascular disease, hypertension, insulin resistance and obesity in later life (Painter 2005). Additionally murine models have demonstrated that protein restriction during conception and pregnancy can have profound consequences for offspring. These animals exhibited numerous features of cardiometabolic disease, including impaired glucose metabolism, dyslipidemia, hypertension, vascular dysfunction and increased fat deposition amongst others. High fat diets have been found to produce similar results (Lillicrop 2011).

While cardiovascular conditions and diabetes are commonly linked to obesity (which may be lifestyle induced), a recent study suggests that the correlation between a disadvantageous fetal environment and metabolic derangement may stand regardless of whether a child becomes overweight (Bush 2011). The study

found that higher maternal glucose concentrations were inversely correlated with insulin sensitivity and beta cell response to glucose in children aged 5 to 10 years regardless of their current weights. Altered sensitivity of the pancreas and insulin target tissues, such as the liver and skeletal muscle, may result from the prenatal nutritional environment.

Essential fatty acids

Low birth weight (from maternal malnutrition) has also been shown to suppress the activity of delta-5 and -6 desaturases in certain populations. This leads to low plasma and tissue concentrations of polyunsaturated fatty acids and their resultant products (Das 2010). These nutrients are known to play a key role in health management and the prevention of metabolic disorders and such altered metabolism may be one of the mechanisms responsible for the high incidence of insulin resistance, metabolic syndrome and ischemic heart disease in such populations (Das 2010).

Vitamin D deficiency

Micronutrient intakes are implicated too. Vitamin D deficiency in perinatal life may predispose a person to an increased susceptibility of early life onset of chronic diseases including heart disease and type 1 diabetes (Kaludjerovic 2010). The Mysore Parthenon Study investigated this connection by measuring serum 25-hydroxyvitamin D in over 500 women at 28-32 week gestation and then followed up by assessing cardiovascular risk markers in their children at 9.5 years of age. The researchers found that the children of vitamin D deficient mothers had far higher fasting insulin resistance than those of mothers with adequate vitamin D serum levels, suggesting that a lack of this nutrient may predispose offspring to risk of both type 1 and type 2 diabetes (Krishnaveni 2011).

Results of studies show not only that rates of vitamin D deficiency are higher among women with impaired glucose tolerance (IGT) and gestational diabetes mellitus (GDM), but that low levels of vitamin D are in themselves associated with an increased risk of GDM (Soheilykhah 2010, Burris 2012). While vitamin D supplementation in women at high risk of vitamin D deficiency has previously been considered to improve neonatal handling of calcium, recent research suggests there is no significant association between infant whole body bone mineral content at 8-21 days of age and feto-maternal vitamin D status (Dror 2012).

Zinc deficiency

Zinc is a highly important nutrient during fetal and early childhood development, playing a role in cell differentiation and division as well as the development of multiple organ tissues including the heart (Stefanidou 2006). It is also considered to be an essential nutrient for the epigenome, due to its roles in enzymes that

control methylation and 'epigenetically modify DNA and histones' (Tomat 2010). Marginal or moderate deficiency induced in rats in utero has been found to correlate to altered activity of zinc finger transcription factors, reduced birth weights and altered growth and maturation of cardiac (and other) tissue. These changes were associated with increases in blood pressure and susceptibility to cardiovascular disease (Tomat 2010).

Neurological function and mental health

Cognitive function and mental health are also susceptible to prenatal influence. Adverse fetal circumstances resulting in low birth weight (such as maternal undernutrition) have been associated with impaired cognitive function, depression and increased stress responsiveness later in life (Broekman 2009, Bale 2010, Jones 2006). Recent studies suggest that this may be due in part to adaptive changes in the activity of brain regions involved in the processing and response to stressful stimuli.

In children who exhibited lower birth weights (adjusted for placental weight), researchers found altered lateralisation of the activity of brain regions involved in the processing and response to stressful stimuli (Jones 2011). This type of asymmetrical activation of cerebral hemispheres is linked in the literature to states of depression and increased vulnerability to stress (Wittling 1997, Hecht 2010). This may be one of the mechanisms by which maternal undernutrition and fetal programming lead to mental health issues in later life.

Essential fatty acids

The most rapid brain growth in humans (the times when it is most vulnerable to nutritional influence and insult) occurs during the third trimester of fetal life and in the first 24 months after birth (De Souza 2011). It is now well acknowledged that undernutrition or malnutrition during this time may be linked to neurointegrative disorder. In particular the role of omega-3 fatty acids, especially docosahexaenoic acid (DHA), in the developing brain has been a primary focus, leading health practitioners to recommend maternal supplementation during pregnancy and breast feeding. DHA is one of the main fatty acids in the grey matter and is required for proper development of the CNS. Deficiency may lead to cognitive impairment and neurological disorders in offspring (De Souza 2011).

Gibson et al (2011) question the use of the n-6 fatty acid linoleic acid (LA, 18:2n-6) in the diet of pregnant women as LA competes with alpha-linolenic acid (18:3n-3) for endogenous conversion to EPA and DHA, and also inhibits incorporation of DHA and EPA into the tissues. Thus high levels of LA in the diet may result in low levels of n-3 long chain polyunsaturated fatty acids (LCPUFAs). The importance of an adequate supply of n-3 LCPUFA for ensuring optimal development of infant brain and visual systems is well established and there is now evidence that the supply of n-3 LCPUFA also

influences a range of growth, metabolic and immune outcomes in childhood (Gibson 2011).

Supplementation of DHA in infants appears to be more effective in improving the neurodevelopmental outcome of preterm children rather than in utero DHA supplementation of pregnant women (Makrides 2012). Infant boys and girls respond differently to DHA supplementation and birth weight may also be important in predicating the DHA responsiveness (Makrides 2012).

Urwin et al (2012) report that women consuming 2 portions of farmed salmon per week from week 20 of pregnancy until delivery provided higher proportions of eicopentaenoic acid (EPA) (80%), docosapentaenoic acid (DPA) (30%) and DHA (90%) compared with controls, thus improving the supply of these fatty acids to the breast fed infant. However a study by Sørensen et al (2012) concluded that there was no support for the hypothesis that higher proportions of maternal EPA or DHA during pregnancy are associated with a lower risk of type 1 diabetes in the offspring.

Caloric deprivation and folate deficiency

Interestingly new research is beginning to identify maternal deficiency as one of the environmental factors implicated in schizophrenic illness. Researchers now believe that this is primarily a neurodevelopmental disorder which may be partially a consequence of brain maturational disruption during the in utero period (Brown 2011). Caloric deprivation along with folate and vitamin D deficiencies are implicated in this process. Data collected from the Dutch Hunger Winter period (referred to earlier) deduced a link between severe famine during the time of pregnancy and/or preconception and a two-fold increase in rates of schizophrenia amongst offspring (Hoek 1998). There was also a peak in neural tube defects amongst the infants of this cohort. Further, epidemiological surveys of the Chinese famine of 1959-61 produced similar results. The accordance of increased neural tube defects and schizophrenia in these studies suggests that a lack of folate may be partially responsible. Some research suggests that the connection may be increased maternal homocysteine levels (due to inadequate remethylation) at various points throughout a pregnancy (Brown 2011).

Vitamin D: an 'optimal window'

Vitamin D has also been receiving considerable press recently for its effect on mental development. There are three major lines of evidence behind the hypothesis linking maternal levels to the development of mental illness. It has been invoked to explain the seasonal correlations between winter births and an increased risk of schizophrenic illness (Davies 2003). In addition murine models where the fetus is deprived of vitamin D produce clear changes in the structure and function of the brain. These changes are similar to those observed in patients with schizophrenia (Brown 2011). Finally darker skin (thus lower levels of vitamin D) has been

linked to higher rates of schizophrenia (Bresnahan 2007). Epidemiological investigation thus far has produced mixed results, leading some researchers to hypothesise that there may be an optimal window of vitamin D levels in newborns with regard to the prevention of the condition (Brown 2011).

Herbal medicine

The use of herbs in pregnancy has been a contentious issue of recent years. As herbalists, most practitioners have been taught to be cautious as many traditional herbal preparations may have adverse effects on the fetus particularly in the early stages of gestation (Wang 2012). That said, there are a number of herbs that have also been shown to be safe and beneficial, notably the well known *Echinacea* spp, *Rubus idaeus* and *Zingiber officinale*. A recent review found that over 72% of midwives surveyed in Canada and New Zealand recommended or offered CAM to their patients (Adams 2011). In addition the most commonly used CAM treatment in the studies reviewed was herbal medicine (85%) (Adams 2011).

Given their sometimes powerful active constituents, herbs have the potential to positively or negatively affect not only maternal physiology but also fetal genetics (as evidenced by the teratogenic effects of some plants). All studies in the past have focused on short term harm that may be caused, rather than the long term benefits. This is understandable given the number of issues that would arise in formulating such studies. As practitioners it is something that should be kept in mind when prescribing herbal medicines in pregnancy. The active constituents may beneficially affect the fetal environment not only directly (e.g. from vitamins contained in the herbs), but also secondarily (e.g. by reducing anxiety in the mother thus reducing the amount of stress hormones to which the fetus is exposed).

Whilst this is a far from comprehensive review of the area, this article highlights some of the emergent research in the area of maternal nutrition and resultant epigenetic programming. The increasing correlations between metabolic and neurologic disorders and the fetal environment has important implications for clinical practice in managing pre- and perinatal nutrition. In the past, health professions have assumed that the major nutritional issues in pregnancy were the provision of sufficient nutrients (i.e. in multivitamins or folate supplements) to prevent obvious infant morbidity (such as neural tube defects).

As we increasingly realise that there are longer term implications, consideration of nutritional and lifestyle advice for pregnancy may need to shift and new recommendations may be required. In particular, physicians and the broader health community should be encouraged to ensure adequate vitamin D levels are received as required. Undoubtedly more information will come to light in the near future as our understanding continues to progress, and for this reason it is important

to be aware of the processes and potentials of nutrition and fetal programming.

As herbalists, practitioners should also keep in mind the potential for phytonutrients and herbal actives to both positively and negatively affect the infant not only in the short term but also long into the future.

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Antiproliferative effect of *Viola tricolor* on neuroblastoma cells in vitro

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Objective: Neuroblastoma is the most common extracranial malignancy in childhood. Although most patients have a good prognosis, some cases experience metastatic tumor development which despite intensive therapy grows progressively. The present study was designed to investigate the antitumor effect of *Viola tricolor* against neuroblastoma N2a cells.

Material and methods: The cells were cultivated and incubated for 24 h with different concentrations (0, 100, 200, 400 and 800 µg/ml) of hydroalcoholic extract of *Viola tricolor* or its fractions: water fraction, ethyl acetate fraction and n-butanol fraction. The cell proliferation was determined using MTT colorimetric assay.

Results: None of the hydroalcoholic extract concentrations or the water fraction significantly changed the cell viability. The ethyl acetate fraction, at 400 and 800 µg/ml, significantly inhibited (38% and 87% respectively) cell proliferation of N2a cells. Similarly 800 µg/ml of n-butanol fraction decreased (76%) the surviving cells.

Conclusion: *V. tricolor* exhibits potent antitumor activity against neuroblastoma N2a cells. The main component/s responsible for this effect are most likely found in the ethyl acetate fraction and may also be present in the n-butanol fraction of this plant.

Key words: *Viola tricolor*, neuroblastoma, N2a, MTT

Introduction

Neuroblastoma is the most common extracranial malignant and deadly solid tumor of childhood (Brodeur 2003, Fisher 2012). It originates from the primordial neural crest cells and arises from either the adrenal or anywhere along the sympathetic chain (Dhir 2010). Although most patients have a good prognosis, some infants with MYCN-amplified (v-myc myelocytomatosis viral related oncogene, neuroblastoma derived (avian) or MYCN) neuroblastoma show poor survival rates. Therefore the search for new alternative or synergistic antitumor agents has continued (Canete 2009).

Medicinal plants have always been a good source for finding new remedies for human health problems. Many traditional medicinal plants have been tested for their antitumor potential in cell culture or in animal models (Eli 2012, Sakarkar 2011, Tavakkol 2006). They show anticancer effects through inhibiting cancer-activating enzymes, promoting DNA repair, stimulating production of protective enzymes, enhancing body immunity and inducing antioxidant action (Sakarkar 2011).

Viola tricolor, a member of Violaceae plant family, is a common horticultural plant in Iran. It has been reported to have a number of medicinal attributes including anti-inflammatory (Toiu 2007), antimicrobial (Witkowska-Banaszczak 2005), antioxidant (Vukics 2008) and diuretic (Toiu 2009) activity.

Materials and methods

Drugs and chemicals

Mouse neuroblastoma (N2a) cell was obtained from Pasteur Institute (Tehran, Iran). Dimethyl sulfoxide (DMSO), penicillin-streptomycin and 3-(4,5-Dimethyl-2-thiazolyl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) were purchased from Sigma (USA). Dulbeccos Modified Eagles medium (DMEM) and fetal bovine serum (FBS) were bought from GIBCO (USA).

Preparation of *V. tricolor* crude extract

The *V. tricolor* aerial parts of the flowering plants were collected from Pardis Campus (Mashhad, Iran). The identity of the plant was confirmed and for future reference a voucher specimen (12568) was deposited at the School of Pharmacy herbarium (Mashhad University of Medical Sciences, Iran). The plant materials were dried, powdered and subjected to extraction with 70% ethanol in a Soxhlet apparatus for 48 h. The hydroalcoholic extract was then dried on a water bath and the yield dissolved in DMSO.

Fractionation of hydroalcoholic extract

For preparation of fractions, the dried hydroalcoholic extract (10 g) was suspended in distilled water and transferred to a separator funnel. With solvent/solvent extraction, it was fractionated using ethyl acetate and n-butanol. The ethyl acetate and n-butanol fractions were separated to obtain water fraction (Ghorbani 2012,

Sadeghnia 2012). The fractions were dried on a water bath and stock solutions made up in DMSO (ethyl acetate and n-butanol fractions) or saline (water fraction).

Cell culture and treatment

The N2a cells were cultivated in high glucose DMEM supplemented with 10% FBS and penicillin (100 units/mL) and streptomycin (100 µg/mL) at 37°C in an atmosphere of 5% CO₂. Trypsin solution was used to passage cultures whenever they were grown to confluence. The cells at subconfluent stage were harvested from culture flask and after checking the viability with trypan blue exclusion technique they were seeded overnight in 96 well culture plate. To test the possible cytotoxicity of *V. tricolor*, the culture media was changed to one containing varying concentrations (100-800 µg/mL) of the hydroalcoholic extract and its fractions. The cells were further incubated for 24 h and observed under light inverted microscope for shape, granulation and suspension (anchorage independency).

MTT assay

The effect of *V. tricolor* on N2a cell proliferation was determined using MTT colorimetric assay as previously described (Hajzadeh 2007, Mousavi 2010). Briefly at the end of treatment the MTT solution was added to each well of culture plate to make a final concentration of 0.5 mg/mL and the reaction mixture incubated for 2 h. The mixture was removed and the resulting formazan dissolved by adding 200 µg/mL L DMSO to each well. The optical density of formazan dye was read at 545 nm (against 620 nm as background). The assay was carried out in triplicate and repeated twice for confirmation. The percentage of viable cells was calculated as the mean ± SEM with controls set to 100%.

Statistics

All results are presented as mean ± standard error of the mean (SEM). The values were compared using the one way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons. The p-values less than 0.05 were considered to be statistically significant.

Results

Effect of hydroalcoholic extract on surviving cells

As shown in Figure 1 the percentage of surviving cells in all concentrations of the hydroalcoholic extract demonstrated no significant change compared with the control cells. In the presence of 0, 100, 200, 400 and 800 µg/mL of this extract, the percentage of cell viability was 100 ± 10, 85 ± 7.6, 100 ± 7, 91 ± 9 and 77 ± 3.5 respectively.

Effect of water fraction on surviving cells

The presence of water fraction in the culture medium led to a concentration dependent decrease in cell viability (Fig 2). But the effect was not statistically significant even at the highest concentration tested in this study.

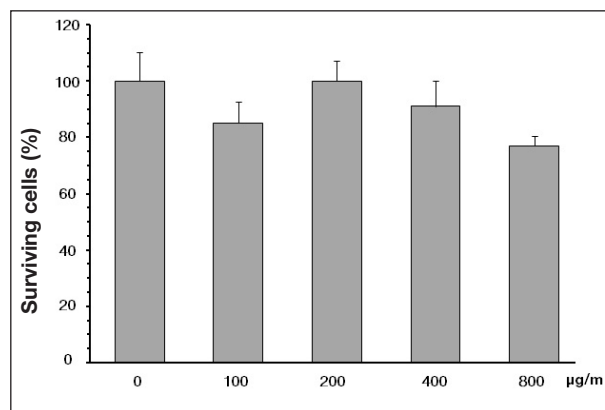


Figure 1: Effects of hydroalcoholic extract of *Viola tricolor* on N2a neuroblastoma cells

Cells were treated with increasing concentrations of hydroalcoholic extract for 24 h.

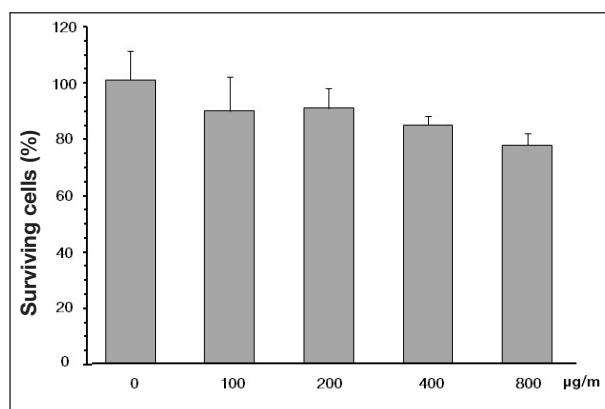


Figure 2: Effects of water fraction of *Viola tricolor* on N2a neuroblastoma cells

Cells were treated with increasing concentrations of water fraction for 24 h.

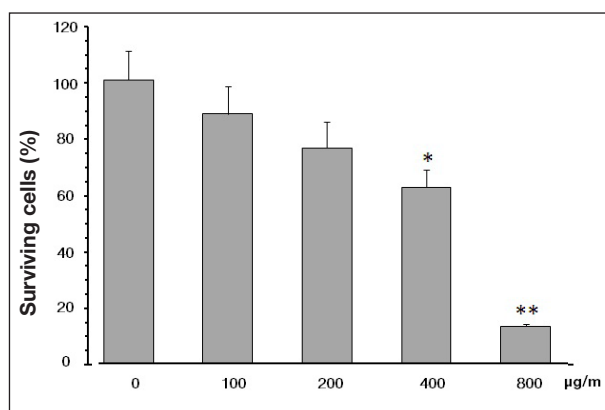


Figure 3: Effects of acetate fraction of *Viola tricolor* on N2a neuroblastoma cells

Cells were treated with increasing concentrations of water fraction for 24 h. *P < 0.05 versus 0, **P < 0.001 versus 0.

The percentage of all surviving cells (quantified by MTT assay) was normalised against untreated control cells (0 µg/mL). Data are mean ± SEM of two independent experiments performed in triplicate.

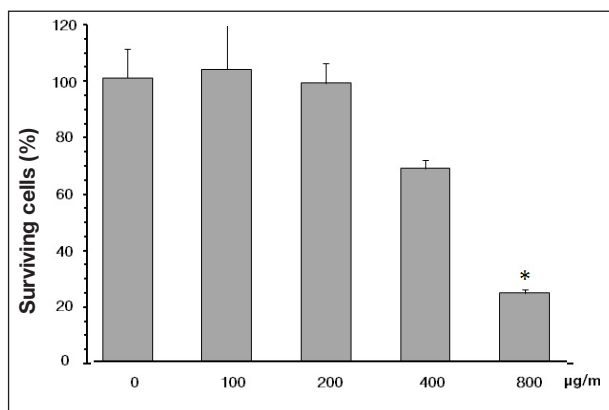


Figure 4: Effects of n-butanol fraction of *Viola tricolor* on N2a neuroblastoma cells

Cells were treated with increasing concentrations of n-butanol fraction for 24 h. * $P < 0.001$ versus 0.

The percentage of surviving cells (quantified by MTT assay) was normalised against untreated control cells (0 µg/mL). Data are mean \pm SEM of two independent experiments performed in triplicate.

Compared with untreated cells ($100 \pm 10\%$), the water fraction at 100, 200, 400 and 800 µg/mL decreased the cell percentage viability to 89 ± 12 , 90 ± 7 , 84 ± 3 , 77 ± 4 respectively.

Effect of ethyl acetate fraction on surviving cells

Figure 3 demonstrates the antiproliferative effect of ethyl acetate fraction of *V. tricolor*. Following incubation of N2a neuroblastoma cells with 100, 200, 400 and 800 µg/mL, approximately 12%, 24%, 38% and 87% inhibition in cell growth was observed respectively compared with untreated cells. The effect of ethyl acetate fraction was concentration dependent and at concentration of 400 µg/ml ($P < 0.05$) and 800 µg/ml ($P < 0.001$) had significant difference compared with that of control cells.

Effect of n-butanol fraction on surviving cells

When the n-butanol fraction was evaluated for in vitro antitumor activity against N2a cells, it was found that after 24 h treatment only high concentrations caused significant cytotoxicity (Fig 4). Exposure of the cells to 800 µg/mL of this fraction showed 76% decrease in cell surviving ($P < 0.001$ compared with untreated cells). No significant effect was found with 100, 200 and 400 µg/mL of n-butanol fraction.

Discussion

Our data for the first time demonstrated that proliferation of neuroblastoma N2a cells is inhibited by *V. tricolor*. This effect was observed only with ethyl acetate and n-butanol fraction, while none of the hydro-alcoholic extract or water fractions could inhibit the cell growth. Regarding the n-butanol fraction, the best antitumor action was observed at the highest dose (800 µg/mL) used

in this study. However the effect of ethyl acetate fraction started at the lower concentration (400 µg/mL). Therefore the main component/s responsible for the antitumor effect of *V. tricolor* are most likely found in ethyl acetate fraction. While the water fraction contains water soluble plant constituents (e.g. glycosides, quaternary alkaloids, tannins) and n-butanol fraction bears low polar agents (e.g. sterols, alkanes, some terpenoids), the ethyl acetate fraction extracts compounds of intermediate polarity such as flavonoids (Seidel 2006, Dominguez 2010, Tian 2011).

Recent studies have characterised high amounts of saponins, mucilages, cyclotides and flavonoids in *V. tricolor* (Toiu 2009, Vukics 2008a,b, Dominguez 2010, Svargard 2004). Between the agents, existing evidences support the possible role for flavonoids.

It has been reported that they exert growth inhibitory effects on tumor cells cultured in vitro (Kanaswami 2005). Deschner et al (1991) indicated that rutin and quercetin, two flavonoid components in *V. tricolor* (Vukics 2008), inhibit azoxymethanol induced colonic neoplasia.

These reports regarding flavonoids are in agreement with our finding that the ethyl acetate fraction has the best antiproliferative effect between the fractions. On the other hand Tang and co-workers (2010) reported that some cyclotides isolated from *V. tricolor* have cytotoxic activities against U251, A549, DU145, MDA-MA-231 and BEL-7402 cancer cell lines, showing that other constituents may also be responsible for the antiproliferative effect of this plant.

Conclusion

This study has shown that the ethyl acetate and n-butanol fractions of *V. tricolor* possess significant antitumor effects against neuroblastoma N2a cells. Isolation and purification of the active compound/s may yield novel anticancer agents.

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Evidence based naturopathic practice literature review: *Hypericum perforatum*

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Introduction

St John's wort has a history of use as a herbal remedy for a variety of ailments and has become a mainstream alternative treatment for depression due to its association with low adverse effects compared with prescription antidepressants. Current research on *Hypericum perforatum*, or St John's wort (SJW) has found several promising results in therapies for cancer, inflammation, bacterial and viral infections, as well as other disorders (Huang 2012, Klemow 2011, Saddiqe 2010). A study conducted by Husain et al (2011) with type 2 diabetic rats strongly suggested that standardised *Hypericum perforatum* extract could be a suitable alternative therapeutic option for prevention, as well as treatment, of co-morbidities caused by or associated with depression, anxiety and diabetes.

The two major constituents of St John's wort, hypericin and hyperforin, have been shown to possess substantial medicinal activity, while other compounds including the flavonoids rutin, quercetin and kaempferol also appear to have medicinal properties (Klemow 2011). This is supported by a recent study conducted by Vissienon et al (2012) that demonstrated quercetin and kaempferol as being the key constituents to the herb's anxiolytic effects.

Some researchers state that hyperforin is responsible for the major antidepressant activity in SJW. A study conducted by Laakmann et al (1998) showed a dose-response relationship between the antidepressant efficacy of *Hypericum* extract and its hyperforin content. Further studies by these researchers stated that extracts with a higher content of hyperforin were particularly effective in patients who were more severely depressed (Laakmann 2002).

Other researchers claim that hyperforin and hypericin are not the only constituents responsible for the antidepressant properties of the plant, but a combination of the chemical constituents within SJW that exerts the antidepressant activity. A study conducted by Butterweck et al (2003) illustrates that a SJW extract free of hyperforin and hypericin exerts antidepressant activity in behavioural models, supporting their hypothesis that the flavonoids are partly responsible for the therapeutic efficacy of SJW. Their results also show that hyperforin contributes to the beneficial properties of SJW extract, following their hypothesis that the crude SJW extract

contains several constituents that contribute to its antidepressant properties.

Despite the evidence on the constituents of SJW showing promising results in producing antidepressive properties, the exact mechanisms of SJW's action still remains unclear. However the available research clearly demonstrates that various bioactive constituents within SJW work in a synergistic manner to contribute to the antidepressant effects.

The use of SJW in treating patients with depressive disorders remains conflicting. A study conducted by Shelton et al (2001) concluded that SJW was not effective in treating major depression. Another larger scale study, a double blind randomised placebo controlled trial conducted in 12 academic and community psychiatric research clinics in the United States, tested the efficacy and safety of a *Hypericum perforatum* extract (LI-160) in major depressive disorder. The study failed to support the efficacy of *Hypericum perforatum* in moderately severe major depression (Hypericum Depression Trial Study Group 2002).

On the other hand a systemic review conducted by Linde, Berner and Kriston (2008) concluded with evidence to suggest not only that *Hypericum* extracts are superior to placebo in patients with major depression and are similarly effective as standard antidepressants, but also have fewer side effects than standard antidepressants. Linde (2009) further states that *Hypericum* extracts have better tolerability in the acute treatment of major depressive episodes.

These findings are supported by a meta-analysis conducted by Rahimi, Nikfar and Abdollahi (2009) whereby their findings maintain that *Hypericum perforatum* is a favourable alternative to antidepressants in the management of depression.

Objective

This literature review aims to critique the scientific literature by Rapaport et al (2011) entitled *The treatment of minor depression with St. John's Wort or citalopram: failure to show benefit over placebo*. This scientific literature was chosen for being the most recent and thorough publication that matches the clinical question. The clinical question was to compare the effects of *Hypericum perforatum* with conventional antidepressants in treating patients with depression.

The search title formulated in PICO was:

Population (P): patients with depression

Intervention (I): *Hypericum perforatum*

Comparison (C): Antidepressants

Outcome (O): efficacy in alleviating symptoms of depression.

Search methods

The search for published studies on the efficacy of *Hypericum perforatum* in treating major depression in comparison with antidepressants was conducted through Pubmed, Cochrane Library, OVID and EMBASE databases. Advanced searches of 'St John's wort' or '*Hypericum perforatum*' and 'major depression' yielded 300-500 articles. That search was then selectively limited to publications from 2011 to 2012, which narrowed to 68 articles. This was due to the fact that the aim of this search was to find the most recent findings and the highest level of evidence pertaining to the clinical question.

Study selection

The key interest in selecting the chosen study was to find a publication that matched the PICO. The publication date was factored into selecting the study and only publications from 2011 and 2012 were considered. There were very few relevant articles published in 2012 with regard to '*Hypericum*' and 'depression'. An article by Sarris, Fava, Schweitzer and Mischoulon (2012) on *St John's wort (Hypericum perforatum) versus sertraline and placebo in major depressive disorder: continuation data from a 26-Week RCT* was considered, however it was a re-analysis and continuation of the Hypericum Depression Trial Study Group published in 2002, and not original research. The search eventually narrowed down to Rapaport et al (2011) *The treatment of minor depression with St. John's wort or citalopram: failure to show benefit over placebo*. This literature was chosen for its match to the clinical question as well as being one of the most recent and thorough studies conducted to test the effects of St John's wort on depression in comparison with antidepressants.

Focused question

The main purpose of the research was to conduct a study comparing and contrasting St. John's wort, citalopram and placebo as treatment for subjects with minor depressive disorder. Their aim was to accomplish two goals:

1. to make a significant contribution to the limited research on placebo controlled trials evaluating the efficacy of an antidepressant as a treatment for minor depressive disorder, and
2. to determine the efficacy of SJW in an acute trial as compared with both an antidepressant approved for the treatment of major depressive disorder (citalopram) and placebo in a population with a milder form of depressive spectrum disorder.

Methodology

Subject selection

The researchers screened 169 prospective subjects of which only 100 were eligible. They were recruited through clinical referrals and community advertising. Subjects with organic mental disorders, substance use disorders, current or within one year of psychotic symptoms or disorders, bipolar disorder or antisocial personality disorder, were excluded. A Structured Clinical Interview for DSM-IV was used for the inclusion and exclusion of diagnoses. All subjects agreed to participate by signing a written informed consent.

Subject assessments and randomisation

The subjects were randomly assigned into three treatment groups: St John's wort, citalopram and placebo. The subjects in these groups were identical in terms of demographics, clinical characteristics, measurement of symptom severity, quality of life and psychological wellbeing. The researchers incorporated validated and structured assessments and interviews in obtaining information from the subjects.

Treatment

The researchers obtained their supply of St John's wort tablets through Cederroth International, a Swedish manufacturer, and followed the dosage of 810 mg per day according to the Swedish authority guideline. The researchers administered 20 mg/day of citalopram and a look-alike placebo. They did not specify which proprietary extract of St John's wort that was administered nor did they specify the exact amount of hypericin and hyperforin that was contained in the tablet.

Results

The results show that St John's wort, citalopram and placebo were equally effective in decreasing symptoms of minor depression, improving quality of life and psychological wellbeing. The researchers claim this lack of differentiation between the treatments was due to a 'large placebo response across all outcome measures'. The research supports the use of St John's wort in managing depression, however there was no certainty as to which treatment would be better indicated for minor depression. All treatments were reported to develop adverse reactions however citalopram scored the highest at 100%, with St John's wort scoring 84.6% and placebo 91.3%. St John's wort was shown to have scored the lowest in adverse reactions in comparison to citalopram and placebo.

Assessment of quality

The overarching strength of this research was the employment of structured, thorough and consistent assessments with the subjects throughout the 12 week study. The researchers adopted an ethical approach to the research by obtaining written informed consent from all subjects. The subjects were constantly monitored for their safety throughout the study. They incorporated a PRISE

(Patient-Related Inventory of Side Effects) evaluation in which the subjects self report side effects by identifying and evaluating the tolerability of each symptom, thus avoiding bias from the researchers.

The main weaknesses in this research was the relatively small sample size (n=79) resulting from the stringent selection criteria which may have omitted many potential subjects. This created a limited range of severity of depression, which may have resulted in the lack of response within the sample size subjected to the active treatment. The researchers admitted that the overall design flaw in the study whereby the design was biased to recruiting subjects who were willing to commit to longer term trials, may have inexplicably created an expectancy to treatment response which then contributed to the high placebo effect. They also failed to include metabolic measurements, further limiting treatment results.

The researchers did not anticipate the challenges that lay ahead of their research, in which they were facing emerging competition from other researchers who were providing huge monetary compensation to their own study subjects. This had a subtle effect on the characteristics of the research subjects. There were significant conflicts of interest in four out of five researchers of this study. There appears to have been an affiliation to the pharmaceutical company that provided the citalopram and placebo for this study.

Implications for practice

The findings presented in this research study comparing St John's wort, citalopram and placebo in treating minor depressive disorder may be significant in clinical practice, despite the confounding results. The placebo group yielding a clinically positive improvement on all outcome measures suggests that subjects with minor depression could potentially benefit from non pharmacological approaches. In a recent systemic review, meta-analysis on the efficacy of antidepressants in treating minor depression shows that even antidepressants do not have an advantage over placebo in treating minor depression (Barbui et al 2011).

These findings show that there currently is no specific pharmacological treatment that has been effective in treating minor depressive disorder.

Future research

Since this research yielded similar results between St John's wort, antidepressants and placebo in the treatment of minor depression, further research should be undertaken to fully elucidate which treatment methods would be superior in treating minor depression. An extensive investigation would also be helpful to find the most effective, non pharmacological treatment for minor depression. Further research into the effectiveness of St John's wort in treating depressive spectrum disorder, not only minor depression, would also be beneficial.

Conclusion

Due to the confounding results, the literature that was analysed did not fully answer the clinical question that was presented which was to compare the effects of *Hypericum perforatum* with pharmaceutical antidepressants in the treatment of patients with depression. The study was not conducted with a superior quality of research due to several limitations in research methods. Therefore further research needs to be conducted to refine the methods and flaws of current and previous studies in order to produce a better research quality as well as to answer the initial question on the effectiveness of *Hypericum perforatum* in treating depression.

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Clinically proven herbs for mental energy and cognitive performance

Michelle Boyd, Herbalist, Lecturer and head of practitioner education at Flordis, focuses on a *specifically clinically proven* herbal option for memory, concentration and mental energy.

Age-related cognitive decline starts early in life

There is great need to address cognitive decline in our ageing population, both for the individual and the ramifications this condition has for our society as a whole. However, authors will argue that cognitive decline is not only a healthcare focus for old age but, young adults also need to be considered. So, when does age-related cognitive decline begin? Research shows that it begins much earlier than we may have thought. Cognitive decline afflicts healthy educated adults from when they are in their 20s and 30s. For example, declines in processing speed are evident from 25 years and short term memory continually declines for most of an adult's lifespan. This knowledge is relevant, not only for addressing cognitive decline in the early stages of its development but also considering implementation of interventions that may prevent or reverse older age-related declines ^(1,2).

Ginkgo biloba and Panax ginseng combination promotes mental energy and cognitive performance

Gincosan[®] is *specifically clinically proven* to enhance cognitive function, improve memory and reduce forgetfulness and mental fatigue in both young and older adults ⁽³⁻¹⁰⁾. Gincosan is a unique combination of specific extracts of *Ginkgo biloba* (GK501) and *Panax ginseng* (G115) that has proven dose-dependent efficacy (in both acute and chronic administration) in high quality clinical trials. One double-blind, placebo-controlled study of young adults (mean age 21 years) suggests that this unique combination may act synergistically to improve both 'speed of performance' and 'accuracy' when there is a need to perform or complete cognitively demanding tasks. Normally, one of these factors improves at the expense of the other. This study evaluated acute administration of Gincosan, demonstrating dose dependent improvement in cognitive function from 1 to 6 hours post dose. Furthermore, results showed that the individual, single extracts of *Panax* and *Ginkgo* did not have the same beneficial effects as the combination product ⁽⁷⁾.

Another study, this time 12 weeks treatment with Gincosan (with 2 week post treatment follow up) involving 256 middle-aged volunteers (mean age 56 years) also demonstrated improvement in both accuracy and speed. Cognitive improvements were demonstrated at each fortnightly

The average effect of Gincosan on the 'Quality of Memory Factor' over a 14-week multi-centre, double-blind, placebo-controlled trial⁵

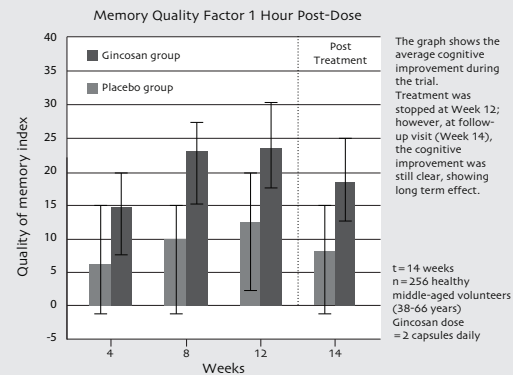


Figure 1. Adapted from Wesnes, et al. 2000⁵

assessment 6 hours post dose and the authors concluded that the level of these effects would be considered desirably noticeable for this age group ⁽⁵⁾.

Overall, clinical trials have demonstrated that Gincosan can:

- Improve 5 measures of cognitive function including both short term and long term measures and mental performance. The measures improved were long-term memory for words and pictures, working memory for numbers and location, the speed of memory processes, the power of concentration and the consistency of concentration. ⁽⁵⁾
- Support cerebral and peripheral blood circulation, improving oxygen supply to the brain ⁽⁸⁻¹⁰⁾
- Increase the supply and utilisation of nutrients in the brain, leading to improved mental energy and cognitive function ⁽⁷⁻¹⁰⁾
- Improve memory quality for middle-aged individuals (40-65 years) ⁽⁷⁾.

This wealth of evidence on Gincosan indicates its use for adults experiencing difficulty with their memory and concentration and those lacking mental energy, particularly when managing a high cognitive load. For these individuals Gincosan can optimise mental function during these times of increased mental demand (overload) enabling faster and more accurate thinking, reduced mental fatigue and an improvement in general mental performance. Studies indicate that optimal benefits are expected after taking Gincosan for 12 weeks.

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Traditional hydrosols and hydro-distillation

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What is a hydrosol?

A herbal infusion or tea is made by pouring boiling water onto the plant. The heat of the water softens the cells releasing the constituents of the plant into the water. The parts that are water soluble and the highly volatile micro molecules of essential oils are caught in the vapour. The hot steam hits the cold lid of the container, forms droplets and falls back into the tea. These precious droplets are called hydrosol, a name coined by Jeanne Rose in 1990.

Hydrosols are real aromatherapy. They can be considered as the homeopathy of aromatic therapy. Just as herbs are to homeopathy so are essential oils to hydrosols. Hydrosols represent the true synergy of herbalism and aromatherapy (Rose 2003).

Up until the Middle Ages the distillation of herbs and flowers was primarily for hydrosols for therapeutic and cosmetic applications. Essential oils were extracted by oil infusions and maceration. It was much later that the techniques of distillation changed to produce greater quantities of essential oils. Of course as they became more popular and commerce and trade was on the move, a tiny vial of an intense aromatic essential oil had a much greater value than a gallon of heavy water.

Mostly the hydrosols have been considered a waste product of the distillation process and discarded. Lavender, rose and orange flower waters have lasted the distance. Many commercial products are synthetic, bearing no relation to the plant or any of its healing properties. Many others are essential oils dissolved in alcohol or glycerine and added to water. However there is now a trend and recognition of the intrinsic value of a true hydrosol.

Hydrosols have many practical applications

Hydrosols contain all the therapeutic qualities of both the plant itself through its water soluble properties (herbal therapy) as well as the therapeutic properties of the essential oils, which are present in the hydrosol in tiny micro drops (essential oil therapy). They can be absorbed by the skin or through the gut or any mucus membrane. They can be:

- used internally by adding 30 mL to a litre of water for a therapeutic refreshing drink
- used externally as eardrops, nose drops, eyewash, douche or suppository

- appropriate for the highly sensitive, elderly and the young
- used as an active ingredient in the aqueous part of creams shampoos and skin tonics
- added directly to the bath, foot baths and compresses
- applied topically for direct application to affected or infected skin or cuts, scratches or any injury
- used in the kitchen as a beverage or cooking ingredient
- used environmentally in the home as a cleaner, insect repellent, room freshener or linen spray
- applied for pet care.

The distillation process

Plants or flowers are put into boiling water or subjected to steam or both. If the plant is in boiling water only it is classed a hydro-distillation. If the plant is steamed and is above or separate from the water it is a steam distillation

We've had a long warm and mostly dry autumn; it's made up for the lack of hot summer. The rosemary in the garden is covered in blue flowers and the leaves have a fresh waxy sticky oily feel. There is plenty of vibrant growth and its strikes upward. It's been bothering me of late, I wake at night it's in my thoughts, the aroma washes by me unexpectedly; I see the blue in many places. Yes, time to replenish the jars and the empty essential oil bottle before the moon wanes, the chill comes in; the flowers turn to seed and the juices of the plant retreat to the roots bedding down for the winter. Last chance before spring!

Making a hydrosol

Rosemary (*Rosmarinus officinalis*) is a hydrosol that can be used daily for many applications including livening the hair, splashing on face, underarms and even as a mouthwash and the essential oil precious. On a more pragmatic note why does this work for me? Rosemary is considered to be an antioxidant, a circulatory stimulant, promotes healthy shiny hair, tones normal to oily skin, helps relieve chest tightness and congestion, eases muscular pain, mild diuretic, stimulates digestions and has a tonic effect on the nervous system.

It is valuable to prepare the two different types distillation to compare results, the steam distillation for essential oil and the hydro-distillation for a hydrosol. A traditional copper Alembic is used for the task.



The still sits on a gas ring, the sterile beakers are ready to catch the distillate and the oil separators are clean and ready. A bowl of rye flour paste is prepared, a pH reader is used for taking regular pH readings of the hydrosol. The pot is 2/3 filled with water and is set to boil.

While the water comes to boil, the leaves are stripped off the stems and any damaged or woody parts of the plant are discarded. There is a fragrant pile of flowers and fresh sticky leaves. The twigs are set aside to dry. These can be used as skewers for kebabs or thrown in the pizza oven. While they are still pliable some are plaited for smudge sticks for cleansing.

When the water is boiling the onion dome is carefully removed and approximately 500 g of the fresh flowers and leaves are placed in the 7 litres of boiling water. The onion dome is quickly replaced and the joins are sealed with the rye flour paste. It is important to prevent any steam from escaping as it has the bulk of the volatile essential oils especially in the first flush of steam.

Why rye flour? Others have tried plumbers tape, silicon and putty. The copper becomes extremely hot during the process and will melt glues and plastic which invariably make a mess of the copper and are difficult to remove. The rye flour is organic and we are not introducing any synthetic complex chemicals. Although it bakes on firm it stays pliable enough to easily remove for a second distillation. It also keeps the copper clean.

As the water comes to a rolling boil, it softens the cells of the rosemary leaves and flowers releasing the volatile essential oils and water soluble constituents. As the steam fills the onion dome and spirals, any particles

and dust will drop back into the water. The steam travels quickly along the copper pipes to the condenser bucket.

The condenser bucket must have cool running water flowing through it during the entire process. This is to cool the coil so the steam condenses to become hydrosol. If the hydrosol feels warm, the coil is too hot and the flow of water needs to be increased. Water can be recirculated through a large big or trickling garden hose can be used into the bucket if water is in good supply.

As the hydrosol begins to trickle out into the beaker, the garden is filled with the aroma of rosemary. A pH reading is taken then 100 mLs has been collected. A good hydrosol has flavour, aroma and a pH between 4.5 and 5.5. With 500 g of rosemary, only 500 mL of good hydrosol would be expected. Distillation is stopped when the pH levels (taken every 100 mL) start rising above the initial reading. At that point it will be mostly water. Flavour and aroma are also checked through the distillation. Less is mostly better than more however every distillation is different depending on the season, soil types, weather, moon, rain or sun. Nature is what makes distillation so interesting.

The pH level of the final product has settled at 5.5, giving 500 mL of hydrosol with a constant pH and a strong, grassy, green, herby flavour of rosemary which is pleasant to taste. Rosemary distilled in the height of

summer before it flowers, has an aroma predominantly of camphor and is quite unpleasant to taste.

In this instance the aroma is sweet, grassy and pleasant to taste, suggesting it will be great in skin and hair care, and internal use. If the camphor is predominating it makes a useful decongestant, antiseptic and cooling skin tonic on hot days. Both types can be used to mist or be added to food at the last minute.



If need be the hydrosol is poured into an oil separator flask. The small amount of collected oil is hardly worth separating and is usually left in the hydrosol.

Simply observing the distillation process gives an insight into how valuable and potent hydrosols are. In some way essential oil is the by-product of distillation, that which cannot be held in suspension. Hydrosols by their nature have a greater complexity than essential oils. Suzanne Catty describes them in her book *Hydrosol's, the next aromatherapy* as 'holograms of the plant'.

When the distillation is complete it is transferred to a clear glass sterile jars labelled with the plant type, date, moon phase, tide (if coastal), type of distillation and ph level. It is then decanted into 100 mL spray mister bottles or a 500 mL amber glass bottle for daily use. The bottles are checked for sediment or cloudiness which

may indicate yeast and microbial blooms. The pH is retested and if it has risen from that on the label there may be microbial activity and the hydrosol is discarded. Provided the hydrosol has a pH between 4.5 and 5.5, is kept in a cool sterile dark place and in sterile containers it can be expected to last for at least 6 months and up to 12 months. This will vary depending on plant type.

Plants can be distilled throughout the year with different results depending on the season. *Eucalyptus* in the middle of a hot dry summer yields double the essential oil compared to a winter distillation, but the winter distillation is softer and sweeter and even makes a pleasant addition to drinking water. The same applies to rosemary. Some plants have a barely noticeable sheen of essential oil – *Melissa*, kawa kawa, cornflowers, *Calendula*, rose and yet they are fragrant, acidic and have flavour. It is important to remember when doing a hydro-distillation that it's not about achieving a high yield of essential oil, its about capturing the whole plant in balance.

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Jill Mulvaney grew up on a New Zealand farm and after spending some time living abroad, moved to Waiheke Island in 1998. Whilst living in Perth WA, Jill 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 now 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.



Rosmarinus officinalis

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LOCATIONS AND DATES

QUEENSLAND

- Caloundra Thursday 4th October
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- Toowoomba Tuesday 9th October
- Mackay Wednesday 10th October
- Townsville Friday 12th October
- Arundel Friday 12th October
- Cairns Saturday 13th October
- Brisbane Sunday 14th October
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- Newcastle Monday 15th October
- Forresters Beach Wednesday 17th October
- Leura Thursday 18th October
- Batemans Bay Friday 19th October
- Wollongong Sunday 21st October
- Cronulla Monday 22nd October
- Sydney Sunday 28th October

ACT

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VICTORIA

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- Takapuna Friday 26th October
- Ellerslie Saturday 27th October
- Tauranga Sunday 28th October
- Napier Tuesday 30th October
- Nelson Wednesday 31st October
- Dunedin Thursday 1st November
- Christchurch Saturday 3rd November
- Wellington Sunday 4th November

ALL SEMINAR TIMES

Registration	2:30 to 3:00 pm
Session 1	3:00 to 4:30 pm
Break	4:30 to 5:00 pm
Session 2	5:00 to 6:30 pm
Dinner	6:30 to 7:30 pm

SPEAKERS

- Roberta Barbiellini
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PCOS with a twist

Melanie Koeman BSc DipHM DipNM MNHAA

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Early in my practice, one of the first patients I ever treated for polycystic ovarian syndrome (PCOS) subsequently developed autoimmune disease of the thyroid (Hashimoto's disease). For many weeks I had given advice and provided prescriptions based on my understanding of the biochemical and metabolic profile of PCOS. It came as a shock to see blood test results with elevated thyroperoxidase (TPO) and thyroglobulin (TG) antibodies, and a TSH around 5.

Given the similarity in many clinical signs and symptoms between the two conditions, it has since been my habit to investigate and consider the thyroid gland in every PCOS patient. It is very clear that PCOS patients are at much greater risk of thyroid disease than women without PCOS. According to one author the risk is 3 times greater (Janssen OE et al. 2004. *Eur J Endocrinol* 150:3;363–9).

Presenting complaint

'Krissy', aged 21, presented with a diagnosis of PCOS made by her GP three years prior through blood test and ultrasound. She was prescribed the oral contraceptive pill (OCP) but found she gained weight on it so stopped it. Since then she had struggled with hirsutism, scalp hair loss, acne and fluctuating weight.

Past medical history

Menarche commenced at 12 years of age, with irregular cycles and episodes of amenorrhoea. She had a history of bloating, constipation and fatty food intolerance. Blood tests confirming PCOS showed classic elevated LH:FSH ratio and elevated androgens and FAI (See Table 1). Of note, her TSH was normal 3 years prior at 2.95 mIU/L.

Social/family history

Krissy has a strong family history of type 2 diabetes.

Physical examination

Krissy was self conscious about her skin, hair and weight. The pattern of acne, hirsutism and weight deposition was consistent with PCOS, however the scalp hair loss and bowel symptoms alerted me to recommend a recheck of her thyroid function.

Treatment

It is widely acknowledged that dietary and lifestyle management of PCOS, which includes a minimum of 150 minutes of exercise weekly (the majority being cardiovascular), is the first line of therapy. Certain

nutrients such as magnesium may also assist in improving insulin resistance.

Herbal treatment

Herb	Conc.	Total
<i>Paeonia lactiflora</i>	1:2	35 mL
<i>Glycyrrhiza glabra</i>	1:1	15 mL
<i>Calendula officinalis</i>	1:2	10 mL
<i>Schisandra chinensis</i>	1:2	20 mL
<i>Cynara scolymus</i>	1:2	20 mL
TOTAL		100 mL

Dose 7.5 mL twice times daily for the next four weeks.

Herbal and nutritional capsule containing extracts equivalent to dry:

<i>Gymnema sylvestra</i> leaf	1.35 g
<i>Cinnamomum cassia</i> stem bark	1.00 g
Chromium total elemental	250 mcg
R,S-alpha Lipoic acid	200 mg
Magnesium	50 mg
Zinc	5 mg
Biotin	1 mg
Vitamin D3	200 IU

Dose 1 capsule with meals three times daily.

I recommended some repeat blood tests.

Follow up

Krissy's blood test results showed elevated insulin consistent with insulin resistance and elevated TSH, with T4 at the lower end of normal, consistent with subclinical hypothyroidism (see blood test table below). Thyroid antibodies were negative.

Herb	Conc.	Total
<i>Paeonia lactiflora</i>	1:2	30 mL
<i>Glycyrrhiza glabra</i>	1:1	15 mL
<i>Fucus vesiculosus</i>	1:1	30 mL
<i>Serenoa serrulata</i>	1:2	25 mL
TOTAL		100 mL

Dose 7.5 mL twice times daily for two months.

Herbal tablet containing extracts equivalent to dry:

<i>Coleus forskohlii</i>	5.61 g
Standardised to forskolin	18.7 mg

Dose 1 tablet twice daily.

Repeat blood tests showed significant improvements in insulin, FAI and LH/FSH ratio (see table below). Krissy's menstrual regularity had settled into a 33 day cycle, her skin had improved, she was noting new growth of hair on her scalp along with a slowing of the loss. However her thyroid readings were still the same. We decided to continue the prescription because she was feeling so much better.

She was committed to her exercise program and low GI diet and felt that she was on track.

Blood test results and reference ranges

Blood parameter ('normal' reference ranges)	Pre treatment	Post treatment
TSH (< 3.5)	4.9 mIU/L*	2.5
Free T4 (10–20)	13 pmol/L	15
Insulin (fasting < 10)	22 mU/L*	10
FAI (< 7.2)	10*	4.3
LH/FSH ratio (1:1)	3.05:1*	0.8:1

* out of reference range

Discussion

I reviewed her clinical signs and symptoms, along with prescription compliance over the following 6 months. All improved significantly and Krissy was rewarded with great blood results and achieving her goal weight all in the same appointment with her GP, who understandably encouraged her to continue her treatment. Krissy demonstrated a commitment to correcting a metabolic problem that is often resistant to change.

She is now maintained on a half dose of her liquid prescription and herbal tablets. She will continue to review twice yearly or as needed and has since encouraged her parents, uncles and aunts to adopt a diet and lifestyle like hers!

Evidence based naturopathic practice literature review: *Hypericum perforatum*

references continued from page 99

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Annual General Meeting



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Reviews of articles on medicinal herbs

Kathleen Murphy, Olga Beilak, Alison Shaw
murphykath@gmail.com

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.

***Withania* may reverse amyloid plaque in Alzheimer's disease**

Sehgal N, Gupta A, Valli RK, Joshi SD, Mills J, Hamel E et al. 2012. *Withania somnifera* reverses Alzheimer's disease pathology by enhancing low-density lipoprotein receptor-related protein in liver. *P Natl A Sci* 109:9;3510–15.

Alzheimer's disease (AD) is a form of dementia which progresses relentlessly and leads to death. There is currently no cure and modern treatment can only slow down the progression. The increased production of beta amyloid which may form amyloid plaques is a main characteristic of AD.

Withania somnifera, ashwagandha, is a well researched herb which has been used in Ayurvedic medicine for about 4000 years. In Ayurvedic tradition it has been known as one of the nootropic herbs that enhances memory and promotes cognition.

The current study examines the effect of 30 days of oral administration of *Withania* root extract on Alzheimer's disease transgenic mice. The researchers used partially purified *Withania* extract consisting of 75% withanolides and 20% withanosides.

Complete regression of amyloid plaques in middle aged mice and significant reduction of plaques in the cortex and hippocampus of old mice were reported at the end of the study. Major Alzheimer marker, beta amyloid 42, was reduced by more than 77% in the brain of middle aged mice and more than 49% in the brain of old mice.

During the course of treatment, plasma level of beta amyloid 42 steadily increased from day 7 to day 14. The density of amyloid plaque decreased significantly after day 14. Researchers believe that those effects could be attributed to clearance of beta amyloid from brain into the circulation. The reduction of plaques manifested in improved behavioural patterns. Researchers reported complete reversal of the behavioural deficits in middle aged mice and enhanced performance of old mice in the radial maze task.

It has been established in earlier studies that low density lipoprotein receptor related protein (LRP) acts as a chaperone in transporting beta amyloid from the brain into the periphery. The researchers measured the expression of LRP in the cortex and in the liver of mice. The expression and catalytic activity of neural endopeptidase (NEP) which is involved in degrading beta amyloid was also measured. Liver NEP and liver

mRNA of LRP demonstrated an increase after 7 days of treatment and continued to increase up to the last day of study. Cortex level of LRP increased only after 14-30 days of *Withania* administration.

The increased expression of hepatic but not brain LRP and NEP was observed in wild type (WT) mice after 7 days of treatment. Decreased plasma beta amyloid level led to the conclusion that high liver LRP and NEP caused a reduction of amyloid load in the circulation. Therefore the therapeutic action of *Withania somnifera* extract could be attributed to its effect on hepatic rather than cortex level of LRP. According to the proposed mechanism of action soluble plasma LRP acts as a peripheral sink for beta amyloid.

Researchers noted that oral administration of *Withania* extract was highly effective. Since *Withania* extract was more complex than the combination of withanolides and withanosides, researchers suggested that more than one pathway contributed to the beneficial outcome of clearing beta amyloid in AD mice.

Curcumin inhibits osteosarcoma cells

Li Y, Zhang J, Ma D et al. 2012. Curcumin inhibits proliferation and invasion of osteosarcoma cells through inactivation of Notch-1 signaling. *FEBS J* 279;2247–59.

Osteosarcoma is a highly malignant bone cancer associated with aggressive local growth and early metastatic potential. While treatment options have improved, patients with metastatic disease at diagnosis, or those who have recurrent disease, have an extremely poor prognosis with only 20% surviving after 5 years.

Curcumin is a naturally occurring phenolic compound shown to have a wide variety of antitumor activities in many different cancers, such as colorectal carcinoma, head and neck squamous cell carcinoma, pancreatic cancer and osteosarcoma. Curcumin modulates the expression of genes involved in cell proliferation, apoptosis, invasion, metastasis, angiogenesis and resistance to chemotherapy.

This in vitro study assessed the potential antitumor activity of curcumin using several osteosarcoma cell lines. This included evaluation of its effect on Notch-1 signalling. The Notch signalling pathway plays an important role in the processes of cell fate determination, including stem cell maintenance, differentiation, proliferation and apoptosis, which may contribute to the carcinogenesis of osteosarcoma.

The cytotoxic effect of curcumin on osteosarcoma cell lines (U2OS, SaOS-2 and MG-63) was evaluated. This demonstrated that curcumin reduced the proliferation of these cells in a dose dependent manner. This data showed that treatment of osteosarcoma cells with curcumin beyond 22.5 μm resulted in statistically significant toxicities at 24, 48 and 72 hours. Subsequently the maximum concentration employed in further investigations was limited to 22.5 μm .

To understand further the molecular mechanism involved in curcumin induced proliferation inhibition, alterations in the cell survival Notch pathway were investigated. Notch-1 and its target genes Hes-1, Hey-1, Hey-2 expression in U2OS and MG-63 cells treated with increasing concentrations of curcumin for 48 hours, were assessed using real time RT-PCR analysis. Compared with control there was a reduction of Notch-1, Hes-1, Hey-1 and Hey-2 mRNA levels after curcumin treatment suggesting that curcumin resulted in the transcriptional inactivation of Notch-1 signalling pathway in osteosarcoma cells. The researchers concluded that curcumin regulated the transcription and translation of the Notch-1 gene.

The study results demonstrated that curcumin elicited a dramatic effect on proliferation, inhibition and G2/M cell cycle arrest in U2OS, SaOS-2 and MG-63 osteosarcoma cells as demonstrated by the MTT assay and flow cytometry analysis respectively. Recent results have shown that Notch signalling is involved in osteosarcoma cell survival and contributes to the pathogenesis of human osteosarcoma.

The results from this study demonstrated that curcumin down regulates the transcription and translation of Notch-1 and its downstream genes, Hes-1, Hey-1, Hey-2 and cyclin D1, which results in osteosarcoma cell growth inhibition. Further research is warranted to investigate the use and dosage in patients with osteosarcoma.

Chamomile for anisakiasis

del Carmen Romero M, Valer A, Martin-Sanchez J et al. 2012. Activity of *Matricaria chamomilla* essential oil against anisakiasis. *Phytomed* 19:6;520-3.

Anisakiasis is a parasitic infection resulting from fish consumption. Clinical symptoms and signs develop as a result of the inflammatory reaction caused by penetration of larvae into the gastrointestinal mucosa. Anisakiasis has high allergenic potency and can induce manifestations of hypersensitivity, ranging from urticaria or angioedema, to anaphylactic shock, along with mixed gastrointestinal and allergic symptoms.

This two armed in vitro and in vivo study sought to measure the effect of *Matricaria chamomilla* essential oil (EO) on the larvae of *Anisakis simplex*.

The EO was extracted from flowering tops of *Matricaria chamomilla* and the composition analysed by gas chromatography/mass spectrometry (GC/MS). Three

concentrations of EO and two of its main components (chamazulene and α -bisabolol) were prepared with 96% ethanol: 125 $\mu\text{g}/\text{mL}$, 65 $\mu\text{g}/\text{mL}$ or 32.5 $\mu\text{g}/\text{mL}$.

The in vitro effect of EO was examined using L3 larvae of *Anisakis* collected by dissecting *Micromesistius poutassou* (blue whiting). Larvae were examined 4 hours, 8 hours, 24 hours and 48 hours following exposure to test the biocidal effect of the compound.

The in vivo effect of EO on larvae was examined using female Wistar rats infested with *Anisakis*. The animals were split into three groups, evaluating two extracts and keeping one control. Regulated necropsy of the rats was performed, recording the locations of the larvae, whether they were alive or dead and the presence of any gastrointestinal lesions (a measure of damage by larvae).

At 125 $\mu\text{g}/\text{mL}$ the EO was effective in vitro against *Anisakis* larvae, achieving 100% mortality after 4 hours. At the remaining concentrations the EO was completely ineffective. Treatment with α -bisabolol achieved 100% mortality at all concentrations. Chamazulene was ineffective at all concentrations.

Only the EO and α -bisabolol were tested in vivo. Gastric lesions were observed in 2.2% \pm 1.8 of EO treated animals, 5.5% \pm 3.2 of α -bisabolol treated animals, and 93.3% \pm 3.9 of controls. This clearly demonstrated the biocidal effect of EO and its component α -bisabolol against *Anisakis* larvae.

The researchers concluded that the data obtained from these in vitro and in vivo observations indicate very low toxicity of *Matricaria chamomilla* EO and support its use in the treatment of anisakiasis. Further studies are warranted to explore use and dosing in humans.

Effect of garlic on commensal bacteria

Filocamo A, Nueno-Palop C, Bisignano C et al. 2012. Effect of garlic powder on the growth of commensal bacteria from the gastrointestinal tract. *Phytomed* 19:8-9;707-11.

The gut microbial ecosystem serves numerous important functions for the human host, including protection against pathogens, nutrient processing, intestinal immune response and regulation of fat storage. The composition of this microbiota can be modified by, amongst other things, changes in diet.

Garlic (*Allium sativum*) is ascribed many therapeutic qualities including antimicrobial and antioxidant activity. This in vitro study evaluated the effects of garlic powder upon the viability of representative elements of human gut microbiota.

The garlic used by the researchers was a commercial garlic product purchased from a UK supermarket; quantitative analysis of allicin and alliin was performed using an HP1100 HPLC system. The garlic powder was found to contain allicin at a concentration of 5.13 mg/g, whereas only trace of alliin was detected. Previous research has identified the compound allicin (allyl 2-propene thiosulfinate) as the main active antimicrobial

agent in garlic. Commercial preparations of garlic may not always contain allicin, which is very unstable and often disappears during processing, quickly transformed into other types of organosulphur compounds.

The following bacterial cultures were tested against garlic: *Lactobacillus casei*, *Clostridium nexile*, *Bifidobacterium longum* and *Bacteroides ovatus*. The effect of garlic on fecal bacteria was also assessed. Fecal samples were obtained from a single human individual. The volunteer was in good health, had not been prescribed antibiotics for at least six months before the study and had no history of gastrointestinal disorders.

Each bacterial culture was prepared and garlic added together in the fermentation tube. A negative control without garlic addition was prepared. Cultures were incubated anaerobically at 37°C. Samples were withdrawn every 2 hours, between 0 and 24 hours. All experiments were performed in duplicate to ensure positive reproducibility.

The inhibitory effect observed was dependent on the bacterial species. Of the four commensal bacteria tested, *Clostridium nexile* was the most sensitive strain, whereas *Lactobacillus casei* was effectively resistant even at the highest (1%) concentration of garlic. In contrast to the growth of *Clostridium nexile*, *Bacteroides ovatus* and *Bifidobacterium longum* were initially inhibited, with a significant drop in viable cell counts, but after 4–8 hours all strains became resistant. The researchers theorised that during exposure a subpopulation of bacteria became resistant and began to multiply resulting in the overall population resistance.

In the fecal sample there was an initial selective reduction in the numbers of bacterial species and again the clostridial group was the most sensitive and the *Lactobacillus casei* was found to be least affected.

Overall the results indicate that garlic powder has an effect on gut commensal bacteria, although this does not appear to be long lasting. While previous research has demonstrated the effect of garlic on pathogens such as streptococci, the researchers concluded that these results support the role of garlic in inhibiting gut pathogens without adversely affecting the commensal microbial community of the human GI tract.

Neuroprotective role of OLE

Rabiei Z, Bigdeli M, Rasouljan B et al. 2012. The neuroprotection effect of pretreatment with olive leaf extract on brain lipidomics in rat stroke model. *Phytomed* 19:10;940–6.

Brain ischemia is profoundly debilitating, inducing the release of excitatory amino acids with subsequent receptor activation leading to calcium influx, metabolic and electrophysiological dysfunction and oxidative stress (including lipid peroxidation). Many phenomena observed during brain ischemia and reperfusion can be accounted for by damage to membrane lipids, specifically by lipolysis during ischemia and by radical mediated

peroxidation of polyunsaturated fatty acids (PUFAs) during reperfusion.

Recent studies suggest that olive extracts suppress inflammation and reduce stress oxidative injury. Oleuropein reduces the amount of superoxide anions and hydroxyl radicals, and inhibits the respiratory burst of neutrophils and related radicals.

This animal study examined the effect of dietary olive leaf extract (OLE) on brain infarct volume, neurological dysfunction and brain lipidomics resulting from middle cerebral artery occlusion (MCAO) in rats.

Four main groups, each of 12 male Wistar rats, received a dietary intervention (11–12.00 h daily) for 30 days. A control received gastric gavage with daily distilled water. The other three groups received 50 mg/kg/day, 75 mg/kg/day, and 100 mg/kg/day gastric gavage of the OLE. Two hours after the last dose, each main group was subdivided to MCAO operated and intact subgroup for assessment of neuropathology (neurologic deficit scores and infarct volume), brain lipid analysis and brain glutathione levels respectively. After assessment the rats were sacrificed and their brain tissue examined.

Pre treatment with 75 mg/kg/day and 100 mg/kg per day OLE for 30 days resulted in a reduction of infarct volume, while the lower dose (50 mg/kg/day) had no effect. Pre treatment with 75 mg/kg/day and 100 mg/kg/day OLE for 30 days also resulted in an increase of glutathione levels in cortex area ($P = 0.01$, $P = 0.000$ respectively) while the lower dose 50 mg/kg/day had no effect ($P = 0.96$). Glutathione is a main component in the antioxidant defences of a cell, acting to directly detoxify reactive oxygen species as well as acting as a substrate for several peroxidase.

The study concluded that pre treatment with dietary OLE may reduce infarct volume neurobehavioural deficit scores in an animal model of cerebral ischemia. The data suggests that alteration of brain lipidomics in ischemic reperfusion can have an impact on neuroprotection mechanism and is thus an important implication in the pathogenesis of stroke. Further research is warranted to investigate these observations.

Use of ginseng in ischemic heart disease

Jia Y, Zhang S, Huang F et al. 2012. Could ginseng-based medicines be better than nitrates in treating ischemic heart disease? A systematic review and meta-analysis of randomized controlled trials. *Comp Ther in Med* 20;155–66.

In China, ginseng based medicines and nitrates are commonly used in treating ischemic heart disease angina pectoris. Ischemic heart disease (IHD) is defined as myocardial impairment due to an imbalance between coronary blood flow and myocardial requirements. There are hundreds of RCTs in the Chinese language documenting the use of ginseng in IHD, however these are not readily accessible to health practitioners and academics outside of China.

This meta-analysis aimed to provide a comprehensive, internationally accessible, systematic review evaluating the role of ginseng based medicines compared with nitrates in treating angina pectoris.

Randomised controlled trials published between 1980 and 2010 comparing ginseng and nitrates in treating angina pectoris were screened and filtered. Specific inclusion criteria were: study designs explicitly described as RCTs; ginseng based medicine was used as experimental and a nitrate drug was used as the control; duration of treatment (follow up) was at least 14 days; participants were suffering from angina pectoris as diagnosed by the criteria consistent with the World Health Organisation guideline; there was at least 50% reduction in frequency of feeling angina chest pain or significant improvement in ST segment in electrocardiogram (ECG) during an exercise test; and outcomes included the data of symptoms improvements.

A total of 467 articles were subjected to manual screening based on titles and abstracts. Based on the eligibility criteria, 18 studies were included for quality assessment and meta-analysis. These studies were RCTs published in Chinese language between 2000 and 2009. The studies involved 1549 participants suffering from angina pectoris, aged between 30 and 83 yrs. The sample sizes were between 45 and 152, with a mean of 86 (95% CI: 70–102). Seventeen studies reported the dosage of ginseng (experimental group) and 16 studies reported the dosage of nitrates (control group). Nine studies adopted single dosage of herbal medicines and the others adopted combined use of herbal medicines and Western medicines in experimental group.

Overall, based on odds ratios of the outcomes, i.e. symptomatic and ECG improvement reported in the included 18 articles, the review concluded that ginseng was more effective than nitrates in treating angina pectoris ($P = 0.001$).

Using these results the researchers recommended that further research be conducted, including high quality multi centre RCTs with longer follow up periods and larger sample size to support the findings of this analysis.

Herbal antiepileptics in earlier centuries

Adams M, Schneider SV, Kluge M, Kessler M, Hamburger M. 2012. Epilepsy in the renaissance: A survey of remedies from 16th and 17th century German herbals. *J Ethnopharmacol* 143:1;1-13.

In the centuries prior to 1882 when the first synthetic anticonvulsant drug, paraldehyde, became available, the people of central Europe depended mainly on plants to treat epileptic seizures. The advent of phenobarbital in 1921 and diphenylhydantoin (dilatant, phenytoin) in 1938, has brought some relief to the 50 million sufferers worldwide but for over 30% of these, uncontrolled seizures continue even with the best available drugs.

Recently a survey was carried out on nine of the most important European herbals of the 16th and 17th century,

including Bock (1577), Fuchs (1543), Mattioli (1590), Lonicerus (1660, 1770), Brunfels (1532), Zwinger (1696) and Tabernaemontanus (1591, 1678). The aim was to systematically explore antiepileptic remedies, identify the plant species, compile them and discuss what is known about their potential effectiveness.

An extensive search of the scientific data bank SciFinder® revealed recent results concerning the phytochemistry and possible anticonvulsive actions of the plants. Some of the plants showing possible antiepileptic potential follow.

Valeriana officinalis: aqueous and ethanolic/aqueous root extracts of valerian were both found to contain GABA and this intrinsic content is believed to be responsible for the ability of both extracts to increase GABA release in rat synaptosomes. An ethanolic extract containing no GABA showed no effects. Isovaleramide, albeit at high doses (100 mg/kg p.o), showed 90% protection against the maximal electroshock seizure in mice compared with sodium phenytoin (100% protection at 20 mg/kg p.o).

Matricaria chamomilla: at doses of 20–80 mg/kg i.p. apigenin significantly delayed the onset of seizures in a mouse model. Another study in rats showed that at 25 and 50 mg/kg i.p. apigenin significantly shortened the latency period of picrotoxin induced fits but did not reduce the incidence of seizures.

Hypericum perforatum: an aqueous fraction of 80% ethanolic extract (100 mg/kg i.m) had a clear antiepileptic effect in rabbits, a butanol fraction was weaker while an ether fraction was proepileptic. In electrophysiological tests, hypericin (10 μ M) lowered NMDA-activated ion currents by 30% and GABA-induced chloride currents by 43%. Pseudohypericin (10 μ M) reduced NMDA-induced ion currents by 20% and GABA-induced chloride currents by 57%.

Lavandula officinalis: an electrophysiological study in rat cortical cells showed that lavender oil at 0.1-1 mg/mL reversibly inhibited the GABA-receptor. Inhibitory and excitatory impulses were suppressed suggesting inhibition of signal transmission between neurons.

Among the other herbal constituents studied, linalool from *Thymus vulgaris* and myoinositol from *Aquilegia vulgaris* showed some promise with regard to anticonvulsive activity. The majority of the plants listed have not been investigated pharmacologically with respect to potential antiepileptic activity. None of the plants have been studied in larger clinical trials. By presenting these herbs to the wider scientific community it is hoped that potentially useful molecules for the treatment of epilepsy will be discovered.

Reviews of medical journal articles

Ann-Maree Bertolli, Sandy Braiuka, Busra Buyukyazici, Melissa Gearing, Sarah Harvey, Naomi Judge, Sarah Kottman, Angela McClelland

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.

Omega-6:omega-3 dietary ratio

Patterson E, Wall R, Fitzgerald GF, Ross RP, Stanton C. 2012. Health implications of high dietary omega-6 polyunsaturated fatty acids. *J Nutr Metab*. Published ahead of print April 5.

Omega-6 (n-6) polyunsaturated fatty acids (PUFA) and omega-3 (n-3) PUFA are precursors to potent lipid mediator signalling molecules, eicosanoids, which have important roles in the regulation of inflammation. Examples of n-6 PUFA are arachidonic acid and n-3 PUFA are eicosapentaenoic acid. In general eicosanoids derived from n-6 PUFA are pro-inflammatory, while eicosanoids derived from n-3 PUFA are anti-inflammatory.

Dietary changes over the past decades show an increase in saturated fat, n-6 PUFA and trans fatty acid intake as well as a decrease in n-3 PUFA intake, which has altered the ratio of n-6 to n-3 PUFA. Coinciding with this is an increase in chronic inflammatory diseases such as non alcoholic fatty liver disease, cardiovascular disease, obesity, inflammatory bowel disease, rheumatoid arthritis and Alzheimer's disease.

By increasing the ratio of n-3 to n-6 PUFA in the Western diet, reductions may be achieved in the incidence of chronic inflammatory diseases. Since the unbalanced dietary consumption of n-6:n-3 PUFA is detrimental to human health, the impact of dietary supplementation with n-3 PUFA upon the alleviation of inflammatory diseases, more specifically, non alcoholic fatty liver disease, needs to be more thoroughly investigated. Increases in the ratio of n-6:n-3 PUFA could potentiate inflammatory processes and consequently predispose to or exacerbate many inflammatory diseases.

Dark chocolate for prevention of cardiovascular disease

Zomer E, Owen A, Magliano DJ, Liew D, Reid CM. 2012. The effectiveness and cost effectiveness of dark chocolate consumption as prevention therapy in people at high risk of cardiovascular disease: Best case scenario analysis using a Markov model. *Brit Med J* 344:e3657.

Metabolic syndrome describes a cluster of risk factors that significantly increase the risk of developing cardiovascular disease and diabetes. Lifestyle change is the first line treatment for people with metabolic syndrome to prevent progression into cardiovascular disease, which now accounts for around 29% of deaths worldwide.

Recently there has been much interest in the beneficial effects of dark chocolate on cardiovascular risk factors, specifically the flavonoid components. Several studies have suggested that the consumption of dark chocolate may alter cardiovascular event risk via antihypertensive and lipid modifying effects, but these studies have only been short term. This paper describes a model to assess the health effects and associated costs of daily consumption of plain dark chocolate (polyphenol content equivalent to 100 g of dark chocolate) compared with no chocolate in a population with metabolic syndrome without diabetes and initially without cardiovascular disease.

Two thousand and thirteen participants were selected from the Australian Diabetes Obesity and Lifestyle study, among whom cardiovascular risk was estimated individually. Specific data such as age, sex, blood pressure and lipids were entered into the algorithm to calculate the risk of cardiac disease and events, along with data from the Reduction of Atherothrombosis for Continued Health (REACH) registry. With each annual cycle the probability of an individual transitioning to other health states was estimated until the period of ten years was reached.

Changes in cardiovascular risk were calculated by the application of expected effects of dark chocolate on systolic blood pressure and lipid levels gathered from meta-analyses of previous clinical trials. The best case scenario, with 100% compliance, showed that dark chocolate consumption could potentially prevent 70 non fatal and 15 fatal cardiovascular events per 10 000 population treated over 10 years.

The estimated incremental cost effectiveness ratio was A\$50 000 per years of life saved when A\$40 per person per year was assumed to have been spent on this particular prevention strategy.

Dietary selenium reduces incidence of type 2 diabetes mellitus

Hu FB, Manson JE, Morris JS, Mozaffarian D, Park K, Rimm EB et al. 2012. Toenail selenium and incidence of type 2 diabetes in US men and women. *Diabetes Care* 35:7:1544-51.

Selenium (Se) is a vital mineral, which serves many roles in the human body, most significantly as a component of selenoproteins and as a cofactor for antioxidant enzymes such as glutathione peroxidases. This study looks at the role of Se as a vital factor in

decreasing free radical activity. Increases in free radical activity may contribute to glucose stimulated insulin secretion which may lead to the onset of type 2 diabetes mellitus (T2DM). This study used toenail samples to give an indication of the effects of long term Se consumption.

Two separate cohort studies took place. Enrolment of cohort 1 began in 1976 and initially comprised 121 700 female registered nurses; cohort 2 began enrolment in 1986 and had the initial numbers of 51 529 male health professionals. Toenail samples were obtained from both cohorts between 1982 and 1983, and 1986 and 1987 respectively. Questionnaires were sent to the cohort members biennially, and were used to determine general health, risk factors, lifestyle, medical history and the incidence of disease.

Toenail samples were taken from all ten toes and were measured for Se content using neutron activation analysis. Those samples with Se levels greater than 1.5 µg/g were excluded due to the implications of contamination, namely from the use of Se supplementation.

These cohort studies were done over a 26 year follow up phase, during which 780 cases of T2DM were identified. Cases of T2DM through both cohorts were identified using the national diabetes data group criteria. The mean concentration Se levels found were 0.84 µg/g in men and 0.77 µg/g in women. It was also found that those who had higher Se levels consumed more whole grains, less saturated fats and less coffee and alcohol.

The cohort studies document that higher toenail Se concentration correlated with less incidence of T2DM. This study is the first to identify the link with Se and T2DM; all other studies have had varied results.

The results of this study highlight the importance of diet and overall health, as well as the importance of micronutrients in the prevention of chronic disease. Although the final outcome could be due to other dietary factors, these large cohort studies are a good indicator of the importance of dietary sources of Se and the prevention of T2DM and potentially other chronic diseases. This needs to be explored further.

Remember your vegetables for prevention of cognitive decline

Loef M, Walach H. 2012. Fruit, vegetables and prevention of cognitive decline or dementia: A systematic review of cohort studies. *J Nutr Health Aging* 16:7:626-30.

The National "Go for 2&5" campaign has been endeavouring to get Australians to increase their fruit and vegetable intake since 2005, in order to reduce their risk of chronic diseases such as cancer, cardiovascular disease and stroke. The evidence for fruit and vegetable intake in reducing disease risk is less clear in other areas.

The authors of this systematic review of cohort studies undertook to clarify the benefits of fruit and vegetable consumption in relation to cognitive decline and dementia.

Nine studies from 1764 publications initially identified were incorporated in the review, providing they met the following inclusion criteria: large sample size with follow up of 6 months or more; the use of tests to measure cognitive changes (such as the Mini-Mental State Examination); and reported risk estimates or number of events for Alzheimer's disease (AD), dementia, mild cognitive impairment or cognitive decline based on measures of fruit and vegetable consumption. All nine studies utilised self reporting measures such as food frequency questionnaires to assess dietary intake of fruit and vegetables.

The review concluded that a higher intake of vegetables is associated with a lower risk of dementia and a slower rate of cognitive decline. The strongest associations were found for cruciferous vegetables, legumes and green leafy vegetables, for example broccoli, cabbage, lettuce, zucchini and squash. In order to prevent AD and cognitive decline with age, there was moderate support for the recommendation to eat three serves or more than 200 grams of vegetables per day. The evidence for such an association is lacking with regard to fruit intake.

The authors speculate that the reason for the greater protective effect of vegetables over fruit may include a higher vitamin E content of vegetables although other compositional differences such as dietary fibres, lycopenes, β-carotenoids and monosaccharides cannot be ruled out. Although this review does not lend unequivocal support to national campaigns to increase fruit and vegetable intake in relation to reducing cognitive decline, the authors highlight the public health importance of this message in other chronic health diseases.

Nicotine exposure in adolescents

Goriounova N, Mansvelder H. 2012. Nicotine exposure during adolescence alters the rules for prefrontal cortical synaptic plasticity during adulthood. *Front Syst Neurosci* 4:3.

This review analyses the effects of nicotine on the adolescent brain. The authors of this paper examined a number of studies in an attempt to understand the impact of nicotine exposure on adolescent brain development.

Tobacco is one of the most socially accepted drugs worldwide. The health risks of nicotine are well known, it being one of the leading causes of premature deaths. Nicotine is an addictive and psychoactive drug that influences the cognitive and emotional processing areas of the brain.

Adolescence is a crucial time for brain development as cognitive maturation is ongoing into adulthood. During adolescence there is an increase in emotional drive that can lead to mood changes, risk taking and impulsivity. This can result in vulnerability and lead to experimenting with drugs of abuse. A study performed across 41 countries noted that 30% of adolescents reported commencing cigarette smoking before the age of 14, with 19% of 15 year olds smoking at least once a week.

Evidence from many clinical trials has indicated that the prefrontal cortex (PFC) is one of the last areas of the brain to develop during adolescence. Smoking during this time can affect the normal pathway of prefrontal development and lead to cognitive dysfunction. In the PFC nicotine affects cognition by regulating the processing of information on several layers by desensitising and activating the nicotine receptors.

According to a recent study the PFC displayed delayed development compared with other cortical areas of the brain during adolescent smoking. Previous studies have suggested that these delays increase the risk for developing psychiatric disorders.

This study shows that cigarette smoking during adolescence has lasting effects on cognitive and developmental behaviour of adolescents. However chronic exposure of nicotine in adults did not have the same effects.

HDL and cardiovascular events in women

Mora S, Buring JE, Ridker PM, Cui Y. 2011. Association of high-density lipoprotein cholesterol with incident cardiovascular events in women, by low-density lipoprotein cholesterol and apolipoprotein B100 Levels: a cohort study. *Ann Intern Med* 155:11;742-50.

Studies have previously found an inverse relationship between high density lipoprotein cholesterol (HDL-C) or apolipoprotein A-1 levels and cardiovascular disease. This prospective cohort study aimed to determine if there is an association between HDL-C or apolipoprotein A-1 levels and cardiovascular disease across a range of low density lipoprotein cholesterol (LDL-C) and apolipoprotein B100 levels in women.

Researchers studied 26 861 healthy healthcare professionals who were enrolled in the US based Women's Health Study. Participants were 45 years old or older at study entrance (between 1992 and 1995). The mean study period was approximately 11 years for each participant. Study participants had baseline lipids and apolipoproteins measured at the time of enrolment. In total 929 confirmed cardiovascular events were reported over the study period, comprising 602 coronary events and 319 strokes.

Researchers concluded that HDL-C and apolipoprotein levels were inversely associated with cardiovascular disease and coronary events regardless of LDL-C levels and independent of other established cardiovascular risk factors. No association was found between HDL-C and apolipoprotein and stroke incidence. Neither were associations noted for HDL-C or apolipoprotein A-1 among women with low apolipoprotein B100 as the number of participants were small in this category and few events were seen.

As women were healthy, mostly white healthcare professionals and considered to be at low risk for CVD on enrolment, it is unclear how these findings can be

extrapolated to women who have established disease or have a higher risk of developing CVD.

Dietary GL and breast cancer risk

Romieu I, Ferrari P, Rinaldi S, Slimani N, Jenab M, Olsen A et al. 2012. Dietary glycemic index and glycemic load and breast cancer risk in the European Prospective Investigation into Cancer and Nutrition (EPIC). *Am J Clin Nutr* 96;2:345-55.

Breast cancer (BC) has been likened to an epidemic in modern society, and likewise diabetes, especially non insulin dependent diabetes (type II diabetes). Chronically elevated insulin concentrations, often found with a typical modern diet consisting of highly refined carbohydrates, have also been linked with many health problems.

This study investigated whether a diet with a high glycemic load (GL) and high total carbohydrate level is associated with an increased risk of BC. Data was taken from the European Prospective Investigation into Cancer and Nutrition with a study population of 11 576 women with invasive BC, among a total of 334 849 women aged 34–66 years. The median follow up was 11.5 years. Dietary glycemic index (GI) and glycemic load were calculated from country specific dietary questionnaires and BC tumours were classified by receptor status as well as progesterone receptor status.

It was found that GI, GL and carbohydrate intake were not related to BC among pre menopausal women, however among post menopausal women GL and carbohydrate intake were significantly associated with an increased risk of estrogen receptor negative BC. Progesterone receptor status showed an even stronger association with estrogen receptor negative/progesterone receptor negative BC. No significant association with estrogen receptor positive BC was found.

This supports the theory that a diet with a high GL and with a high total carbohydrate level, which is associated with higher insulin levels, is a risk factor for BC.

Vitamin D deficiency and critically ill children

Madden K, Feldman H, Ellen M. Smith, BS, Gordon CM et al. 2012. Vitamin D deficiency in critically ill children. *Pediatrics* 2011–3328. Published ahead of print August 6.

Vitamin D is a fat soluble vitamin which is synthesised from cholesterol on adequate sun exposure and is important for the proper functioning of multiple organ systems, as well as being essential in healthy bone development and the immune system. Recent studies have found that vitamin D deficiency may be a contributing factor in the recovery and outcome of children who are admitted to pediatric intensive care units (PICUS) for life threatening illnesses.

One of these studies tested 511 children who were admitted for severe or critical illnesses from November 2009 to 2010 and found that 71.2% of these children had insufficient levels of vitamin D. The median

25(OH)D level of the children studied was 22.5 ng/mL (interquartile range 16.4-31.3 ng/mL). Of the 71.2% of children who had low levels of vitamin D, 40.1% had a deficiency (33.1% 10-19.9 ng/ml and <10 ng/ml in 7%). Levels were found to be lower in children who had septic shock, however there seemed to be no difference in children who had life threatening illnesses from those who did not. Unfortunately 13 of the children died in hospital during the study - the levels of these children were on average 19.4 ng/mL.

In this and other similar studies a high rate of children who are critically ill are found to be vitamin D deficient. These studies suggest that critically ill children admitted to hospital be tested for vitamin D levels and treated accordingly as it can be hypothesised that vitamin D supplementation may improve recovery outcomes.

Breast cancer and use of soya foods for congenital heart disease

Messina M, Messina V, Jenkins DJ. 2012. Can breast cancer patients use soya foods to help reduce risk of congenital heart disease (CHD)? *Brit J Nutr* 3:1-10.

There has been some controversy recently about the benefits and/or risks of soyafoods consumption. In 1999 the US Food and Drug Administration approved the claim that soya foods have hypocholesterolemic effects and are thus beneficial for CHD. Since that time it has been claimed that the phytoestrogen (isoflavone) content may be contraindicated for women with breast cancer or at high risk of breast cancer.

Recent meta-analyses support the hypocholesterolemic action indicating that soya protein directly lowers circulating LDL-cholesterol by approximately 4%. Furthermore one trial showed that when soya foods replace commonly consumed sources of animal protein, LDL-cholesterol is reduced by 3-6%, which significantly reduces heart disease risk.

There is clinical evidence that soya foods exert coronary benefits independent of their effect on lipid levels. For example four meta-analyses have found that soya lowers blood pressure, although further research in this area is needed.

Recently published clinical and epidemiological data does not support observations in rodents that soyabean isoflavones increase breast cancer risk. Concerns that soya foods are contraindicated for women with a history of breast cancer and women at high risk of developing this disease are based on the oestrogen like effects of isoflavones and a series of studies which show that isoflavones and isoflavone-containing soya products stimulate the growth of existing mammary tumours in athymic ovariectomised mice. However another study failed to confirm the tumour stimulatory effect despite the use of almost identical models. It was noted that before implantation, the specific cells were cultured in an oestrogen free environment, whereas in the studies in

which tumour stimulation occurred, the cells were exposed to a high concentration of oestrogen before implantation. It was claimed that this high oestrogen concentration is unphysiological and makes the cells hypersensitive to oestrogenic stimuli. Recent work suggests that because of differences in isoflavone metabolism between mice and humans, the former may not be an appropriate model for predicting effects in the latter.

In post menopausal women isoflavone exposure does not adversely affect breast tissue density or breast cell proliferation. Both US and Chinese prospective epidemiological studies show that post diagnosis soya consumption is associated with an improved prognosis. Therefore soya foods should be considered by women as health foods to include in diets aimed at reducing the risk of CHD, regardless of their breast cancer status.

Eating disorders not otherwise specified are the real concern

Field AE, Sonneville KR, Micali N, Crosby RD, Swanson SA, Laird NM et al. 2012. Prospective association of common eating disorders and adverse outcomes. *Pediatrics* 130:2;289-95.

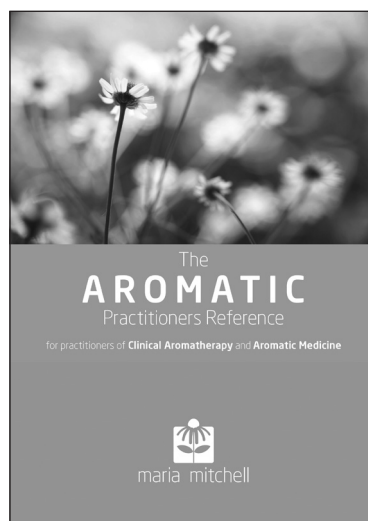
Both anorexia nervosa and bulimia nervosa are considered rare, however eating disorders not otherwise specified (EDNOS) are a common occurrence with adolescent girls. This study evaluated whether bulimia nervosa and subtypes of EDNOS in females were predictive of developing adverse outcomes long term.

Included were 8594 females who participated by answering annual questionnaires from 1996 to 2001, then biennially from 2007 to 2008. Participants were classified as having bulimia nervosa (BN) (\geq weekly binge eating and purging), binge eating disorder (BED) (\geq weekly binge eating, infrequent purging), purging disorder (PD) (\geq weekly purging, infrequent binge eating), other EDNOS (binge eating and/or purging monthly), or nondisordered.

The results showed that 1% of adolescent girls were BN affected, 2-3% had PD and 2-3% had BED. Those with BED were almost twice as likely as the nondisordered group to become overweight or obese or develop high depressive symptoms. Those with PD had a significantly increased risk of starting to use drugs or starting to binge drink frequently.

The authors concluded that both PD and BED are common and predict a range of adverse outcomes. They state that the 'primary care clinicians should be made aware of these disorders, which may be underrepresented in eating disorder clinic samples' and that 'efforts to prevent eating disorders should focus on cases of subthreshold severity'.

Book review



The Aromatic Practitioners Reference

By Maria Mitchell

Maria Mitchell 2011

ISBN 978 0 646 558561

Reviewed by Sandra Walton

The Aromatic Practitioners Reference is a quick and easy reference guide to 100 essential oils. The guide is set out in 3 sections, Materia Medica, Formulations and Dosages and is a good review of the current literature. I especially liked the inclusion of essential oils native

to Australia and New Zealand and also those used in Ayuveydic practice.

It includes information on popular plants where the essential oil is not used therapeutically, such as vanilla and wormwood. Vanilla oil has little therapeutic action and is mostly used in perfumery, while wormwood, a well known plant to practitioners, is toxic in its essential oil form. The latter demonstrates how you cannot assume the herb and essential oils can be used in the same way.

The double page layout for each essential oil provides a clear guide to a lot of information. As many practitioners know, there can be many therapeutic properties and actions linked to medicinal plants. A quick visual guide using crosses denotes the importance of the properties of each oil. The indications, dosages and toxicity are in a good format and the reference section allows the practitioner to follow up more information.

As a herbalist I found the information in the Formulations and Dosages section, which includes internal dosages, gives the practitioner confidence to use various essential oils in different combinations. Maria Mitchell also encourages the use of other treatments such as teas, herbs, nutrition and supplements together with the use of essential oils.

This is an enjoyable, easy, accessible guide to 100 essential oils which provides the practitioner relevant information in a well sourced review to help extend the treatments available in practice.

Available for loan from the NHAA library or purchase from www.mariamitchell.com.au.



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AJHM based CPE Questionnaire

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(3)

From the information in this journal, which statement is most correct?

1. PCOS

- The use of insulin-modulating herbs will assist in treatment of PCOS.
- Herbal treatment of hyperprolactinemia in PCOS may aggravate symptoms.
- Herbal treatment of the adrenal system appears to aggravate PCOS symptoms.
- Tribulus terrestris* is most effective for PCOS in women during the week prior to the menstrual cycle.

2. Consequences of maternal nutrition

- Evidence shows that *Echinacea* spp should not be used during pregnancy.
- Some herbal medicines may be unsafe in pregnancy for the mother but will have no effect on the fetus.
- Herbal medicines may affect the fetus but will have no long term effects.
- Herbal medicines may affect the fetus and may have long term effects.
- a) and d)

3. Review of *Hypericum*

- Evidence suggests that *Hypericum* extracts have no effect on major depression.
- Evidence suggests that *Hypericum* extracts may have value in acute treatment of major depression.
- The literature review clearly shows that in some cases *Hypericum* has superior action to pharmaceutical antidepressants in treatment of depression.
- Pharmaceutical antidepressants have a clear advantage over *Hypericum* in treatment of minor depression.

4. *Withania* and Alzheimer's disease

In using *Withania* for treatment of Alzheimer's disease in animal models:

- Effects were greater in older subjects than middle aged subjects.
- The most significant decrease in beta amyloid 42 was between day 7 and day 14.
- The reduction in amyloid plaque resulted in improved behavioural patterns.

- Cortex levels of lipoprotein receptor related protein increased between day 7 and day 14.

5. Effect of garlic on commensal bacteria

- Garlic shows long lasting eradication of multiple strains of bacteria.
- Garlic shows short term detrimental effect on some strains.
- Overall garlic had no adverse effects.
- Both b) and c)

Medical science questions - AJHM 24(3)

1. Dark chocolate in cardiovascular disease

The amount of dark chocolate consumed daily that shows a reduction in cardiovascular risk factors is:

- 20 gm
- 50 gm
- 100 gm
- 125 gm

2. Remember your vegetables

- Alzheimer's disease is associated with low zinc levels.
- High intake of both fruit and vegetables prevents the onset of dementia.
- Consumption of orange and red vegetables is particularly associated with a lower risk of dementia.
- Daily consumption of fruit slows the rate of cognitive decline in the elderly.
- Vegetables may have greater protective effects for dementia than fruit because of the higher content of vitamin E.

3. Dietary GL and breast cancer risk

- GI, GL and carbohydrate intake are not related to breast cancer in post menopausal women.
- A diet consisting of mainly high GL carbohydrates and high total carbohydrate level is a risk factor for breast cancer amongst women of any age.
- This study should not be considered valid because the median follow up was only 1.5 years.
- Pre menopausal women do not have an increased risk of breast cancer if they consume a diet with a high glycemic load.



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.
- Accepted articles become the property of the Australian Journal of Herbal Medicine.
- 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.

Books: Author's surname Author's initials. Year. *Book title* edition. City of publication: Publisher. (Snooks R. 2002. *Style Manual* 6th edn. Sydney: Wiley & Sons.)

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