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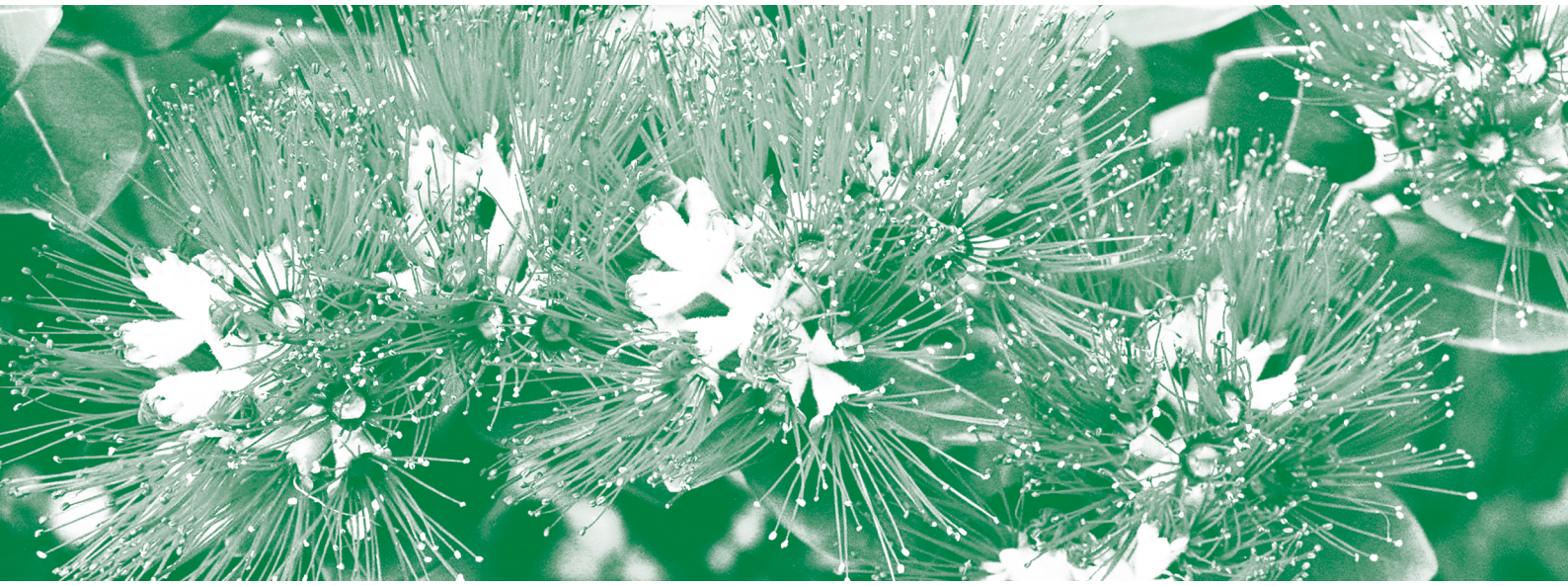

Concerns Surrounding Black Cohosh & Liver Damage

Following a recent ruling by the UK Medicines and Healthcare products Regulatory Agency (MHRA), all black cohosh-containing products being sold in the UK market are required to be labelled with a warning statement that they may harm the liver. This step follows a similar move by the Australian TGA in February of this year.

Concerns regarding potential hepatotoxicity of black cohosh *(Cimicifuga racemosa)* were first raised in Australia following publication of two Australian case reports in 2002[1, 2]. This was followed by a further single Australian case report in 2003[3].

Since that time, there have been a further three case reports published in the peer-reviewed scientific literature[4,5,6], all from the U.S.

Of the Australian cases, one involved a 47 year old woman who developed jaundice and subsequently required a liver transplant two weeks after taking a menopausal product containing black cohosh for a one week period. Histological examination of her liver confirmed severe hepatitis and early fibrosis, but no information about the dose of black cohosh taken was provided. The fact that this patient's liver showed signs of early fibrosis,

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something that generally takes months rather than days to develop, implicates the presence of other causes other than the black cohosh.

The second Australian case, a 43 year old woman, developed nausea, vomiting, diarrhoea and jaundice after taking a product containing black cohosh and several other herbs for an unspecified period of time. These included skullcap, a herb for which substitution problems involving *Teucrium* species, a known hepatotoxic plant family[7], have occurred. She was also diagnosed with ovarian adenocarcinoma shortly after presentation[1].

The third Australian case report described a 52 year old woman, who developed acute liver failure after taking a herbal formula containing liquid extracts of several herbs for three months, but ceased four weeks before hospital admission. Clinical details supplied for this case however were very scanty, and positive identification of two of the other herbs in her formula was unsuccessful[3].

The fourth case report involved a 57 year old diabetic woman who developed autoimmune hepatitis, three weeks after starting to take an unknown brand and unknown dose of black cohosh[4]. While the authors attributed this to the herbal preparation, it is significant that this patient was also taking the drugs labetalol, fosinopril, verapamil, metformin, aspirin and insulin. Their statement that 'none of the woman's other medications have ever been implicated as triggers for autoimmune hepatitis', is however highly debatable, given the fact that a simple search of the literature shows that hepatitis has been associated in at least 13 published case reports for labetalol[8,9,10], several also for verapamil[11,12,13] and metformin[14,15,16,17], and one for fosinopril[18].

The fifth case report was published in March 2005, and involved a 50 year old American woman who developed acute jaundice, and was given a provisional diagnosis of autoimmune hepatitis. She failed to respond to prednisone treatment and subsequently underwent a liver transplant, from which she had an uneventful recovery[5]. During the five months prior to the onset of jaundice, she had taken black cohosh at a dose of 500mg daily.

The sixth and most recent case report involved a 54 year old woman who developed liver failure after taking black cohosh for eight months[6]. She was hospitalised and prescribed prednisone for possible autoimmune hepatitis, but after 15 days her condition worsened. She subsequently underwent liver transplantation 39 days after admission, but died following an uncontrollable haemorrhage during the operation.

After publication of the two Australian reports, Phytomed raised concerns regarding the methodology of evaluation used, including failure to analyse or verify the ingredients in the products implicated, failure to provide sufficient information about the past or present medical history, use of over-the-counter or recreational drugs, and other lifestyle factors which could have been involved[2].

In only one of the six cases was the presence of black cohosh in the products conclusively established. In none of the case reports was a rechallenge with black cohosh or a lymphocyte stimulation test undertaken. These tests are normally recommended to help provide more conclusive evidence of a causative link between exposure to a particular substance and adverse effects.

With the evidence-based weighting of an individual case report being regarded as relatively low, it is extremely important to ascertain and fully evaluate all potentially confounding factors in a totally impartial manner. Authors of some of the U.S. studies used the published Australian case reports to justify their identification of black cohosh as the cause of their patient's liver problems. The fact that they did this highlights the risk of a self-fulfilling prophecy, unless these issues are fully addressed.

From our experience evaluating similar reports of alleged hepatotoxicity, particularly those associated with Kava, the number of actual cases claimed to have been associated with black cohosh ingestion, as reported by both the Therapeutics Goods Administration (TGA) and MHRA, is likely to be somewhat more than the numbers actually involved. At the time of writing only these six actual case reports had been published in the peer-reviewed literature, considerably less than the nine Australian cases reported to the TGA, and 22 cases to date reported also to the MHRA.

We have requested full information about all reported cases from the MHRA for further evaluation, but are still awaiting receipt of this.

In late August, the Canadian regulatory agency Health Canada also issued an advisory about a possible link between black cohosh and liver damage[19]. Their statement reported that three cases of liver damage allegedly associated with the use of black cohosh had been notified, but that "case reports of liver damage are rare and the link between black cohosh and liver toxicity is unclear". Unlike the previous announcements in the UK and Australia, Health Canada does not require products carrying black cohosh to carry warning labels.

Of interest also was a US District Court's dismissal of a product liability lawsuit against two manufacturers of black cohosh products in September of this year[20]. This case, known as Grant and Beck vs Pharmavite and Nutraceutical Corporation, heard in the US District Court of Nebraska, involved a plaintiff from one of the published US case reports who developed autoimmune hepatitis and required a liver transplant within months of starting to take black cohosh-containing products. While in the published case report it was stated that the patient did not drink alcohol and was not taking any other medications, during the trial it emerged that she had regularly drank wine, took ibuprofen, and had been prescribed the antiviral drug valacyclovir. Several case reports of ibuprofen and valacyclovir-induced hepatitis have been published[21,22,23,24].

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A further development of potential importance to this situation was a paper published in the May issue of the *Journal of Agricultural and Food Chemistry*, which evaluated the botanical authenticity of 11 different black cohosh products on sale in New York between 2002 and 2004[25]. Of the 11 products, three were found to contain an Asian species of *Cimicifuga (Actaea)*, while one contained both *Cimicifuga racemosa* and a cheaper Asian *Cimicifuga* species. With herbal medicine manufacturing operations in the U.S. being subject to few regulatory requirements, these findings highlight the importance of verification of the correct botanical species before assigning blame for an alleged adverse effect. While hepatotoxic reactions are not known for the likely Asian species involved (*Cimicifuca foetida, Cimicifuga dahurica*, or *Cimicifuga heracleifolia*), this subject clearly needs more investigation.

Until further detailed information concerning the UK case reports is received, formation of a definitive opinion is perhaps somewhat premature. The millions of women taking black cohosh daily throughout the 'developed' world, particularly since negative findings on hormone replacement therapy were published a couple of years ago, mean that such rare, idiosyncratic reactions could occasionally occur. A study by hepatologists in Canada, also draws attention to the frequency of acute liver failure where no aetiology is revealed, this being of unknown origin in 27% of 81 consecutive patients admitted to their hospital between 1991 and 1999[26]. This highlights the fact that many cases of spontaneous liver toxicity can occur in large population groups, due to as yet unidentified viruses or other unknown causes.

Black cohosh now has a long history of safe use in many countries[27,28,29], and the substantial amount of research undertaken to date on this agent, as well as the individual constituents it contains, has not provided any evidence of hepatotoxicity until recently. Based upon the evidence available, we believe that the cases reported to date are either unrelated to true black cohosh ingestion and due to other unknown causes of idiopathic hepatitis, or attributable to black cohosh ingestion, but an extremely rare, idiosyncratic reaction such as those that occur in a very small number of individuals taking any medicament. An objective attempt to quantify the frequency of any hepatotoxic reactions to black cohosh comes to the conclusion that at best, they appear to be extremely rare, and certainly much less than that for widely consumed pharmaceuticals such as paracetamol.

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Safety of Ethanolic Kava Extract Following Chronic Use

In June 2002, the German Federal Institute for Drugs and Medical Devices (BrArM) withdrew the marketing authorizations of all products containing the herb kava (*Piper methysticum*), after reports of adverse liver reactions in Germany and Switzerland. This was followed by various degrees of restrictions in the UK, Canada, Australia and other countries. In the U.S. and New Zealand, no such restrictions have been made to date, although safety assessments are in progress.

These restrictions on the availability of kava products have however remained controversial amongst herbal practitioners and industry groups[1,2,3]. While nearly 80 cases of liver damage reportedly associated with kava use have now been documented worldwide[1,4,5], a causative link with kava for most of these has been challenged[1,5,6]. Using the number of case reports and sales figures of kava extracts, an estimated incidence rate of one potential case in 60 to 125 million patients has been calculated by various authors[1,6,7]. This is substantially lower than the incidence of hepatotoxic reactions to widely available drugs such as paracetamol[8,9], and implies that any such reactions are idiosyncratic and very rare.

It has been suggested that there is an increased risk of hepatotoxicity through use of products made from ethanolic (alcoholic) or acetone extracts, rather than the more traditionally used aqueous extract [10,11]. These organic solvents enable extraction of higher levels of active kavalactones, but it has been speculated that kava components found in ethanolic extracts may undergo some kind of metabolic degradation to toxic compounds in humans. This premise is also based partly on the fact that traditional aqueous kava infusions contain higher levels of the hepatoprotective compound glutathione than extracts obtained from organic solvents[10]. Failure of aqueous kava extract to produce liver damage in a study on rats, and lack of case reports of liver toxicity in population groups using traditionally prepared kava extracts, has also been cited as evidence to support this premise[11]. Aqueous but not ethanolic or acetone extracts of kava, have subsequently been approved by the Australian medicines regulator, the Therapeutic Goods Administration (TGA). The perception of non-aqueous forms of kava being associated with an intolerable risk of liver damage does however remain contentious. A paper by a team of Italian and German researchers, published in the September issue of the journal *Phytomedicine*, provides further information. These researchers conducted a long term toxicological study in rats, using a commercial ethanol-extracted kava preparation, standardised to contain 47.4% total kavalactones[12]. Their data had previously been submitted for product registration requirements only in 1989.

Doses of 7.3 or 73mg kavalactones per kg body weight were given to rats daily for six months. Haematological and biochemical parameters were then measured, and histological examination of major organs, including the liver, was undertaken.

Despite the use of these very large doses of an ethanol extract of kava known to be high in kavalactone concentrations, no effect on body weight development, haematological or biochemical parameters or organ weights, was measured. Previous toxicological testing in animals has also found kavalactones and various other types of kava extracts to have a low level of toxicity[13,14,15,16].

Other possible causes of these rare cases of kava-associated liver toxicity include the use of the cheaper but phytochemically different (and traditionally discarded) stem peelings rather than the root as the starting material for kavalactone extraction, or a genetic predisposition in some Caucasian groups.

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As virtually all cases of liver damage linked to kava consumption have involved Caucasian rather than Polynesian populations, a possible contribution of the kavalactone metabolising enzyme cytochrome P450 2D6 (CYP2D6), has been proposed. Approximately 5 to 10% of Caucasians are deficient in CYP2D6, whereas those of Polynesian descent are much less likely to lack this particular enzyme[17].

Another recent study which separated kava extracts derived from root, leaves and stem peelings into various fractions using a variety of solvents, and tested these for potential cytotoxicity to human liver cells *in vitro*, found the compound flavokavain B, a type of flavonoid shown to be extracted more effectively with organic solvents rather than water, to be particularly toxic to liver cells[18]. However, the extract concentrations associated with this *in vitro* toxicity, seem to be significantly greater than those likely to be achievable in a clinical situation.

Publication of these two recent studies has come soon after a February update to a Cochrane Review on the subject of kava for the treatment of anxiety[19]. The conclusion from this meta-analysis of only seven trials was that kava is an effective symptomatic treatment for anxiety, and appears to be relatively safe when used for one to 24 weeks, although longer term studies are required. Another meta-analysis of six trials conducted by German researchers and published last year, also concluded that the particular acetone extract of kava used in these trials was an effective and safe treatment for anxiety disorders[20].

Regulatory reviews in other countries apart from Australia have also taken place since the initial ban, and in May last year the BfArM repealed the ban of registered kava drug products in Germany. However, while a process by which kava products can be appropriately reconsidered for future registration and sale was thus reactivated, the kava registrations in question are still inactivated until further notice, and kava remains unavailable on the German market[21].

Despite a review of Kava safety conducted in the UK, the MHRA announced in July of this year that it was upholding a full restriction on the use of all types of kava preparations[22].

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Cardiovascular Properties of White Horehound

While aerial parts of White horehound (*Marrubium vulgare*) are best known as a respiratory herb for bronchial conditions such as coughing and congestion, other less well known traditional uses of this plant are of interest. These include its use in liver and digestive disorders, and as an analgesic[1].

Cardiovascular properties of White horehound in particular, have been investigated during recent years[2]. Vasorelaxant and antihypertensive[3,4,5], antioxidant and anti-inflammatory effects have been reported[6,7,8,9].

Hypotensive effects were first reported in 2001 by Morrocan and French researchers following oral administration of a water extract of White horehound to spontaneously hypertensive but not normotensive rats[3]. The same researchers later observed a similar reduction in systolic blood pressure to the calcium antagonist drug amlodipine, following a ten week treatment period to hypertensive rats. A reduction in aorta hypertrophy, was also seen during White horehound treatment[10].

These actions have been linked with vascular effects including inhibition of the contractile responses of rat aorta to noradrenaline and potassium chloride. While crude extracts of the aerial parts of White horehound had some activity, diterpene constituents including marrubenol and marrubiin were subsequently shown to be the most active vasorelaxant constituents[4]. Calcium antagonist activity by marrubenol is thought to be contributory to these vasorelaxant effects[5], although other non-calcium antagonist effects by White horehound constituents have also been implicated[10].

Further investigations into the cardiovascular benefits of White horehound have recently been made by Canadian researchers, with positive findings[11]. A water-methanolic extract of White horehound was tested for its effects on human low-density lipoproteins (LDL) using human LDL *in vitro*. With oxidation of LDL being implicated as an early stage in the formation of atherosclerosis lesions, agents which inhibit human LDL oxidation may help protect against atherogenesis and thus reduce the risks of cardiovascular disease.

Human LDL cells obtained from 12 healthy volunteers aged 20-25 years were oxidised by incubation with copper sulphate, and incubated with or without White horehound extract in concentrations ranging from 0-100ug/ml, for up to eight hours. Formation of conjugated dienes, biochemical markers of lipid peroxidation, were also monitored during these experiments, as were levels of LDL endogenous vitamin E. Determination of the free radical scavenging activity of the herbal extract and its ability to chelate copper was also undertaken, as was its effect on high density protein (HDL)-mediated cholesterol efflux using human THP-1 macrophages.

In these experiments, White horehound incubation was found to cause a significant inhibition of lipid peroxidation of human LDL and formation of conjugated diene formation, in a dose-dependent manner. These protective effects against lipid peroxidation of human LDL were correlated with antioxidant effects, including a protective effect measured against vitamin E degradation and chelation of copper irons. Antioxidant effects including protection against LDL peroxidation in bovine aortic endothelial cells has previously been reported for White horehound[6,12], but this is the first study using human LDL.

The capacity of HDL to remove free cholesterol from cells is one of the mechanisms of its protective effects against cardiovascular disease. An additional finding in these studies that White horehound enhanced HDL-mediated cholesterol efflux in a dose-dependent manner, thus provides further evidence of the possible use of this herb as a protective agent against atherosclerosis and cardiovascular disease development

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Non-steroidal Anti-inflammatory Drugs Increase the Risk of Heart Attack

Non-steroidal anti-inflammatory drugs (NSAID's) have become some of the most widely available and commonly used analgesic drugs in the past twenty years. Drugs such as ibuprofen, naproxen and ketoprofen are routinely taken for arthritic or musculoskeletal pain, and have been available 'over the counter' in most countries for many years.

Gastrointestinal side effects including irritation and an increased risk of bleeding are well known for this class of drugs, and they contribute to a large percentage of patients presenting to emergency clinics with gastrointestinal haemorrhage. Asthmatic patients are also generally advised to avoid NSAID's, due to possible exacerbation of asthma symptoms.

So-called selective cyclooxygenase-2 (COX-2) inhibitors such as rofecoxib (*Vioxx*®), were developed with the aim of being less likely to produce such gastrointestinal side effects, but these have recently been largely withdrawn from use due to findings that they cause a roughly twofold increase in the risk of myocardial infarction and other vascular events[1,2,3]. Alarmingly, a European review of the safety of the older and more established NSAID group of analgesic and anti-inflammatory drugs published in the June 3 issue of the *British Medical Journal*, has revealed that high doses of these drugs can also increase the risk of heart attacks if taken long term[4].

The review took the form of a meta-analysis of 138 trials involving 145,373 participants, where selective COX-2 inhibitors had been compared to placebo or a traditional NSAID, for a minimum of four weeks. In these trials, the incidence of so called serious vascular events (defined as myocardial infarction, stroke, or vascular death) in the drug treatment groups was compared to that in the placebo control groups.

Overall, the incidence of serious vascular events was found to be similar for treatment with a selective COX-2 inhibitor or an NSAID (1.0% a year versus 0.9% a year). Increased risk of myocardial infarction was chiefly responsible, the findings indicating that for every 1000 people taking a NSAID, three a year would suffer a related heart attack.

The relative risk of such vascular events appears to differ depending on the particular NSAID and dosage taken, with high dose regimens of ibuprofen and diclofenac appearing to have the greatest risk. High doses of one particular NSAID drug naproxen however did not appear to be associated with such risks.

Such risks associated with the use of NSAID's were reported also by an independent group of Danish cardiologists in the June 27 issue of the journal *Circulation*[5]. Their analysis of the risk of rehospitalisation for myocardial infarction and death in a total of 58,432 patients taking these drugs, found the use of all doses of selective COX-2 inhibitors or high dosages of NSAID's, to be associated with an increased mortality in patients with previous myocardial infarction.

As a result of these studies, the Commission on Human Medicines (CHM) has written to every doctor in the UK advising them to prescribe "the lowest effective dose for the shortest time necessary". A review in October by the European Medicines Agency ruled that NSAID's were more beneficial than harmful, provided they are not given to patients with cardiovascular or gastrointestinal problems[6].

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Liquorice Protective Against Azathioprine Hepatotoxicity

The immunosuppressant drug azathioprine is widely used in the treatment of autoimmune diseases, such as severe rheumatoid arthritis and systemic lupus erythematosus, as well as in organ transplants. Side effects are quite common however, and include bone marrow suppression, gastrointestinal upset, an increase in risk of infections and malignancies, as well as hepatotoxicity. An estimated 10-30% of patients are unable to tolerate these adverse effects[1].

Hepatotoxicity is evident in clinical practice by a dose dependent increase in liver enzyme levels, as well as centrilobular hepatocyte damage in patients following azathioprine use[2,3]. Azathioprine hepatotoxicity has also been widely studied *in vitro* using rat hepatocytes, and this has shown severe cell death involving depletion in glutathione (GSH), mitochondrial injury as well as a decrease in ATP levels[4].

Extracts of liquorice, derived from the roots of *Glycyrrhiza glabra*, *Glycyrrhiza uralensis* or *Glycyrrhiza inflata*, have been used in Chinese medicine for the treatment of liver problems for over 2000 years[5]. Glycyrrhizin, the principle active component of liquorice, has also been given intravenously in Japan for many years as part of the treatment of chronic Hepatitis B and C[6,7]. This treatment has been reported to increase the effectiveness of interferon for chonic Hepatitis C[8,9], although larger scale trials are needed to confirm this. Various mechanisms of action may contribute to these effects, including free radical scavenging, protection of hepatocyte membranes by preventing a change in cell permeability[5,10], or inhibitory effects on cytochrome P450 (CYP450) 2E1 metabolising enzymes[11].

To date few laboratory studies have examined the effects of liquorice or glycyrhizzin on human as opposed to rat hepatocytes, but a team of Chinese researchers have recently undertaken such studies, as well as measured the effects of liquorice and glycyrrhizic acid (the major metabolite of glycyrrhizin formed by human intestinal bacteria), on liver toxicity produced by azathioprine[12]. In these studies, the Chinese form of liquorice, *Glycyrrhiza uralensis*, was extracted with water then incubated in different concentrations with either rat or human hepatocytes exposed also to azathioprine in low concentrations. These experiments found that rat hepatocytes were sensitive to low and clinically relevant concentrations of azathioprine, resulting in a concentration-dependent toxicity accompanied by corresponding glutathione depletion. Human hepatocytes were however less likely to be adversely affected by clinically relevant concentrations of azathioprine (0.5-10μM), although the intracellular glutathione content of human hepatocytes was depleted to a greater extent. As glutathione is involved in the conjugation and elimination of toxic drug metabolites, such depletion could be significant.

In the rat hepatocytes, pre-treatment with glycyrrhizic acid as well as liquorice extract itself exhibited hepatoprotective effects. In the human hepatocytes, glycyrrhizic acid gave protection against the intracellular glutathione depletion of human hepatocytes resulting from azathioprine hepatotoxicity, at a concentration of 0.175-0.25g/L glycyrrhizic acid. While this concentration seems somewhat greater than that likely to be achieved at hepatocytes in a clinical situation following oral liquorice administration, the finding that low

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concentrations were more hepatoprotective than high ones is of interest.

Continuous, high dose ingestion of liquorice is well documented as being associated with reversible minerolacorticoid-like side effects such as hypertension and fluid retention, due to inhibition of cortisol inactivation. Such adverse effects do not appear to have been problematic with the Japanese parenteral preparation of glycyrrhizin used in a twice weekly treatment regimen however. Another recent study which attempted to quantify safe levels of glycyrrhizin ingestion following oral administration in humans, has recommended a safe daily dose range of 0.015 to 0.229mg glycyrrhizin per kilogram body weight[13]. These estimations were hampered however by reduced glycyrrhizin bioavailability when consumed as liquorice.

To summarise, the Chinese study provides further evidence of possible hepatoprotective actions of both liquorice and glycyrrhizin, which in this case applies to the prevention of liver damage associated with use of the immunosuppressive drug azathioprine. It also highlights species differences in terms of different individual sensitivities of human and rat hepatocytes to azathioprine-induced hepatotoxicity. Such species differences in toxicological profiles as well as substrate specificity and activity of CYP450 metabolising enzymes, and thus likely clinical effects in different animals, has become increasingly recognised in recent years. This reinforces the importance of using human as opposed to animal tissue cells where possible when evaluating pharmacological effects.

The risk of onset of azathioprine-induced liver toxicity is currently managed through liver enzyme monitoring and withdrawal of azathioprine should these become elevated. The encouraging results from this study do however support further *in vitro* studies using lower and perhaps more clinically relevant concentrations of these herbal ingredients, to further explore the possible value of liquorice or glycyrrhizin as hepatoprotective therapy during azathioprine treatment. A small scale placebo-controlled clinical trial involving adjunctive liquorice treatment in humans being prescribed azathioprine may also be useful.

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Apples Protective Against Alzheimers?

Neurodegenerative conditions such as Alzheimer's disease are known to be associated with increased oxidative stress, and dietary intake of large levels of fruits and vegetables with antioxidant properties may partially compensate for these effects and therefore be associated with a reduced incidence of such conditions. Protective effects against the adverse influence of oxidative stress to acetylcholine (Ach) muscarinic receptor subtypes implicated in Alzheimers, have also been shown for a range of antioxidant-rich fruit extracts[1,2]. Epidemiological studies finding lower levels of Alzheimer's disease in vegetarian populations also support this view[3,4,5].

Neuroprotective effects of apple juice concentrate have previously been demonstrated in vitamin-deficient (folate and vitamin E-lacking) mice challenged with excess iron as a pro-oxidant[6], a regimen shown to induce impaired cognitive performance[7]. Subsequent work by the same American team of researchers found apple juice concentrate to have similar neuroprotective effects in normal, aged mice, whose performance was somewhat impaired by this deficient diet[8].

A further paper recently published by the Massachusetts University researchers, funded by the United States Apple Association and the Apple Products Research and Education Council, has further explored these neuroprotective effects of apple juice concentrate[9]. This study measured levels of the neurotransmitter ACh in the cortex and hippocampus of the brains of mice who had been fed on the folate and vitamin E-deficient but high iron diet, or normal diet. Cholinergic neuron activity and ACh levels decline with normal aging and Alzheimer's disease, and enhancement of ACh levels in the central nervous system is the mechanism of action of a number of drugs developed as treatments for Alzheimers disease, such as tacrine and donepezil.

The type of apple used to prepare the 70 brix apple juice concentrate was unfortunately unspecified, but had a high 70% brix level and was stored frozen until used. This was administered *ad libitum* at a final concentration of 0.5% v/v as the sole source of drinking water, a concentration previously shown to provide maximal neuroprotection to mice.

As expected, ACh levels in mice fed the deficient diet were shown to be less than those fed a normal diet, as they were in aged compared with younger mice. In mice given apple juice concentrate *ad libitum* for a period of one month however, this decline in ACh levels was prevented. Effects were most marked in aged mice with resulting brain ACh levels being higher than in aged mice fed a complete diet, but improvement in ACh levels was also measured in normal mice following apple juice concentrate ingestion.

Screening of plants for possible activity as inhibitors of the ACh metabolising enzyme cholinesterase, has found herbs such as Sage and Lemon balm to have such properties, and these have been linked to cognitive enhancing and possible protective effects against Alzheimer's disease. Such effects have also been reported using these herbs in clinical studies[10,11,12,13]. These latest American studies suggest that daily consumption of a readily available fruit, and one which perhaps has been somewhat under-rated during recent years, could have similar benefits in preventing what is set to become a major health epidemic during coming decades.

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Anti-cancer Properties of Elecampane

Elecampane (*Inula helenium*) is a popular herb used by Western phytotherapists, mainly for its benefits in the treatment of coughs and a range of other lung conditions. Originally native to Southeast Europe but introduced to Central Europe, parts of Asia and the USA, Elecampane root preparations have been used by various traditions as part of the management of upper respiratory tract infections, bronchitis, asthma and tuberculosis[1,2,3]. Gastrointestinal conditions such as indigestion, chronic enterogastritis and helminth infections have also been treated with this herb, and one of its principle sesquiterpene constituents, alantolactone, has strong anthelmintic and antibacterial activities, including activity against *Mycobacterium tuberculosis*[3].

Screening programmes involving the testing of plants for anti-cancer activities have lead to the discovery of a number of revolutionary new chemotherapeutic agents in recent decades, including taxol (paclitaxel) and docetaxel from the Pacific and Yew trees, and vinca alkaloids (vincristine and vinblastine) from the periwinkle plant, *Vinca rosea*.

As part of such a screening of various plants for antiproliferative activity, Japanese researchers reported in 2002 that a methanolic extract of Elecampane root from Tibet had significant activity against three tumour cell lines, namely human uterus and gastric adenocarcinoma, and murine melanoma[4]. The water extract of this herb however exhibited no activity. Several sesquiterpene lactones were identified as contributing to antiproliferative activity.

A separate team of researchers in Taiwan have also recently reported activity against human melanoma cells for isocostunolide, one of the sesquiterpene lactones found in Elecampane root[16]. Cytotoxic activity of compounds extracted from the aerial parts of a related species *Inula verbascifolia*, has also been reported by Greek workers[5], while antitumour sesquiterpene lactones have been identified in three other related Asian species, *Inula Britannica*[6,7,8], *Inula hupehensis*[9], and *Helenium microcephalum*[10,11].

Researchers based in New York and Germany have recently investigated the possible cytotoxic properties of Elecampane further, by studying the effects of an extract of dried Elecampane root on four different human carcinoma cell lines[12]. This *in vitro* study involved incubating various amounts of the Elecampane extract (a '1 in 3' strength extract prepared using acetone and methanol as solvents), with tumour cell suspensions over a 24-hour period, then measuring cell viability. Both positive and negative controls were used, and each experiment was repeated twice. As well as the human cancer cell lines used (colon, breast, pancreatic and astrocytoma), normal peripheral blood mononuclear cells taken from healthy donors, were incubated with the Elecampane extract in different concentrations.

All four different human carcinoma cell lines (HT-29, MCF-7, Capan-2 and G1) had their growth inhibited by the Elecampane extract in a concentration-dependent manner. The measured LD50 (lethal dose to 50% of cells) concentrations of extract in the four cancer cell lines ranged from a low 0.015 to 0.020 microlitre of extract, whereas concentrations required to inhibit the growth of healthy human peripheral blood lymphocytes were more than 100-fold higher. These results reveal a highly selective toxicity towards the four different tumour cell lines, but a much lower toxicity against normal cells.

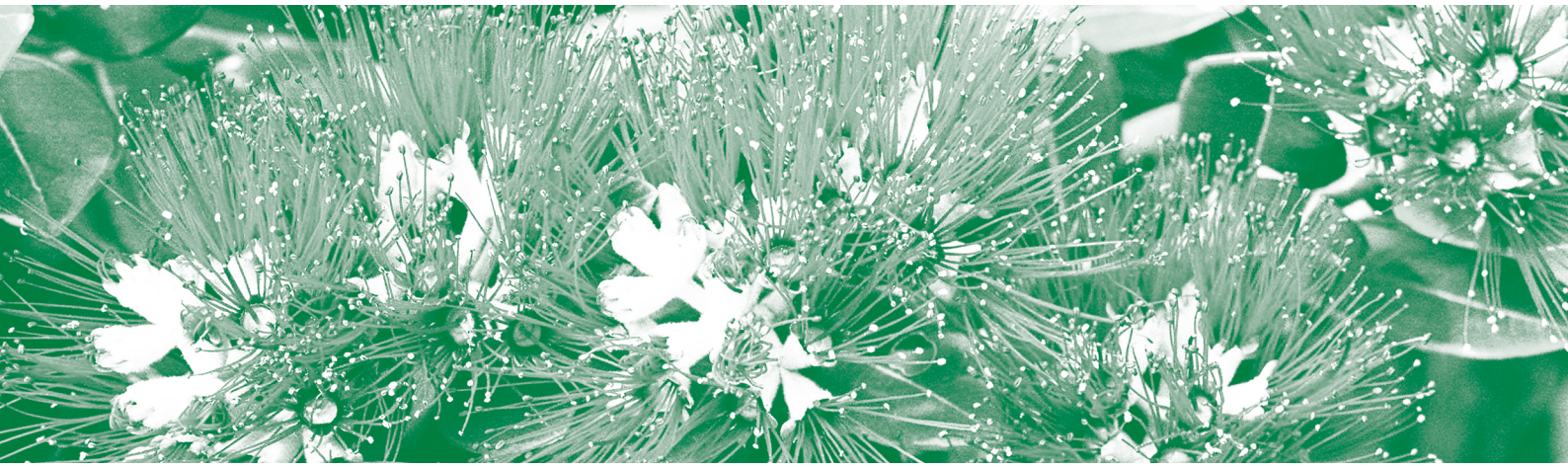
As an additional investigation into the cytotoxic properties of Elecampane, it was tested for possible mutagenicity in the Ames test. In these experiments, none of the Elecampane extracts used in the cytotoxicity studies were found to be mutagenic, thus further showing the selectivity of cytotoxic effects for cancerous rather than normal cells.

During these experiments, the course of cell death was monitored using various physiological and morphological parameters, including extensive electron microscope studies. These indicated that programmed cell death took place in all cases through a necrotic reaction in the cancer cells, in which the mitochondria adopted pathological features, lysosomal activity increased, and cellular disintegration occurred.

This appeared rather different to the usual mechanism of action through chemical or phytochemical treatment of tumour cells, which generally involves induction of

Continued page 12.

Anti-cancer Properties of Elecampane



Continued from page 11.

apoptosis (programmed cell death involving a complex network of biochemical pathways, that normally ensure a homoeostatic balance between cellular proliferation and turnover in nearly all tissues).

A similar screening programme involving Russian plants traditionally used either in anti-cancer preparations or as adaptogens, has also recently identified Elecampane as having particular promise as an anti-cancer agent[13]. Activity comparable to that of the chemotherapy drug fluorouracil against human lymphoblastoid Raji cells, was found for five out of the 61 plant species tested, including *Inula helenium*. Subsequent purification of the Elecampane extract found it to have greater cytotoxic activity than fluorouracil and cyclophosphamide, two widely used chemotherapy drugs.

In conclusion, the results from these various recent laboratory studies are highly promising to date. While further investigations are required, it would appear that indications for the use of Elecampane root could perhaps be extended to include potential benefits in the prevention or treatment of a number of different types of cancer in humans.

Over the past three years, Russian scientists have demonstrated several protective effects against stress by preparations of Elecampane [14,15]. Possible adaptogenic activity, likely to be attributable at least in part to the antioxidant activity of this plant, is therefore a further area where further research will hopefully provide useful findings.

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