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Avian Influenza Update

The risk of a global pandemic of avian influenza (H5N1, or "bird flu") has become hot news over recent months, and the discovery of the virus in poultry in Turkey, Romania and Greece in mid-October, has raised its profile in Europe. It is likely that wild birds migrating from the Ural mountains in Russia, on route to Africa, brought the virus from Asia to Europe.

While avian influenza is highly contagious among birds, it has yet to develop the ability to easily transmit from person to person. It has however crossed the species barrier in Asia, where it has caused at least 60 known human deaths to date in Vietnam, Thailand and Indonesia. Of these nearly all had a history of direct contact with poultry, although some evidence of limited, non-sustained human-to-human transmission has appeared (see *Phytonews 21*[1]). With its rapid evolution from a once benign virus found in birds to a highly pathogenic one however, development of a human-to-human transmission vector seems likely. Historical patterns also suggest the next major influenza pandemic is well overdue[2].

In the wake of this development, bird flu has been described as "public health enemy number one", and just as many infectious disease experts have been saying for many months, public health officials are now warning that a bird flu pandemic is inevitable.

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Avian Influenza Update

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Nurses, GP's and pharmacists in most countries are currently being trained to prepare for a massive influx of patients and increased demands on health services once an outbreak occurs. In New Zealand alone, up to 300 people across the health sector and other government departments and agencies are currently working on preparing the country for an avian influenza outbreak[3]. Businesses are also being urged to make contingency plans to cope with a reduced workforce if such a pandemic occurs.

Despite these preparations however, the message coming across loud and clear is that should an outbreak of avian influenza in humans occur, people would have to largely look after themselves and each other. The anticipated sudden surge in demands for finite health service resources, combined with limited supplies of efficacious antiviral drugs, would quite simply result in health services being unable to cope with the large numbers of people likely to be affected.

The neuraminidase inhibitor antiviral drugs oseltamivir (Tamiflu®) and zanamivir (Relenza®) could help to contain the virus and reduce illness and death[4,5], but would need to reach the site(s) of any pandemic outbreak quickly and in sufficient quantities, in order to be effective. With use of oseltamivir for influenza being common in Japan and the first cases of resistance to it being reported recently[7,8], not to mention the fact that the virus itself is still evolving rapidly, the rationale for building up national stores of this expensive drug has not gone uncriticised[9].

While most wealthier countries are currently building up their stockpiles of this and other antiviral drugs, poor countries simply cannot afford to do this. The World Health Organisation is currently trying to raise US$260 million from the international community to adequately fight the disease in Cambodia, Indonesia, Laos and Vietnam, countries regarded by most experts as forming the most likely epicentre of a human epidemic. At the time of writing however, only approximately US$20 million of this had been committed, and with the recent outbreak in Europe, concerns are mounting that money will now be diverted there instead.

Meanwhile, as the migratory route of the birds which have brought the disease to Europe ends in East Africa, it is probable the disease will arrive there. The relatively underdeveloped East African countries of Kenya, Tanzania, Uganda, Ethiopia and Somalia are presently totally unprepared to cope with the arrival of the virus, and an appropriate early response and early detection such as seen in Turkey or Greece, would be very difficult to implement. Poor African countries are also highly unlikely to be able to afford to stockpile oseltamivir, making the risks of an outbreak there cause for grave concern[10].

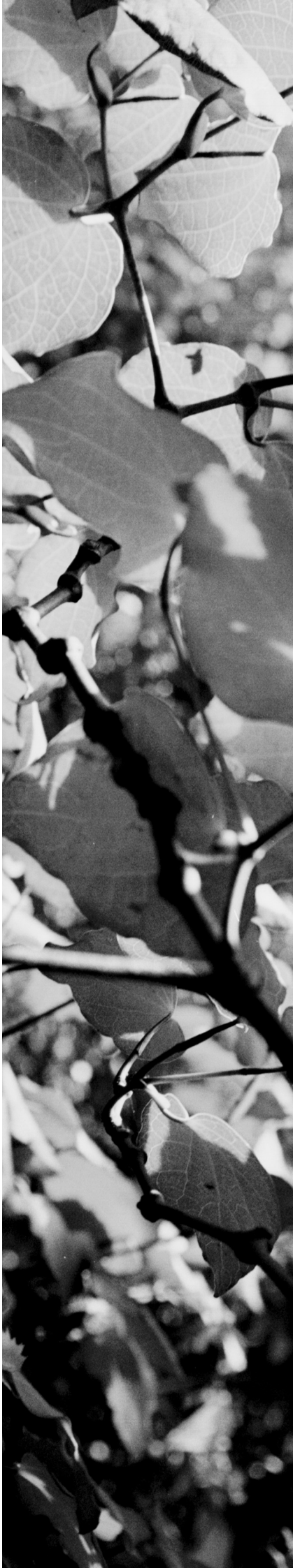
Further bad news was reported recently through the discovery by U.S. researchers that only massive doses of a H5N1 vaccine will provide definite protection against the disease[11]. Due to the evolving nature of the H5N1 virus and its frequent mutation to new genotypes, it would also be necessary to wait until it actually appears in humans before growing the pandemic strain into an effective vaccine[12]. While research towards vaccine development continues, it now seems doubtful that there will be enough time to produce sufficient quantities of an efficacious vaccine, to prevent a global pandemic from occurring.

With all this bad news and a pandemic on the way sooner or later, identification of other possible methods to help prevent its spread or lessen its impact in individuals and whole populations, need urgent attention.

Prior to the introduction of chemical antimicrobial agents and antibiotics, herbal preparations were the principle methods used to prevent and treat infectious diseases in humans. Common sense as well as an objective appraisal of ethnopharmacology and the use of antimicrobial herbal medicines by many traditional communities, suggests a possible contribution of phytotherapy to managing the risks of avian influenza in humans.

H5N1 virulence and immune response

The H5N1 virus enters the body through mucous membranes in the throat, nose or eyes, then takes up residence in the



respiratory tract and begins to replicate. In general, it does not seem to move outside of the respiratory tract, although the high frequency of diarrhoea and detection of viral RNA in faecal samples, suggests gastrointestinal tract involvement. Some evidence of CNS replication has also been documented[13].

Avian influenza has killed just over half the 120 or so people it has infected to date in south-east Asia, making it much more virulent than other influenza viruses. The 1918-1920 influenza virus, also thought to have originated from an avian source, became increasingly virulent over time, and is estimated to have killed around 40 to 50 million people[2,14,15].

While still in their infancy, studies to better understand the molecular mechanisms underlying the pathogenesis and strongly virulent nature of H5N1 infections are beginning to produce some interesting findings.

Evidence from these studies suggests that the severity of H5N1 influenza virus-induced disease in humans, could correlate with the ability of virulent strains to induce proinflammatory cytokines in macrophages[16,17]. Acute infection with a highly virulent H5N1 strain seems to produce an excessive immune response of cytokines, including induction of high levels of tumor necrosis factor alpha (TNFα) and other pro-inflammatory cytokines such as macrophage inflammatory proteins, interleukins 1ß, 6,12 and 18, & granulocyte colony stimulating factor, in monocyte-derived macrophages[16,17,18] . The virus also seems to have a mechanism that dampens other immune responses, such as decreasing levels of the anti-inflammatory cytokine IL-10.

This results in a type of inflammatory cascade known sometimes as a 'cytokine storm' or cytokine dysregulation whereby the release of inflammatory cytokines is unrestrained by normal feedback mechanisms. Upregulation of genes involved in apoptosis, tissue injury, and oxidative damage, are additional potentially damaging effects observed for recombinant virulent H5N1 viruses[19].

The combined effect of these processes is the promotion of granulocyte infiltration into the lungs, resulting in acute lung injury and an unusually severe disease. This type of immune response is implicated by several studies in animals as well as the clinical presentation of severe lung symptoms progressing to multiorgan failure often seen during acute H5N1 infection in human victims[16,18,19,20,21]. This shutdown of several different organs in the body, in conjunction with respiratory distress, can result in rapid death[20].

Such immune dysregulation following viral infection of the respiratory tract is known to occur for other highly pathogenic viruses[22,23]. The virulence of SARS, a coronavirus-associated severe acute respiratory syndrome which caused 774 deaths between July and September 2003, was also associated with persistence of lung inflammation and high levels of lung cytokines, due to delayed clearance of the aetiological coronavirus[23].

Enhancing resistance

Many unanswered questions remain with regard to how an efficacious immune response is promoted while at the same time sparing the host from excessive damage to the respiratory tract. As with all infectious diseases however, the level of pre-existing immunity to the microbial pathogen is a key factor known to influence the severity of an influenza pandemic or epidemic. By far the most important contributory factor in immunity to influenza viruses seem to be serum antibodies to the viral HA glycoproteins[24]. These are induced by prior infection or vaccination, and impart a strong and disease specific host resistance to the virus.

With the likelihood of an effective vaccine against a pandemic strain becomming available in time being remote, other possible methods of enhancing the immune system's ability to either ward off or successfully resist H5N1 infection, should be considered.

Echinacea:
Echinacea has been shown in animal studies to impart protection against mortality from various viruses[25,26], and several clinical trials have shown beneficial effects of Echinacea during the treatment of colds and influenza[27,28,29,30,31].

Increased numbers of circulating white blood cells, monocytes, neutrophils and natural killer (NK) cells, and the phagocytotic abilities of these, are the principle immunological changes associated with Echinacea root usage. These effects are all a reflection of enhancement of the non-specific immune response, whereby the body's ability to maintain immunosurveillance against a variety of potential viral or bacterial pathogens or spontaneous-developing tumours, is increased. This aspect of the immune response is quite different to that of the specific immune system as provoked by vaccination, whereby production of specific disease-related antibodies and a subsequent immune response occurs.

Certain of the above effects of Echinacea may at first seem counterproductive during the acute stage of an established H5N1 infection, in which much of the immune system could well be over-activated as described earlier. While activation of innate immune mechanisms such as NK cell activity is useful in the early immune response to most types of influenza[32,33], excessive enhancement of NK cell activity during the acute stage of a H5N1 infection, could perhaps be associated with a theoretical worsening of lung function[34].

At this point however, it is perhaps important to recognise that Echinacea root exhibits pharmacological actions better summarised as being immunomodulatory and anti-inflammatory rather than simply immunostimulant. Its use by experienced practitioners for autoimmune conditions such as asthma and eczema, is also due to its perceived actions as a modulator or regulator of a poorly functioning overall immune system. Additionally, while activation of several inflammatory as well as anti-inflammatory cytokines has been shown for Echinacea both *in vitro* and *in vivo*, the specific actions and potencies in this regard vary depending on the type of Echinacea product concerned.

Anti-inflammatory effects of Echinacea root are well established from both its traditional use to treat snake bites and major abscesses, as well as modern

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pharmacological studies[35,36,37,38]. Recent studies have also shown that Echinacea alkamides produce a dual modulatory rather than simple stimulant effect on TNF-a expression in humans[39]. These and other effects of Echinacea on gene expressions indicate a broad spectrum anti-inflammatory and immunomodulatory response[40], including reduced expression of IL-1ß, IL-8 and TNF-α. Such effects could be helpful during the acute stages of H5N1 infection.

While speculative, modulation of TNF-α and other cytokine expression by Echinacea could therefore be useful in a situation of H5N1 infection during which TNF-α and cytokine production in general, is dysregulated. In addition to these anti-inflammatory and immunomodulatory effects, antioxidant actions by Echinacea could also be helpful during this situation[41].

However, while adequate doses of a good quality Echinacea preparation have been shown to enhance the capability of the body's immune system to combat existing upper respiratory tract infections, whether such effects would occur to help hasten elimination of a virulent H5N1 infection, remain unknown.

The question also arises as to whether Echinacea is best used as a possible preventative agent to optimise immune defences ready for when an avian influenza pandemic occurs, rather than a treatment when an individual infection has occurred. Optimising immunosurveillance and activity of the non-specific immune system against such a virulent pathogen, while less likely to ensure complete protection than appropriate vaccination, may prevent such a virulent and life-threatening response to its presence.

Other phytomedicines:

Shikimic acid is a phenolic acid compound used as a key starting material in the manufacture of oseltamivir, and found in many plants including Chinese Star Anise *(Illicium verum)*[42]. While this and other shikimic acid rich plants have been increasingly sought after since fears of a pandemic escalated, little evidence exists that shikimic acid itself or plants containing it, could be useful.

American ginseng (*Panax quinqefolium*) root has a protective effect against winter influenza in institutionalised older adults (see *Phytonews 20*[43]). More recently, a preventative effect against winter colds has been reported following daily use for 4 months, in a Canadian study involving 323 subjects aged 18-65 years of age[44]. A higher immune response from influenza vaccination, has also been produced by concurrent administration of the closely related Panax ginseng[45].

The popular Chinese herb ***Astragalus membranaceous***, traditionally used to help manage viral infections such as the common cold, has also shown some evidence of immune stimulation and antiviral effects[46,47,48].

***Elderflower* (*Sambucus nigra*)** is generally used for its diaphoretic and decongestant properties, particularly during hay fever as well as catarrh and fever associated with the common cold or influenza. A clinical trial has found elderberry to have *in vitro* antiviral effects and reduce the duration and severity of influenza symptoms[49], and possible immunostimulant properties have been reported[50].

The root of **Ginger (*Zingiber officinale*)** has immunomodulatory[51] and antiviral properties[52,53,54], which along with its anti-inflammatory actions, may be useful. The fungus **Reishi (*Ganoderma lucidum*)**, has established immunomodulatory effects and enhances innate immunity by activating NF-kappaB[55].

Olive leaf (*Olea europaea*) has become popular over recent years as an alleged treatment for winter influenza, although little published research has appeared until recently[56].

Propolis, the resinous material manufactured by bees from plants and rich in flavonoids, shows *in vitro* activity against various viruses[57,58], and has proven immunomodulatory and anti-inflammatory effects including down-regulation of inflammatory cytokine production[59,60].

Various plants contain compounds which act as neuraminidase inhibitors in vitro in the same manner as oseltamivir. The best studied of these is 5,7,4"-trihydroxy-8-methoxyflavone[61,62], a flavone closely related to baicalin and baicalein and found in roots of the popular Chinese herb **Baical Skullcap (*Scutellaria baicalensis*)**, as well as aerial parts of the well-known European Skullcap (*Scutellaria lateriflora*).

A study just reported using a Chinese formula containing a large amount of baicalin in a pre-clinical animal model of endotoxin-induced lung injury, found a marked reduction in elevated plasma levels and the expressions of several inflammatory cytokines, in lung tissues. The formulation itself (San-Huang-Xie-Xin-Tang), as well as baicalin alone, also reduced plasma concentrations of IL-1ß, TNF-α, and expressions of other cytokines associated with lung injury and lethality[63].

This study as well as another finding *in vitro* antiviral activity against SARS coronavirus for baicalin[64], provides strong support for a potential role for baicalin-containing phytomedicines in the treatment of acute H5N1 infection. The well-established anti-inflammatory, antioxidant and antimicrobial activities of Baical Skullcap[65], would seem to make further research on this phytomedicine in particular, highly justified.

Other neuraminidase inhibitors include resveratrol (found in red grapes) and emodin derivatives, anthraquinone compounds found in Aloe vera and a number of other laxative phytomedicines[66]. The strong laxative effects of these would probably be major limitations to their clinical usefulness at this stage. As for all such compounds, considerations of bioavailability and dosage of each of these herbal medicines that would be required for significant activities need to be made.

With excessive inflammation in the lungs being increasingly related to the virulence of H5N1, treatment of infected patients with anti-inflammatory drugs or herbal medicines may in some cases be useful. However while the use of corticosteroids together with antiviral medication has been reported to confer clinical benefits in some cases of SARS[23], clinical trials are lacking and such steroid therapy has been linked with residual lung damage following treatment. There nevertheless remains a theoretical potential role for anti-inflammatory phytomedicines or phytochemicals such as curcumin, a key constituent of the cheap spice Turmeric, which is a significant inhibitor of TNF and could help reduce the adverse effects of excessive cytokine release.

While in all cases it is at this stage conjectural as to whether these or other various natural treatments would be

helpful during an H5N1 pandemic, the seriousness of the situation now arising warrants a systematic evaluation of these and other herbal medicines as possible alternative or adjunctive treatments to anti-viral drug or vaccine therapy.

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Echinacea root prolongs longevity?

Some very interesting and potentially hugely significant research involving an Echinacea root preparation which did not make headlines in the popular press when published, has recently come out of Canada[1].

Despite its popularity, debate continues on whether Echinacea is most efficacious when taken intermittently, continuously, or only at the start of an infection. Researchers in Montreal set out to investigate the effects of continuous Echinacea ingestion on the life expectancy and immune parameters of normal mice. They administered a daily dose of 2mg of Echinacea root contained within the diet of a group of mice from puberty at 7 weeks of age until late middle-age, when the mice were just beyond 13 months old. A control group of mice which were identically housed and maintained, received the same diet but lacking in Echinacea root.

Mice who consumed the untreated diet had a 79% survival rate at 10 months of age, while 100% of those consuming Echinacea daily were still alive at this age. At approximately 13 months of age only 46% of the untreated mice were still alive, while the survival rate of those consuming Echinacea was 74%.

These outcomes were accompanied by a significant elevation in levels of natural killer (NK) cells in both the bone marrow and spleen. Natural killer cells are thought to be the key immune cells acting as the first line of defense against developing neoplasms in mice and humans. An elevated level of NK cells is thought to be a prime element in immunosurveillance against spontaneous tumour development, something which increases in frequency with progressing aging in both mice and humans.

This study clearly has hugely exciting implications for use of Echinacea root, and strongly indicates the possibility of a similar benefit to prolong the life expectancy of humans who consume this herbal medicine in sufficient doses on a daily basis. To design and conduct a robust clinical trial to test this possibility on humans would however be very difficult and take many years, making the results from this non harmful study using animals known to be 85% identical to humans from a genetic perspective, of great significance. The dosage of 2mg per day used in this study on mice translates to approximately 8000mg (8grams) per day in an average adult human.

This is not the first study to find that Echinacea can prolong the life span of mice, with two earlier studies finding that mice with leukaemia also benefited from an improved survival rate accompanied by enhancement of NK cell activity, when given regular Echinacea root extract[2,3].

It also provides support for long term regular usage of Echinacea root, as a chemopreventative and longevity-promoting agent.

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More negative press on Echinacea

Negative findings from yet another poorly designed clinical trial involving an Echinacea preparation received widespread publicity around the world during late July. As often occurs when trials involving popular herbal products fail to find efficacy, the media had a field day as a result, and numerous news items appeared claiming that as a result of this study, "Echinacea doesn't work" as an immunostimulant and anti-influenza treatment.

As with several similar studies previously reported *(Phytonews 15 & 18*[1,2]*)*, I therefore find myself once again defending the therapeutic validity of using a medicinal herb about which much nonsense has been written over recent years, much in the name of supposedly good science.

The study concerned, whose findings were published in the July 28th issue of the *New England Journal of Medicine*[3], involved an evaluation of the efficacy of three different tincture preparations made using *Echinacea angustifolia* root, against an experimental type of rhinovirus infection. All of the 437 participants were inoculated with infectious doses of the virus then isolated in individual hotel rooms for the remainder of the study. Participants received either a prophylactic treatment consisting of an Echinacea tincture beginning seven days before the virus challenge, or treatment beginning at the time of the challenge, with one of the three different preparations or placebo. They were then monitored for five days, and the rates of infection and severity of symptoms measured. Nasal secretion volumes, as well as polymorphonuclear leukocyte and interleukin-8 concentrations in nasal lavage specimens, were also quantified.

The three preparations involved all consisted of '1 in 5' strength liquid *Echinacea angustifolia* root extracts manufactured using different methods, namely supercritical carbon dioxide extraction, 60% ethanol, or 20% ethanol. A daily dosage of 1.5mls was taken by each participant. No statistically significant effects of the three Echinacea extracts on rates of infection or severity of symptoms, as well as the nasal secretion measurements, were found.

To experienced medical herbalists and well-informed clinicians, these results are no surprise when the dosage given to each participant is considered. This amounted to a total daily dosage of the equivalent of 900mg of Echinacea angustifolia root given in three doses of 300mg each, although the authors fail to document whether this was based upon dried or fresh herb weight. This dose is some five to tenfold less, than that normally recommended by phytotherapists in New Zealand and Australia.

Like all medicinal substances, the need for adequate Echinacea doses in order to achieve efficacy is implicated by the earliest published data on its traditional use by North American Indians, and subsequently the Eclectic physicians, who first brought Echinacea to Europe during the 19th century. These early practitioners prescribed massive dosages of up to half an ounce (i.e. 14 grams) of Echinacea root (which is higher in active alkamides than aerial parts) every two to three hours during the acute stages of severe infections such as tetanus, diphtheria and meningitis[4,5,6].

As a prophylactic against influenza and upper respiratory tract infections, Australasian medical herbalists generally recommend a dosage equivalent to 2500 to 3000mg dried Echinacea root, extracted using ethanol or similar solvents which produce a high alkamide-containing preparation. This dosage recommendation is generally increased when the person is in a high risk situation or infection has occurred such as that which forthcoming inoculation with a rhinovirus would entail. If such an infection is already in existence, daily dosages of 5,000 to 10,000mg well-extracted Echinacea root are normally recommended.

This is the third clinical trial involving subtherapeutic doses or weak extracts of Echinacea to receive prominent media coverage in the past three years[7,8], and one must ask how such fundamental considerations of dosage are overlooked or given such poor attention when designing a clinical trial in today's world. Clinical trials involving 10 to 20% of the recommended dosage of any medicinal agent, are obviously doomed to failure. The phlethora of poor quality and low strength Echinacea preparations that have been marketed heavily to consumers over the past twenty years, compounded by the virtual disappearance of ethnopharmacological knowledge and understanding about how this phytomedicine was used in its traditional setting by American Indians, are also factors which undoubtedly help to foster a continuing failure to address the key issue of Echinacea dosage.

Another minor contributing factor in this trial may have been poor blinding due to the difficulty in masking the distinctive taste of alkamide-containing Echinacea extracts. This is evident from the fact that the proportion of participants who believed they were taking the active medication during the treatment phase of the study was 41 to 50% in the active-treatment groups, while only 36% in the placebo group believed they were receiving the active medication.

In an editorial of the same issue of the *New England Journal of Medicine*, a damning attack appeared on the rationale of clinical trials to investigate "implausible remedies", and criticism of the U.S. National Centre for Complementary and Alternative Medicine (NCCAM) who contributed funding to this and other trials as part of a programme to investigate a number of popular herbal medicines[9].

This whole debacle has potentially dire consequences as consumer usage of Echinacea will in all likelihood be dampened by the enormous adverse publicity generated by this study. Meanwhile, widespread overprescribing of antibiotics continues against a background of steadily increasing mutation of microbes towards new potentially devastating, highly pathogenic infectious organisms.

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Effects of ginkgo on platelet function in diabetes

Ginkgo biloba is primarily known for its benefits on mental function and the cardiovascular system, but recent research has revealed several new potential applications for this phytomedicine .

Vascular disease and secondary conditions such as retinopathy and glomerulonephropathy are common complications of long term diabetes. As these are associated with small blood vessel occlusions and often platelet hyper-reactivity, investigations into the potential effects of ginkgo on platelet function in diabetes mellitus are warranted.

Reduced platelet aggregation has previously been observed following three months ginkgo ingestion by both non-diabetic and type 2 diabetic subjects[1]. While antagonism of platelet activating factor (PAF) has been widely reported as contributing to these anti-platelet effects, the clinical significance of this as a mechanism of anti-platelet activity for ginkgo has recently been challenged[2,3]. No effects on PAF-mediated platelet aggregation were measured during this study, and other mechanisms of antiplatelet action were implicated. These include a nonselective inhibition of cyclooxygenase-1 (COX-1) mediated thromboxane a(2) in platelets and COX-2-mediated prostaglandin PGI(2) production by the endothelial cells, and perhaps platelet-enriched levels of arachidonic acid or COX-1 activity, or both[1].

Further findings by the same investigators into the effects of ginkgo on platelet function in diabetes were published in the April issue of *Diabetes Research in Clinical Practice*[4]. This reported a reduction in platelet malondialdehyde-thiobarbituric acid reacting substances (TBARS) in type 2 diabetic patients with both normal or high cholesterol levels, indicating reduced platelet hyperactivity as well as antioxidant activity. These effects were not associated with a change in platelet counts, but rather inhibition of cyclooxygenase (COX)-1 catalysed arachidonic acid oxygenation, or a reduction in the arachidonic acid pool by ginkgo flavonoids[4].

Taiwanese researchers have reported improvement in haemorrheological parameters following 3 months administration of ginkgo to 25 diabetes patients with retinopathy. These benefits included a significant reduction in blood viscosity and viscoelasticity, a reduction in erythrocyte membrane malondialdehyde (MDA) levels, and an increase in retinal capillary blood flow from 3.23 to 3.67 cm per minute[5].

Other reported effects of ginkgo with potential benefits in diabetes include possible hypoglycaemic activity by the flavonoid fraction[6], stimulation of pancreatic beta-cell function in type 2 diabetic subjects with pancreatic exhaustion[7], and protection against oxidative renal injury[8,9,10]. Preliminary clinical reports associating regular use of oral or parenteral ginkgo with possible protective effects against diabetic retinopathy and nephropathy, have also been made by Polish and Chinese researchers during the past 2 years[11,12].

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Ginkgo potentially protects against oral cancer

Many flavonoids are known to have antioxidant, anticarcinogenic and antimutagenic effects[1,2,]. These include ginkgo flavonoids such as quercetin, kaempferol and isorhamnetin, which exhibit suppressive activity against cyclooxygenase-2 (COX-2) enzyme promoter activity, and thus colon carcinogenesis[3]. Increased rates of apoptosis, inhibition of cell proliferation and angiogenesis, are also produced by flavonoids in cell cultures, and intestinal protective enzymes such as glutathione transferases have their activities enhanced by flavonoids *in vivo*[2].

Various studies conducted with molecular, cellular and animal models have implicated anticancer properties for Ginkgo biloba. These properties are likely to be related to antioxidant, anti-angiogenic, cyclooxygenase-2 and nitric oxide synthase (NOS) suppressive mechanisms, as well as modulation of gene expression[4]. Both flavonoid and terpenoid constituents appear to be involved in these activities.

Chemopreventive effects of ginkgo against digestive tract carcinogenesis, and protection against adverse effects of the chemotherapy drug doxorubicin, have been reported[5]. A clinical study involving combined parenteral ginkgo treatment with the chemotherapy drug 5-fluorouracil in patients with advanced colorectal cancer, found a good benefit-risk ratio of the combined treatment as a second line treatment in this condition[6].

Treatment of human hepatocellular carcinoma cell lines with ginkgo has also been found to produce a marked inhibitory effect on their cell proliferation and increased cytotoxic effects[7]. Anti-angiogenic activity following ginkgo use has also been reported[8].

Korean researchers based at Yonsei University College of Medicine in Seoul, have now investigated the effects of ginkgo on oral cancer cells[9]. Human SCC 1483 cell lines were treated with various doses of ginkgo extract for 24 hours, and apoptosis measured by flow cytometry. A dose dependent increase in apoptosis and inhibition of cell proliferation occurred, with cancer cell proliferation being inhibited by more than 50% at a concentration of 250mg/ml ginkgo extract.

Oral cavity cancer has a tendency to produce a second primary tumour following successful treatment of an initial lesion, and this is a major cause of death in such patients. The use of chemopreventive agents such as ginkgo is therefore a potentially useful approach which may prevent the development of second primary cancers in oral cancer patients.

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Acute versus chronic ginkgo administration in healthy volunteers

While ginkgo is best known for its beneficial effects in clinical and, to a lesser extent, healthy ageing populations, evidence has emerged over recent years of improved cognition in younger populations[1,2].

Two separate studies initially reported improvement in reaction times in short-term memory tests in healthy young participants, following administration of larger than usual (600mg) doses of ginkgo extract[3,4] . In both of these studies, improvement occurred one hour following ginkgo administration. These possible effects were investigated more systematically by studies conducted at the Human Cognitive Neuroscience Unit at the University of Northumbria in 2000[5]. Four primary cognitive 'factors', recognised as useful measurements of cognitive function changes and corresponding to speed of attention, accuracy of attention, speed of memory and quality of memory, were assessed in a group of twenty student volunteers. A placebo-controlled, multi-dose, double-blind crossover design was utilised, and single doses of 120mg, 240mg and 360mg of a standardised extract of ginkgo or placebo given.

Measurement of cognitive performance at regular intervals for the next 6 hours, found it to be enhanced by acute ginkgo administration. In particular, the speed of performance on tasks assessing attention was improved, in a dose and time dependent manner. Significant improvements were seen only for the two highest doses, between 2.5 and 6 hours following ingestion. The only other cognitive factor observed to be affected was the 'quality of memory', for which performance was enhanced for the lowest dose (120mg), at 1 and 4 hours following ingestion[5].

Three further studies have investigated the effects of acute/subchronic doses of ginkgo in university students. Administration of a single dose of each of the above amounts of ginkgo was found to improve performance on serial subtraction tasks[6]. The higher 360mg dose also improved performance on a factor of 'secondary memory', and, to a lesser extent, on a factor of attention[7].

Whether similar effects are produced following chronic rather than acute treatment in healthy young persons however, has been little investigated to date. A trial conducted in 2001 where male college students were treated with 120mg ginkgo extract for only 5 days showed little effects[8]. In another trial however, improvement in working memory and memory consolidation was reported in participants aged 18 to 40 years, following treatment with the same dose over a 30 day period[9].

Most recently, a study was conducted by researchers at the Psychopharmacology Research Unit and the School of Pharmacy at Kings College in London, to compare the effects of ginkgo after acute and chronic treatment on tests of attention, memory and executive function or planning ability, in healthy university students[10].

Two experiments were conducted, the first in which 52 students were randomly allocated to receive a single dose of ginkgo (120mg) or placebo, then tested 4 hours later. In the second experiment, forty students received either ginkgo 120mg or placebo each day for 6 weeks, before testing.

As with the earlier studies, acute administration of ginkgo was observed to improve performance in tests of attention and memory. Short-term visual recognition memory, and performance in complex attention tasks that involve working memory, were particularly affected. No effects at all however, were seen on the various memory and attention tests following six weeks of ginkgo treatment.

Possible reasons for this difference between effects after single dose or chronic treatment include the effect of practice on the tests, as the practice effect on certain tests undertaken was shown to be particularly marked despite the gap of six weeks between testing sessions. The other possible explanation is that tolerance may develop to these effects of ginkgo after chronic treatment in healthy young individuals. This is inferred also by the fact that other studies which have reported improvements in attention, short-term memory and mental flexibility after six weeks treatment, are limited to older aged individuals with a more impaired performance[11].

This study implicates that while acute dosing with ginkgo (120mg) improves sustained attention and episodic memory in healthy young volunteers, these effects were not apparent after six weeks of regular treatment. While factors such as the dosage used and potential influences of the practice effect for some tests may have influenced the results, based upon current information, ginkgo is probably best used on an occasional or 'as required' basis by young healthy students in situations such as sitting exams or cramming sessions, rather than being taken every day. Recommendations for older people whose cognitive functions are less robust than 20 year old students however, remain in favour of more regular, chronic treatment.

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Influences of Ginseng, Ginkgo and Garlic on cytochrome P450 phenotypes in the elderly

Elderly patients are more likely to take regular herbal medicines in conjunction with prescription drugs, and evaluation of the potential for adverse herb-drug interactions in the elderly is important. With modulation of cytochrome P450 (CYP) activity being a principle mechanism of interaction between drugs and phytomedicines, tests to determine whether long term use of popular herbs by the elderly can affect CYP activity, are warranted.

In a recent American study[1], twelve healthy volunteers between the ages of 60 and 76 years, were each given a 28 day course of St John's wort, garlic oil, Panax ginseng or Ginkgo biloba, one at a time, followed by a 30 day washout period. Single doses of various probe drugs known to be metabolised by particular CYP isoforms (midazolam, caffeine, chlorzoxazone and debrisoquine) were then given at the end of each herbal treatment period, and the rate and degree of their metabolism, as measured by urinary and serum levels of particular metabolites, was measured. Levels of particular active or marker phytochemicals in each phytomedicine were also determined.

As expected, significant induction of CYP3A4 and CYP2E1 activity occurred during 28 days treatment with St Johns wort, with these enzyme isoforms being increased by approximately 140% and 28% respectively. While garlic oil inhibited CYP2E1 by approximately 22%, and Panax ginseng inhibited CYP2D6 by approximately 7%, the latter did not appear to be clinically relevant. No influence of Ginkgo on CYP isoforms, was measured.

These findings confirm those of a similar study involving younger subjects using the same phytomedicines, which found no effects for Panax ginseng or Ginkgo on CYP activity[2].

In conclusion, this study indicates that concurrent usage of Ginkgo or Panax ginseng with a wide range of other medications, is not likely to be associated with adverse interactions involving potentiation or inhibition in the metabolism, and thus changes in the plasma levels of these.

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Thyme and Baical Skullcap synergistic antibacterial effects with antibiotics

With bacterial resistance to antibiotics reaching alarming levels, researchers have been routinely screening a wide range of herbal medicines for the ability to potentiate antibacterial activities of various antibiotics. During one such screening programme to identify compounds capable of potentiating the antibacterial effects of antibiotics against methicillin-resistant Staphylococcus aureus (MRSA), an extract of Thyme leaves (*Thymus vulgaris*) was found to greatly reduce the minimum inhibitory concentration of the antibiotic tetracycline against MRSA[1]. Subsequent fractionation studies identified baicalein (5,6,7-trihydroxyflavone) as the most active compound involved in this potentiation of antibiotic activity.

Antibiotic resistance is largely due to the ability of bacteria to keep antibiotics out of their cells through an efflux pump mechanism. Because of this, the effect of baicalein on the efflux of tetracycline from Esherichia coli bacteria was investigated, and strong inhibition of transport of tetracycline efflux was observed. Tests involving MRSA strains that did not have this efflux pump ability, also showed a synergistic antibacterial effect for a combination of baicalein and tetracycline, indicating the presence of more than one mechanism of this action.

These *in vitro* studies, like others conducted using various other plant extracts over recent years, support the adjunctive use of herbal medicines (in this case leaves of Thyme and the root of Baical Skullcap (*Scutellaria baicalensis*), which also contains baicalein), as treatment agents for bacterial infections. These are likely to potentiate the clinical efficacy of antibiotic drugs against certain pathogens thus reducing the dosages of such drugs required, as well as help slow down the growing problem of bacterial resistance developing to more and more antibiotics.

The antibacterial and antifungal properties of Thyme are well documented[2,3]. Baical skullcap (Huang qin) is also widely used in the treatment of infectious diseases in China, and antimicrobial activity for both aqueous extracts[4], as well as the essential oil[5] of this herb, have been reported. An ethanolic extract of *Scutallaria baicalensis* has also been recently shown by a separate group of Chinese workers, to potentiate the antimicrobial activity of four different antibiotics against MRSA[6].

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Agrimony for Hepatitis B

Several investigations into potential new treatments for Hepatitis B virus infection have been undertaken over recent years, catalysed in part by the high cost, adverse effects and limited efficacy of conventional drug treatments[1, 2]. Inhibition of antigenic components such as hepatitis surface antigen (HBsAg) has been reported for a number of plants, including *Phyllanthus amarus*[2], *Plantago asiatica*[3] and *Terminalis chebula*[4].

Antiviral activity has been reported for Agrimonia species in the past[5], and Korean researchers recently investigated various species of the Agrimonia genus for potential inhibitory activity against HBsAg secretion by hepatitis B cells (HepG2.2.15) *in vitro*[6].

Significant inhibition of HBsAg release by several aqueous extracts of *Agrimonia eupatoria* (Agrimony) prepared using variable extraction temperatures was observed. This effect was found to be best for extracts prepared from plant material harvested during the flowering stages, and made using moderate rather than high temperatures. An agrimony extract extracted at 60 degrees had an EC50 (extract concentration required to produce 50% inhibition) of 74ug/ml. Inhibitory activity was very low in the aerial parts of agrimony collected 3 months after the flowering season.

This initial study indicates a potential role for Agrimony as a treatment or source of potential drug compounds against Hepatitis B, and shows the importance of harvesting and extraction considerations to optimise bioactivity from phytomedicines.

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