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White Horehound


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# Gastroprotective and other lesser known activities of White Horehound

*Marrubium vulgare* (White Horehound) is a herb originally found in Europe and northern Africa, but now naturalised in most continents of the world. Leaves of White Horehound are traditionally used in many countries for the treatment of inflammatory conditions, and are primarily regarded by western medical herbalists as being useful for infectious and congestive respiratory conditions. In the traditional medical systems of Latin countries such as Brazil, however, White Horehound is also used as a tea for the treatment of gastrointestinal ailments and headaches[1].

Peptic ulcer is a common health problem, usually caused by an imbalance between aggressive and protective factors in the stomach. These factors include acid secretion, *Helicobacter pylori* infection, mucus secretion, blood flow, cell regeneration and prostaglandins. Drug treatments are aimed at inhibiting acid production by the gastric wall, through mechanisms such as inhibiting the proton pump or histamine-2 receptors. However these show limited efficacy and adverse effects are reasonably common.

Based upon its traditional digestive system use, and the fact that the diterpene labdane marrubiin is prevalent in White Horehound and many terpenes exhibit protective effects on the digestive tract[2-4], a team of Brazilian researchers recently assessed this plant for possible gastroprotective activity[5].

Extract of White Horehound leaves and the diterpene marrubiin obtained from it, were tested for potential anti-ulcer activity in various protocols in mice. Gastroprotective activity was first assessed against ulcers induced by ethanol and indomethacin, common screening tests for anti-ulcer activity. The methanol extract of White Horehound given at doses of 50 and 100mg/kg, produced a significant reduction in the lesion index, total and percentage injured area when compared with the control group. Marrubiin also significantly reduced these parameters when given at a dose of 25mg/kg. These effects were similar to those produced by the anti-ulcer drug omeprazole, used in a dose of 30mg/kg as a positive control.

Anti-ulcer activity was also evaluated using the model of ulcers induced by non-steroidal antiinflammatory drugs (NSAIDs). In this model also, the White Horehound extract at doses of 25, 50 and 100mg/kg caused a dose dependent reduction in all parameters, as did the drug cimetidine used as a positive control at a dose of 100mg/kg.

An increase in gastric pH and decreased concentration of H+ ions was measured using both White Horehound extract or marrubiin in these experiments, suggesting interference with gastric acid secretion. Both substances also caused an increase in production of protective mucus from the gastric epithelium. Further investigations found influences of White Horehound and marubiin on endogenous nitric oxide activity and endogenous sulfhydryls, both involved in gastric mucosal protection against damaging agents.

In summary, a pronounced cytoprotective activity was shown for the White Horehound extract, this being correlated at least in part with the presence of marrubiin, and more than one mechanism of action.

Other reported beneficial properties of White Horehound relevant to the digestive system include hypoglycaemic, hypolipidaemic, and antispasmodic activities[6-8]. Antispasmodic, antinociceptive and analgesic effects have been reported for White Horehound and marrubiin in different experimental models, in some cases being more potent than some well-known analgesic drugs[2,9].

The finding that White Horehound exhibits antinociceptive and gastroprotective properties in addition to its anti-inflammatory ones is interesting, given that many anti-inflammatory drugs produce irritant rather than protective effects upon the gastrointestinal tract.

Antihepatotoxic activity of marrubicacid, a monoterpene acid constituent, has also been reported recently[10], as has activity against methicillin resistant *Staphylococcus aureus*[11] and other Gram positive pathogenic bacteria[12,13].

Inhibitory activity against digestive enzymes related to diabetes (alpha-amylase and alpha-glucosidase) has been shown for the Lebanese plant *Marrubium radiatum*,[14] and White Horehound is also used in Mexican traditional medicine for the control of type 2 diabetes. Ingestion of White Horehound tea for 21 days, however, had little effect on fasting blood glucose, cholesterol and triglyceride values of poorly controlled diabetic patients in a small clinical trial[8].

An inhibitory effect against microvascular leakage and the development of oedema has been reported through intraperitoneal administration of marrubiin in animal studies[15]. Potential hypotensive, calcium channel blocking and angiotensin converting enzyme (ACE) inhibitory activities have also been revealed in animal studies [16-19].

White Horehound leaves have also shown a high level of antioxidant activity[20]. Antioxidant and antigenotoxic properties, including inhibition of nitrofurantoin-induced mutagenicity, have also been recently reported for compounds derived from the botanically related *Marrubium deserti de Noe*, which also occur in *Marrubium vulgare*[21]. These effects extend to inhibition of LDL oxidation and reversal of cholesterol transport, and thus the possible prevention of cardiovascular disease development[22,23].

The diverse activities with which White Horehound is now attributed could make it useful to help prevent a variety of health ailments associated with the aging process, or living in the modern world. As an endemic and easily grown or noxious plant across several continents, such diverse pharmacological activities make it worthy of further human clinical trial evaluation.

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White Horehound

# Echinacea Improves Liver Health?

Medicinal plants have been used for treating liver disease for many centuries, and several phytomedicines and phytochemicals have been shown to have significant hepatoprotective activity, with minimal adverse effects.

Milk Thistle *(Silybum marianum)* was the first of these, with the flavonolignan complex known as silymarin having hepatoprotective, anti-inflammatory, antioxidant, and chemopreventative activities[1, 2]. Silymarin is now widely used in alcoholic liver diseases, liver cirrhosis, viral hepatitis, toxic and drug induced liver diseases, and as an everyday health supplement.

Mechanisms of hepatoprotective action generally appear to relate to strong antioxidant activities, including induction of antioxidant enzymes like superoxide dismutase, and reversal of reduced glutathione and catalase. Additional mechanisms include inhibition of nitric oxide production, hepatocyte apoptosis and nuclear factor κB activation, and stimulation of the enzyme heme oxygenase-1 (HO-1)[3].

Heme oxygenase (HO) catalyses the degradation of heme to biliverdin, carbon monoxide (CO) and iron. Different isoforms of HO are expressed in the liver, with HO- 1 being found mainly in Kupffer cells, and HO-2 in the hepatocytes.

HO-1 and CO may play a regulatory role in the resolution of inflammation, and HO-1 is a potential therapeutic target for treating inflammatory diseases, and encouraging hepatoprotection[4]. Overexpression of HO-1 protects against paracetamol, endotoxin and carbon tetrachloride-mediated hepatobiliary dysfunction in animal models[5-7], and has been implicated in preventing cytokine and CD95-mediated liver damage in mice[8]. A number of chemopreventive agents including curcumin, dietary polyphenols and quercetin, appear to produce antioxidant and cytoprotective effects at least partially via induction of HO-1[9].

Echinacea root was traditionally used by North American Indians as an anti-inflammatory and anti-infective treatment, and its effects on gene expressions indicate a broad spectrum anti-inflammatory and immunomodulatory response. This includes reduced expression of inflammatory cytokines such as IL-1β, IL-8 and TNF-α[10, 11], and inhibition of cyclooxygenase (COX) activity [12]. While alkylamides, caffeic acid derivatives and polysaccharides are all major groups of phytochemicals found in Echinacea, the alkylamides are probably most responsible for these immunomodulatory and anti-inflammatory effects[13-15].

A research team in Taiwan has recently published results from various different experiments that investigated the effects of key alkylamides found in *Echinacea purpurea* root, on hepatic injury in mice[16].

The major alkylamides dodeca-2E,4E,8Z,10Z(E)-tetraenoic acid isobutylamides (Alk-8/9), were extracted from roots of *Echinacea purpurea,* and their effects on lipopolysaccharide/D-galactosamine LPS/D-GalN)- induced fulminant hepatitis in mice were evaluated. This model is widely used to investigate the underlying mechanisms of clinical fulminant hepatic failure, and to develop effective therapeutic strategies.

Four groups of 6 mice each were used, one receiving treatment vehicle only, one Echinacea alkylamides only, one LPS/D-GalN only, and one both LPS/D-GalN as well as Echinacea alkyamides. Echinacea alkylamides were given intraperitoneally at 48, 24 and 1 hour before LPS/D-GalN administration. Blood samples were then analysed, and liver tissues were removed 4 hours after LPS/n-GalN treatment and microscopically examined.

Echinacea alkylamide pre-treatment produced a reduction in LPS/D-GalN-induced acute hepatitis and liver injury, serum AST and ALT activities, TNF-αα protein level, hepatic inflammation, and necrotic tissue injury. Incubation of macrophage cells with Echinacea alkylamides also inhibited expression of the inflammatory cytokine TNF-α by LPS, an effect which was also related to HO-1 protein expression and its catalytic product CO. Further experiments showed that certain mitogen-activated protein kinases (MAPKs) are involved in induction of HO-1 gene expression in response to alkylamides.

While an enhanced risk of hepatotoxicity has been suggested when administering Echinacea with drugs such as paracetamol[17, 18], no clinical or experimental evidence exists in support of this premise. Instead, this latest study provides convincing evidence that intraperitoneal administration of Echinacea alkylamides 8/9 produces an hepatic protective effect in mice, and suggests that anti-inflammatory activity through inhibition of the pro-inflammatory cytokine TNF-αα by MAPK dependent expression of HO-1, contributes significantly to this hepatoprotective activity. It also suggests a potential application for high alkylamide-containing *Echinacea purpurea* products in protecting against inflammatory hepatitis in experimental animals and perhaps humans.

This is not the first study to implicate hepatoprotective effects for Echinacea. A previous study in Egypt investigated Echinacea's effects on liver toxicity in rats treated with cyproterone acetate[19]. Echinacea treatment for 2 and 4 weeks reversed the cyproterone induced fall in glutathione peroxidise and superoxide dismutase, suggesting an antioxidant-related protective effect on the liver against this drug.

*Echinacea purpurea* has also been shown to decrease the damaging effects on mice liver cells of the industrial pollutant cadmium [20], and to significantly ameliorate changes in liver tissues observed in mice as a result of gamma-irradiation[21].

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Echinacea

# Anti-Influenza Properties of Silymarin

The influenza A virus is a common cause of seasonal respiratory illness, which can sometimes reach a serious level particularly in the young and elderly[1]. Seasonal influenza kills 250,000 to 500,000 people each year, and can place enormous strains on hospital and community healthcare services. Especially virulent genotypes can also be responsible for the outbreak of pandemic forms of influenza A, often with high rates of death. Several influenza A pandemics have occurred last century, including the H1N1 ('swine flu') pandemic of 2009-2010[2,3].

Apart from public health measures, treatment options for influenza A are currently limited. The neuraminidase inhibitor drug oseltamivir (Tamiflu®) is considered the drug of choice for patients with pandemic influenza, but routine use of this for seasonal influenza has increased the incidence of drug resistance, and can produce unwanted side effects. Zanamivir, the other available neuraminidase inhibitor, is also contraindicated in those with underlying respiratory conditions and is difficult to administer in younger children[4].

Silymarin is a flavonolignan complex containing a mixture of silybin, isosilybin, silydianin and silychristin found in seeds of *Silybum marianum,* known commonly as Milk or St Mary's Thistle. Best known for its liver-protective effects and applications in liver conditions such as mushroom poisoning and viral hepatitis, silymarin exhibits antioxidant, anti-inflammatory, immunomodulatory, antiproliferative and antifibrotic activities[5,6].

Evaluation of other possible indications has been an active area of research in recent years. This has revealed evidence of potential applications as a preventative and treatment agent for various cancers, due to anti-neoplastic effects shown *in vitro* and *in vivo* for models of skin, breast, lung, colon, bladder, prostate and kidney carcinomas[7-12].

Multiple effects have also been reported for silymarin on the hepatitis C virus (HCV) lifecycle, including inhibition of virus entry, RNA and protein expression, and infectious virus production, in an HCV cell culture infection[13]. Inhibition of the proliferation and proinflammatory cytokine secretion of T cells in blood derived from patients with HCV infection has also been reported for silymarin[5,14].

Encouraged by these findings, and the role of inflammation in the pathogenesis of influenza A viral infections, a team of Korean researchers has now further extended our knowledge of silymarin through measuring its antiviral activity against influenza A/PR/8/34 virus, and comparing this to that of the neuraminidase inhibitor oseltamivir[15].

Silymarin was purchased as a phytochemically characterised compound from Sigma-Aldrich, and oseltamivir from a pharmacy in Korea. An *in vitro* cellular proliferation reduction effect method was used to measure antiviral activity, with each antiviral compound being dissolved in dimethyl sulfoxide (DMSO) and subsequently diluted in appropriate culture media. Oseltamivir was used as a positive, and DMSO as a negative control. A reverse transcriptase-polymerase chain reaction (PCR) analysis was also used to assess viral mRNA synthesis after 48hrs infection of Madin-Darby canine kidney (MDCK) cells.

A strong antiviral activity of about 98% against the influenza A/PR/8/34 virus was demonstrated for silymarin at a concentration of 100µg/ml, and activity of about 45% for a silymarin concentration of 10µg/ml. Oseltamivir by comparison showed antiviral activity of about 52% at a 100µg/ml concentration, and activity at less than 40% at a 10µg/ml concentration. Neither substance was toxic to MDCK cells. The viral cellular proliferation effect was also prevented by 100µg/ml silymarin, and this inhibitory response was also stronger than that observed for oseltamivir.

PCR analysis showed the viral mRNA syntheses to be completely inhibited by silymarin at 100µg/ml after 48hrs infection, this also being greater than the inhibitory response shown by oseltamivir at the same concentration. The same researchers have previously reported similar effects for the flavonoid quercetin-3-rhamnoside[16,17], and many other phytomedicines are now thought to

Milk Thistle

have a useful role in the treatment or prevention of influenza A virus[18].

With the ongoing emergence of antiviral resistance to oseltamivir, and the appearance of new pandemic strains of influenza A virus[19], the development of new antiviral agents for influenza is an important area of research. This initial study suggests a potential anti-influenza effect by a readily available and comparatively cheap phytomedicine, and further *in vivo* studies are warranted.

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# Kava Hepatotoxicity and Raw Material Quality

Availability of phytomedicines made from Kava *(Piper methysticum)* has been restricted in many countries for several years, following reports of hepatotoxicity associated with its use particularly in Europe[1,2].

Initially these reports all involved modern versions of Kava extracts prepared by organic solvents including acetone or ethanol, and it was observed that liver toxicity had not been documented with traditional water based extracts used in Pacific countries. This lead to a proposal that while western formulations of Kava may be hepatotoxic, traditional Kava use is safe[3,4]. In support of this hypothesis was the absence of reports of hepatotoxicity found in studies involving traditional use by Australian[5-7], New Caledonian[8], and Hawaiian[9] populations.

In a move which gave significant weight to this so called 'Pacific Kava paradox', in 2005 the Australian TGA approved the manufacture and sale of aqueous Kava extracts only, while continuing restrictions on non-aqueous extracts. Production and use of hydroethanolic Kava products is still permitted in New Zealand and the United States.

Soon after the Pacific Kava paradox was proposed, however, case reports began to appear associating hepatotoxicity with use of Kava as a traditionally prepared water extract. The first of these, in 2003, described severe hepatotoxicity following the use of traditional aqueous extracts derived from Kava imported from Vanuatu[8]. Further case reports emerged from Australia[10,12], the U.S.[11,12], and Germany[12-14]. In all these reports, causality for Kava was implicated through use of the same structured, quantitative, liver specific and updated CIOMS scale[15,16] that was used to assess cases of liver disease associated with acetonic and ethanolic extracts[13,14]. Also, in 2007 the World Health Organisation (WHO) reported five cases of hepatotoxicity associated with use of aqueous Kava extracts, although only two were from traditionally prepared Kava[17].

Another study compared typical clinical features of Kava hepatotoxicity associated with five case reports involving aqueous extract use, with those associated with nine cases of acetonic or ethanolic extract use from Germany and Switzerland. This found a similar clinical picture in all fourteen patients, independent of which type of extract was used[12,14].

While the above numbers and the total number of reported cases of hepatotoxic reactions linked with traditional aqueous extract use to date are small, these studies suggest that the solvents used to produce Kava extracts may not contribute to this problem as was previously thought. Other potential causes of hepatotoxicity related to the herb itself, or a rare idiosyncratic reaction, therefore seem more likely.

To date there is little experimental evidence that Kava lactones themselves may be hepatotoxic[18-20]. Two non-Kava lactone constituents have been identified as potential culprits, the alkaloid pipermethystine[21] and the chalcone flavokavain B[22]. However, pipermethystine failed to cause experimental liver injury even in high doses, and was not found in commercial Kava extracts[23]. Flavokavain B produces modest signs of hepatotoxicity in experimental animals, but only at doses far in excess of those found in ethanolic Kava extracts[22].

While traditionally the peelings of rhizomes and roots were not used to prepare customary Kava drinks, during the lead-up to the first reports of liver toxicity from Europe, high demand led to these peelings being sometimes supplied to product manufacturers[24, 25]. Two new papers on this subject by Professor Rolf Teschke, a renowned German researcher into Kava hepatotoxicity, now suggest that contamination of raw material during storage with hepatotoxic fungi and moulds, could be at least partly contributory to these cases[26, 27].

The high temperatures and humidity in many South Pacific countries are ideal conditions for growth of moulds, which can develop soon after harvest, particularly during the poor drying and storage conditions often prevalent in these countries. This could encourage the development of moulds within the bags and containers in which Kava is exported. Without adherence to good agricultural and manufacturing practices, or robust sampling and quality assurance systems by manufacturers, this problem may not be picked up prior to extraction taking place. Mould growth can lead to the production of hepatotoxins such as aflatoxins and ochratoxin A, which could conceivably be carried through into Kava finished products, particularly if peelings are used.

Aflatoxins are a group of mycotoxins principally produced by *Aspergillus flavus* and *A. parasiticus*, both natural contaminants of food and feedstuff. These toxins can have various negative health effects including acting as liver carcinogens, especially in combination with chronic hepatitis C virus infection[28]. Epidemic outbreaks of hepatitis due to consumption of maize heavily contaminated with *Aspergillus flavus* and aflatoxins, have previously been reported in western India[29].

While the potential role of mycotoxins in Kava-associated hepatotoxicity seems quite plausible, a wide range of studies are required in order to further investigate this as well as other potential aetiological factors. Professor Teschke calls for toxicological studies into various Kava cultivars, possible adulterants, suspected contaminants including fungi and bacteria, and diseases of the Kava plant such as Kava dieback[17, 26, 30]. However, even prior to the results from these studies becoming known, more attention should be given by regulators and manufacturers to ensure properly dried and processed raw materials, free of mould contamination, are used.

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Kava

Ginkgo

# Ginkgo Improves Memory in Middle-Aged Healthy Volunteers

*Ginkgo biloba* extracts are widely used for their perceived preventative and treatment benefits in dementia and cognitive decline associated with ageing. Several clinical trials have shown benefits in patients with mild to moderate dementia[1-4], although recent meta-analyses summarise the evidence for this as somewhat inconclusive and difficult to determine[5,6].

Research to determine whether Ginkgo can also help prevent dementia has attracted attention in recent years[7]. A large clinical trial involving 3069 participants found that 120mg daily dose of the usual 50:1 strength Ginkgo extract taken over a 6 year period had no such protective effects[8]. However, the fact that participants didn't commence treatment until they were aged 75 years or older may well have contributed to these negative findings[9].

Ginkgo has also been reported to improve reaction time and short term memory in young students who received single Ginkgo doses much larger than usual[10-12]. These effects were not replicated following 6 weeks of daily administration of 120mg Ginkgo extract[13], however criticism of these studies and their methods has also been made[14].

Despite its popularity and there being nearly 3000 scientific papers involving Ginkgo published in peer-reviewed journals, many of these have methodological flaws. In fact most studies have involved elderly participants, and Ginkgo's effects in middle-aged healthy subjects have not been well investigated. Results from a recent clinical trial sponsored by the German manufacturers of the well known Ginkgo extract EGb761, which aimed to investigate its effects on memory and the specificity of such effects in healthy subjects aged 45-56, are therefore of interest[15].

A total of 188 healthy subjects took part in the trial, which involved taking a single daily dose of 240mg Ginkgo extract or placebo daily for 6 weeks, then undergoing various assessments of long-term memory. All subjects had high-level secondary education and were mentally healthy. An extensive list of exclusion criteria was applied, including previous participation in a Ginkgo clinical trial, hospitalisation, ischaemic stroke within the last three months, cognitive impairment due to any neurological origin or psychiatric disorder, and history of recurrent major depression or recurrent anxiety disorder.

Main outcome measures at completion were two memory tests described as 'relevant to daily living' and one subjective memory questionnaire. General well-being and mood were also assessed as secondary outcome measures.

One memory test was for free recall (8 complex appointments to be learnt by heart within 2 minutes, followed by an unaided short-term free recall and another free recall test after 45 minutes delay). The other was a test of less complex learning and remembering operations, based on a driving route recognition task. This measures the level of retention of a verbal explanation of actions to be performed in the future, and is designed in a way such that even cognitively intact persons do not achieve the maximum possible score. The third test involved a standardised memory self-rating questionnaire, which assessed the ability of memory performance and occurrence of memory failures.

In the quantitative measure of correctly recalled appointment items, Ginkgo-treated subjects improved significantly in quantity of recall (i.e. number of correctly recalled appointments). These improvements were more apparent, however, in the long-term delayed recall ($p=0.01$), than when recall was immediate ($p=0.092$).

In the driving route recognition task, Ginkgo produced no significant difference to placebo. Both treatment groups reported slight improvements over the 6 week trial in the subjective memory questionnaire, although no between-group difference was measured.

As has been the case in other well designed trials involving Ginkgo, reported adverse events were infrequent and mild, and their incidence similar in the treatment and placebo groups. Also no significant mood changes took place as a result of Ginkgo treatment.

In summary, effects were found only in complex parameters of the

highly demanding appointments remembrance task, but not in the easier driving route recognition task. These findings are in concordance with those of previous studies that have reported either effects on complex attentional or long-term memory parameters[16-19].

The improvement in long-term memory shown in this study involved somewhat younger subjects (mean age 54.5 years) than those who participated in previous trials[16,17,19]. This study and another involving subjects aged 18-40 years[18], challenge the notion that effects of Ginkgo are lacking in individuals younger than 60 years[14]. Methodological challenges in assessing cognitive functioning in healthy volunteers, as opposed to measures used in dementia, make it difficult to demonstrate efficacy of any cognition-enhancing intervention. However, this study appears to have tried to adequately address these issues, and adds to evidence that Ginkgo may be useful in middle aged subjects wanting to optimise their memory and performance when challenged with highly demanding mental tasks.

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# Propolis Improves Fracture Healing

Propolis is a sticky substance made by honeybees from resins collected from the bark and buds of trees, which are mixed with wax to be used as a beehive protectant and many other purposes in the hive. It contains more than 300 different chemical compounds, with flavonoids being predominant.

Propolis has attracted much interest from researchers, and antimicrobial, antiviral, antifungal, anti-inflammatory, antihepatotoxic, anticancer, antioxidant, anti-ulcer, immunostimulant, and local anaesthetic effects have been reported[1].

The natural function of the resins used by bees to make propolis seems largely directed at helping facilitate bark or stem healing following injuries or breaks, and a role for propolis in wound and fracture healing in humans is therefore postulated.

Propolis was first shown to have useful effects in wound healing by Russian researchers[2, 3]. Wound healing promotional effects have since been observed for topical propolis in both diabetic and normal rats[4, 5]. Propolis administration also demonstrates a protective effect against radiotherapy[6] and chemotherapy[7] induced oral mucositis in rats.

Australian researchers have reported protective effects of a topical application of propolis against UV-radiation-induced inflammation, lipid peroxidation and immune suppression in mouse skin[8]. Antimicrobial and anti-inflammatory effects comparable to those of silver sulfadiazine were observed following propolis cream application to minor burns, in a burn clinic in Brazil[9]. Anti-inflammatory effects comparable to those of dexamethasone, have also been measured for a topical 1% ethanolic extract of propolis, in chemical corneal injury[10].

Propolis application is being researched as a possible tool in dental and oral surgery, and topical application of a 10% hydroethanolic propolis solution has been reported to accelerate epithelial repair after tooth extraction[11].

In terms of fracture healing, an accelerated rate of ossification including an enhanced rate of bone tissue regeneration following application of an ethanolic propolis extract, was first documented by German researchers in 1978[12]. Such applications have been further explored recently, by a study which evaluated the effects of propolis administration on the healing of femur fractures in rats[13].

The animals in this Turkish study received propolis administration over a 3 or 6 week period following experimental femur fracture then retrograde fixation under anaesthesia[13]. Four groups of rats were used, two control groups who received no propolis, one given 200mg/kg propolis orally for 3 weeks,

and the other given this dose for 6 weeks post-fracture. Propolis of mainly black poplar tree origin in Turkey was used to first prepare an ethanolic extract, then a propylene glycol extract which was used in the study.

Animals were sacrificed at the end of the 3 or 6 week treatment period, then their femurs removed for histopathological examination as well as measurement of tissue antioxidant levels. Before killing the rats, anteroposterior and lateral radiographies of the femur were obtained, whole body bone mineral density was measured, and blood was drawn for analysis.

Propolis treatment was associated with a higher bone mineral density at both the 3 and 6 week treatment period (0.181 vs 0.147, and 0.196 vs 0.150 g/cm$^2$ respectively). These differences were highly significant ($p<0.001$). Radiological evaluation scores were also better among propolis-treated rats compared with the controls at both timepoints. More favourable histological assessment scores (based upon the amounts of fibrous, cartilage and immature bone tissues), were evident after 3 weeks propolis treatment, but were no different from the placebo group after 6 weeks treatment.

Various biochemical changes suggesting improvement in antioxidative processes were also measured as a result of propolis administration. Plasma and tissue levels of the endogenous antioxidants superoxide dismutase, myeloperoxidase and glutathione were lower among propolis-treated than control rats at both 3 and 6 weeks. While these responses warrant further investigation, they may be explained by a reduced need for endogenous antioxidants due to the effect of propolis, a substance which exhibits powerful antioxidant activity.

In summary, the results from these various tests suggest an accelerated rate of bone fracture healing attributable to internal propolis ingestion, particularly during the first 3 weeks following fracture. Furthermore, significant changes in endogenous antioxidant levels took place compared with controls, both in blood and bone tissue.

Bone fracture is associated with oxidative stress[14, 15, 16], and this is most prominent during the early phases of fracture healing. A strong antioxidant effect of propolis has previously been demonstrated in tissues other than bone[17-19], and this action probably explains at least in part why the healing effects of propolis were most apparent during the early phases.

The authors conclude that their study shows time-dependent beneficial effects of propolis on fracture healing as assessed by several tools, including bone mineral density measurements, radiographic and histopathological evaluations. These findings were supported by changes in the level of endogenous molecules taking part in antioxidative process when compared with the controls, indicating a potential role for propolis in the treatment of fractures through the facilitation of bone and tissue healing and union.

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