Public Health Nutrition: 3(4A), 473–485

Efficacy and safety of ginseng

DD Kitts* and C Hu

Food, Nutrition and Health, Faculty of Agricultural Sciences, University of British Columbia, 6650 N.W. Marine Drive, Vancouver, B.C. V6T-1Z4, Canada

Abstract

Ginseng (*Panax ginseng*, C.A. Meyer) has been a popular herbal remedy used in eastern Asian cultures for thousands of years. In North America, the ginseng species indigenous to both Canada and the United States (*Panax quinquefolium*) represents an important industry for both domestic and export markets. There are numerous theories and claims describing the efficacy of ginseng, which can combat stress, enhance both the central and immune systems and contribute towards maintaining optimal oxidative status against certain chronic disease states and aging. Risk issues concerning the safety of ginseng at recommended dosages are less prominent and scientifically based. While some epidemiological or clinical studies have reported indications of efficacy for specific health benefits or potential toxicity, there are an equal number of studies that provide contradictory evidence. This situation has led to questionable conclusions concerning specific health benefits or risks associated with ginseng. Recent advances in the development of standardized extracts for both *Panax ginseng* (G-115) and *Panax quinquefolius* (CNT-2000) have and will continue to assist in the assessment of efficacy and safety standards for ginseng products. This paper reviews the scientific literature and evidence for ginseng efficacy and safety derived mostly from *in vitro* and animal studies and places emphasis on the need for more randomized, double-blinded, placebo clinical studies that can provide unequivocal conclusions. An example of the efficacy and safety of ginseng is provided with the description of biological activity of a North American ginseng extract (NAGE), which includes illustrating mechanisms for antioxidant activity without prooxidant properties.

Keywords
Ginseng
Safety
Efficacy
Antioxidant activity

Ginseng is a perennial herb which is indigenous to Korea, China (*Panax ginseng* C.A. Meyer), Himalaya (*Panax pseudo-ginseng*), Vietnam (e.g. *Panax vietnamensis*), Japan (e.g. *Panax japonicus*) and North America (*Panax quinquefolium*). In North America, ginseng is grown commercially mainly in the Canadian provinces of British Columbia and Ontario. Wisconsin is the largest producer of North American ginseng in the United States. Ginseng, which is present in the Pharmacopoeas of China, UK and Germany, is regarded as a tonic with adaptogenic, stimulant and aphrodisiac properties[1–3]. There is a considerable body of evidence to suggest that ginseng may also have important roles in maintaining oxidative status, by possessing both direct or indirect antioxidant functions. The validity of many of these claims should be determined by demonstrating the efficacy of the product, which supports the causal relationship between the bioactive constituent(s) and the proposed benefit. Closely associated with this evaluation process is the assurance that no adverse reactions exist with nutritional or toxicological endpoint parameters associated with consumption. In a recent systematic review of the literature, Vogler *et al.*[4] remarked on the paucity of controlled or randomized human studies that limits sound conclusions concerning the efficacy and safety of ginseng. The purpose of this paper is to review some of the clinical and epidemiological evidence concerning the efficacy and safety of ginseng, a common herb which can be found as an easily accessible food supplement. In addition, we have examined the *in vitro* chelating and reducing activity of a North American ginseng extract (NAGE; CNT-2000) and extended these experiments to establish standards of evidence for antioxidant activity associated with the inhibition of hydroxyl radical formation. Specific examples of biochemical activity related to the *in vitro* antioxidant properties of ginseng are presented to further demonstrate the potential efficacy of ginseng.

Ginseng composition

The recognized primary active components of ginseng are a group of 30 different triterpene saponins, also referred to as ginsenosides, which vary in content and relative

**Corresponding author*: Email ddkitts@interchange.ubc.ca

proportions among different species of ginseng[1,3]. Noting the differences in ginsenoside composition between North American and *Panax ginseng* C.A. Meyer ginsengs is important in assessing and interpreting efficacy and understanding safety. For example, traditionally recognized differences in pharmacological properties exist between different species of ginseng, as proposed by the ancient Asian concept of the complementary forces of *ying* and *yang*. In this example, it is the North American ginseng that provides the *ying*, or the cooling effect to offset stress, while *Panax ginseng* C.A. Meyer provides warmth, or the *yang* conditions to counter-balance stress. These apparent differences in cause and effect properties of ginseng may be related to the different composition of ginsenosides present in these two sources of ginseng. However, in addition to the bioactive ginsenosides, recent studies have also identified an acidic polysaccharide, referred to as 'Ginsan', with noted immunostimulatory activity[5,6]. To alleviate the difficulty in assessing the bioactive properties of ginseng, relatively recent ginseng research has employed the use of standardized extracts of both *Panax ginseng* C.A. Meyer (G115, marketed as Ginsana) and North American ginseng (*Panax quinquefolium*, extract CNT-2000 from Chai-Na-Ta Corp., Langley, B.C.). These products hold the potential to enable improvements for assessing both quality control of ginseng products and efficacy of proposed activity from this herb. Of the numerous ginsenosides that have been identified from ginseng, six (namely Rb1, Re, Rc, Rd, Rb2 and Rg1) have been chosen for reference standards for ginseng products[7].

It is noteworthy that although the *Panax quinquefolium* species is grown in both North America and Asia, pharmacological activity with specific organ systems has been reported to be stronger for the North American variety than the Asian counterpart[8]. Although the mechanisms underlying these differences in biological activity are not fully understood, subtle compositional differences are a likely explanation. Similar differences in bioactive properties have also been observed between cultivated and wild *Panax ginseng*[9]. Moreover, although the traditional source of ginsenoside from ginseng is the root, both the leaf and berry parts of this plant also contain significant quantities of ginsenoside[10], thus adding to another potential variable in product source. In light of these facts, the employment of standardized extracts of ginseng with specified bioactive potential relative to known composition of active constituents will be the first step to assess efficacy and safety as well as ensuring optimal quality control of ginseng-based products. A good example of this is the proposal to include ginseng powder in multivitamin and mineral supplements for possible synergistic effect[11,12].

Ginseng metabolism

Central to the difficulties in explaining, in scientific terms, the underlying biochemical mechanisms for various biological functions proposed for ginseng is the paucity of information concerning the absorption and metabolism of ginseng constituent(s) (e.g. primarily the ginsenosides, see Fig. 1). Pharmacokinetic studies conducted in rats have reported only 23% absorption of ginsenoside (Rb1) after a period of 2.5 h[13]. Very small recoveries of this ginsensoside were made in the liver (e.g. 0.25% dose) and heart (e.g. <0.1% dose), while the majority of the material was recovered in the small intestine. The bioavailability of bioactive ginseng constituents appears very limited, as evidenced by the low absorption rates for orally administered Rg1 (e.g. 0.1% dose) and Rg2 (e.g. 1.9% dose)[14]. Considering the fact that very little of the original ginsenoside material was recovered in the faecal matter (e.g. <1% dose), these results would appear to indicate that either metabolic or bacterial transformation of the parent ginsenoside constituents occurs in the small intestine. Support for this suggestion comes from studies conducted with bacteria collected from human intestines, which hydrolyse ginsenosides Rb1 and Rb2 to specific metabolites[15], and which in turn are the likely forms absorbed from the intestine. Bacterial metabolites of ginseng have also been reported to have antigenotoxic properties[16]. The potential for the transformation of parent ginseng constituents to specific products of bacterial metabolism has made it difficult to directly associate various physiological effects noted for ginseng with standard pharmacokinetic parameters of specific ginsenoside absorption.

Adaptogenic effects of ginseng

Some adaptogenic effects of ginseng, initially reported by Brekhman and Dardymov[17] are listed in Table 1, and probably involve changes in tissue oxygen uptake and transitory alterations in carbohydrate and lipid utilization. Pieralisi *et al.*[18] demonstrated increased oxygen uptake and related work load in normal subjects consuming ginseng. Earlier studies also reported increased oxygen uptake and transport in elderly subjects as well as enhanced energy levels in athletes[19]. These results are in agreement with other studies that have reported a dose-dependent *in vitro* increase in glucose uptake in brain tissue and associated decreases in lactate and pyruvate concentration ratios attributed to the presence of the standardized ginseng G115 extract[20]. Reduced lactic acid metabolism by ginseng has also been reported in athletes undergoing intense exercise after consumption of 4% ginsenoside[21]. The enhancement of tissue glucose uptake and reduced production of anaerobic metabolic products have been explained by ginseng-related changes in tissue oxygen uptake.

The ergogenic effects of ginseng are less clearly observed in non-stressed experimental animal or human subjects, compared with counterparts which were challenged with an intense physical activity. For example, studies designed

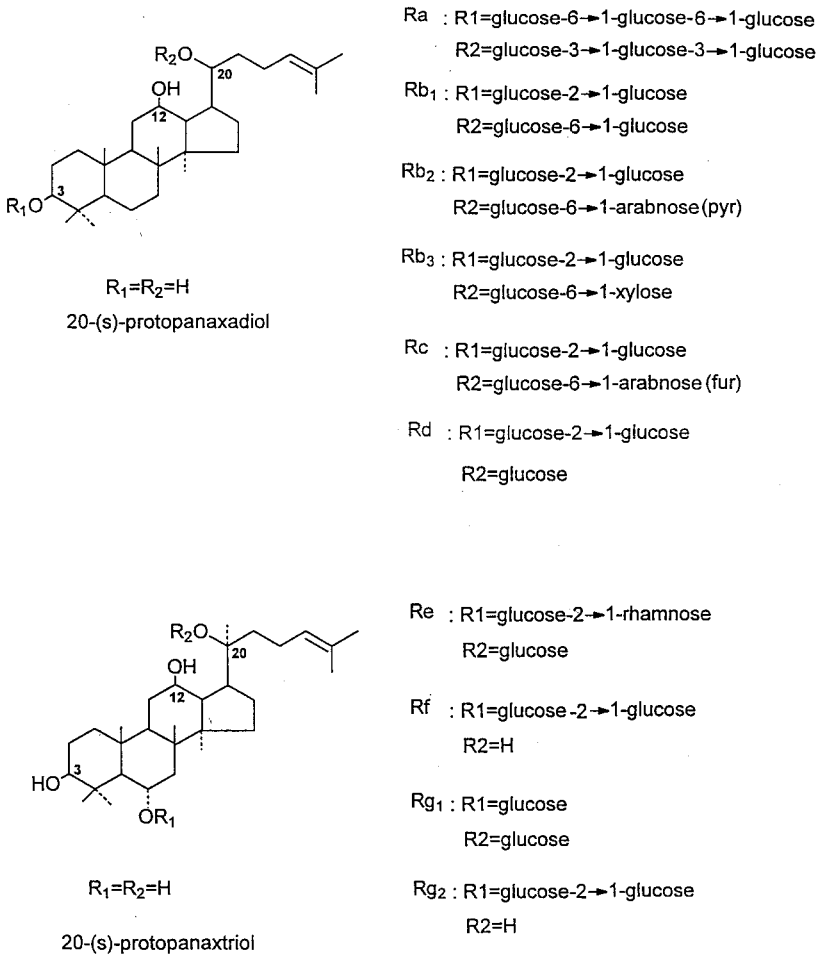

Fig. 1 Characteristic ginsenosides present in Asian and North American ginseng

to evaluate exercise performance due to ginseng supplementation have reported a marked stimulating effect of ginseng on carbohydrate and lipid mobilization and utilization[22]. Rats given a ginseng extract devoid of ginsenosides Rb1 and Rg1, prior to exercise, exhibited a preference for utilization of free fatty acids, thereby sparing glucose and glycogen levels. As a result of these animal studies, ginseng has recently been evaluated in human subjects for efficacy in numerous exercise performance studies[22–28] and incorporated in sports drinks to enhance athletic performance[23]. Many of these studies, however, have questioned the proposed ergogenic effect of ginseng[24–26]. In a clinical study comprising two dosage treatment groups receiving 8 and 16 mg kg^{-1} body weight of both a placebo and ginseng before an intense cycling exercise, no effect of ginseng ingestion for a 1 week period was observed in enhanced physical endurance compared with placebo treatment[25]. A randomized, double-blinded, placebo controlled study of healthy men receiving 200 and 400 mg day^{-1} of a *Panax ginseng* extract (G115) drew similar conclusions. The study showed no effect on oxygen consumption, respiratory exchange ratio, blood lactic acid and perceived exertion in men receiving ginseng[26]. Similar results have been reported in healthy adult females using a randomized, double-blind placebo-controlled experimental design[25].

The fact that many of the metabolic effects attributed to ginseng relate to adaptation of homeostatic mechanisms resulting from exposure to stress, suggests some interaction between ginseng constituents and endocrine activity (Table 1). Ginseng has been reported to bring about changes in circulating levels of adrenocorticotrophin hormone[27] and corticosterone concentrations[28]. Specifically, two ginsenosides, Rb1 and Rg1, have been reported to possess glucocorticosteroid activity due to a tendency to behave as functional ligands to the glucocorticoid receptor[29]. The tendency of ginseng to exhibit apparent ergogenic, or non-specific resistant effects, is possibly due to the increased adrenal responsiveness afforded by ginseng constituents which ultimately enhances the activity of the pituitary–adrenal axis.

Significant increases in plasma corticosterone attributed to both intraperitoneal and oral administration of ginseng have also been shown to parallel increases in plasma glucose and decrease plasma immunoreactive insulin[30]. Other studies have reported ginseng constituents to lower

Table 1 Reported metabolic and adaptogenic effects of ginseng

	Observed effect	Reference
Physiological system		
Metabolic	Enhanced oxygen uptake	19
	Enhanced cellular glucose uptake	20,21
	No effect on plasma cholesterol level	24
	Activates DNA polymerase	79
	Stimulatory effect on brain neuronal activity	9
	Lowers blood glucose in non-insulin diabetic patients	31,32
	No ergogenic effects	24–26
Endocrine	Enhanced adrenocorticotrophin secretion	27,28
	Rb1, Rc, Rd-induced increase in plasma corticosterone	28,30
	Reduced Ach-evoked catecholamine release	80
Immune	Enhanced function of peripheral blood mononuclear cells in immune compromised subjects	81
	Rg1-induced increase in T-helper cells	82
	T-cell and macrophage cytokine induction	6
	Rb1-induced reduction of leuokotriene release	83
	Immunostimulatory activity in the aged	84,85
Chronic disease condition		
Cancer	Anti-neoplastic immunostimulatory activity	5
	Specific anti-mutagenic and anti-tumour activity	86–91*
	Protection from radiation-induced DNA damage	92
	Rb2-induced inhibition of tumour metastasis	93
	Epidemiological evidence of protection against cancer	94,95
	Enhanced recovery of cardiac ischaemia injury	48,96
Cardiovascular	Enhanced recovery of brain ischaemia injury	41
	Inhibition of platelet aggregation	97
	Protection by ginsenoside-induced nitric oxide release	44,98

* Ref. 87 – no interference with therapeutic cancer agents.

blood glucose and stimulate insulin release in diabetic animals[31]. Similar findings have been reported in non-insulin-dependent subjects administered 100 mg and 200 mg ginseng in a random study that continued for 8 weeks[32]. Reduced fasting blood glucose corresponded to an increased physical activity and body weight reduction. Future clinical studies are needed with accompanying information on the specific ginsenoside content to confirm efficacy and safety of the proposed adaptogenic properties of ginseng.

Ginseng and oxidative status

The abundance of studies that have shown a strong association between ginseng intake and increased levels of activated oxygen species in various pathological states, or during natural processes such as exercise[33], demonstrates the importance of antioxidant mechanisms which function to preserve oxidative status. The oxidative damage to carbohydrates, proteins, nucleic acids and lipids resulting from contact with free radicals is believed to be the source of early detrimental cellular changes that influence the aetiology of chronic disease (e.g. cancer, atherosclerosis) and aging. The oxidative status of the individual is balanced by the activity of both non-enzymatic antioxidant compounds (e.g. tocopherols, β-carotene, glutathione) and antioxidant tissue enzymes (e.g. superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px)), which together prevent reactive oxygen species formation, or work to mitigate the damage caused to cells by various sources of free radicals[33].

The role of ginseng constituents in supporting antioxidant defence mechanisms has also been shown to involve direct stimulation of cell defence mechanisms (Table 2). Prolonged administration of standardized ginseng G115 extract to rats reduces oxidative stress in certain tissues by altering specific antioxidant enzyme activities that are required to eliminate free radicals, thus reducing specific end-products of tissue peroxidation reactions[34–36]. Common to these studies has been the demonstration of a dose-dependent, ginseng-induced increase in GSH-P_x activity and an associated decrease in tissue malonaldehyde. This effect is particularly evident in stress-induced states, such as that reported with the intense exercising of rats that resulted in both an increase in cytosolic and mitochondrial GSH-P_x and SOD activities[36]. Marked protective effects of ginseng against chemical induced hepatotoxicity has also been reported[37], thus demonstrating a role for ginseng in protecting against xenobiotic toxin-induced peroxidation reactions. Specific ginsenosides have also been shown to protect against cellular toxicity induced from oxygen and lipid derived free radicals in dogs with haemorrhagic shock[38], and characterized in a ferrous/cysteine peroxide induction model system using hepatic microsomes[39]. The

Table 2 Standards of evidence for ginseng antioxidant activity

Evidence	Reference
In vitro model mechanisms	
Inhibition of Fenton reaction induced radicals	
(a) Fe^{2+}-cysteine induced peroxidation of cultured liver, brain microsomal assay	99
(b) Membrane fatty acid peroxidation assay	53
(c) Site-specific deoxyribose degradation assay	54
(d) DNA scission assay	54,58
(e) Electron spin resonance analysis	56
In vivo mechanisms	
Tissue antioxidant enzyme activity	
(a) Reduced hepatic GSH-Px activity and MDA levels	34
(b) Reduced hepatic GSH-Px and SOD activities (exercised)	36
(c) Rb1 & Rb2-induced increased hepatic GSH-Px activity	100
(d) Rc-induced decrease in Cu/Zn SOD activity	100
(e) Rh2-induced increase in catalase activity	100
(f) Nitric oxide-like activity in pre-contracted aortic rings	48
(g) Rb2 induced expression of Cu/Zn SOD1 gene	101

affinity of Rb1 and Rg1, in particular, to scavenge reactive oxygen species in hepatic and brain microsome preparations exposed to Fenton type oxidation reactants, has been reported to occur at ginsenoside concentrations of 10^{-3} to 10^{-4} M[39]. These same workers observed significant reductions (28%) in malonaldehyde and associated increases in GSH-P_x (96.4%) and CAT (47%) after administering 50 mg kg^{-1} day^{-1} Rb1 to rats.

The protection against oxidative stress afforded by ginseng consumption has also been associated with the regulation of Cu/Zn SOD at the molecular level. Kim and coworkers[40] demonstrated that both total saponins and panaxatriol were ineffective at inducing SOD levels, whereas Rb2 triggered a specific induction of SOD transcription. The interaction of Rb2 with an activator protein (AP2) in a promoter region of the SOD gene resulted in the induction mechanism for antioxidant enzyme activity. Using a heterotropic heart transplantation model for myocardial ischaemia and re-perfusion, Liu and Xiao[1] observed that ginsenosides both stimulated myocardial SOD activity and reduced malonaldehyde, a product of lipid peroxidation. Similar antioxidant properties have also been reported in studies using ischaemica re-perfusion injury in the rat brain[41]. Additional evidence of an indirect *in vivo* antioxidant role for ginseng has come from studies that proposed the preservation of tissue SOD activity, resulting from the consequence of ginseng-induced stimulation of nitric oxide production[2,42]. The tendency of ginseng to prevent the effects of oxygen free radical induced injury by promoting nitric oxide release has been confirmed in studies using pulmonary and endothelial organ systems[42–44]. Collectively, the findings that nitric oxide protects against Chinese hamster fibroblast V79 cytotoxicity induced by generation of reactive oxygen species[45], and the fact that ginseng increases both nitrite and cGMP levels in rat serum and urine[46], as well as facilitating endogenous nitric oxide release[47], is strong evidence for an indirect role for ginseng antioxidant activity that involves enhanced nitric oxide synthesis. Further confirmation of this suggestion has recently been reported with the finding that *Panax quinquefolium* inhibited thrombin-induced endothelin release similar to the pharmacological action noted for nitric oxide[44]. Ginseng extracts derived from *Panax ginseng* have also been shown to exhibit a dose-dependent protection against free radical induced injury in pulmonary[35] and myocardial[48] tissue. The fact that crude extracts of ginseng were found to be more effective at inducing this effect, compared with purified ginsenosides (e.g. Rb1 and Rg1), demonstrates a possible synergistic action among different ginseng constituents which occurs only when an extract of ginseng is employed[35].

In recent years, numerous components derived from fruit[49], vegetable[50], oilseed[51] and herb[52] plant sources have demonstrated *in vitro* antioxidant activity. *In vitro* studies conducted with *Panax ginseng* have shown a strong tendency to prevent peroxidation of polyunsaturated fatty acids using a transition metal induced lipid oxidation model[53]. Recent *in vitro* studies using a standardized North American ginseng extract (CNT-2000 from *Panax quinquefolium*) reported direct free radical scavenging of stable radicals as well as the prevention of both site specific and non site specific hydroxyl radical mediated deoxyribose and DNA degradation[54]. This study demonstrated the free radical scavenging activity of North American ginseng using a number of model systems. The question concerning which one, or more, of the specific ginsenoside(s) present in ginseng contributes directly to antioxidant activity, or in concert with other bioactive components, has not been clearly established. Studies conducted with *Panax vietnamesis* root extract showed inhibition of TBARS formation in mouse tissue homogenate[55], further supporting antioxidant activity reported by other workers. These workers also showed that ginsenosides Rg1 and Rb1 failed to inhibit hydroxyl radical formation. However, recent studies conducted in our laboratory with Rb1 have led to the

conclusion that this ginsenoside inhibits peroxyl radical induced DNA breakage (Hu and Kitts, unpublished data). Ginsenosides Rb1, Rb2, Rb3 and Rc, but not Rd, have also been shown to protect against superoxide radical induced damage of cultured cardiac myocytes[56]. Similar *in vitro* studies conducted with individual ginsenosides have indicated some degree of protection against free radical induced endothelial cell damage[57]. Maltol, a principal plant phenolic present in ginseng (e.g. Korean red ginseng) has also been shown to contribute to antioxidant, and little prooxidant activity, as assessed using an iron-catalysed free radical DNA damage model[58]. More research is required to define the source of antioxidant activity characteristic of specific ginseng constituents.

To demonstrate the mechanisms of antioxidant activity of a North American ginseng extract (CNT-2000), a number of *in vitro* model systems were used to characterize ginseng antioxidant activity, thereby suggesting efficacy towards enhancing the stability of different biological macromolecules. Statistical analysis of the experimental data was performed using Student's *t*-test with significant difference set at $P < 0.05$.

Assessing ginseng antioxidant activity *in vitro*

The absorption spectra of 1 mg ml^{-1} NAGE ginseng (line I) and 100 μM $CuCl_2$ (line II) in phosphate buffer (pH 7.4) are shown in Fig. 2. Maximum absorbance of 217 nm was common for both the NAGE and $CuCl_2$ solutions. A shift in absorption maxima from 217 nm to 245 nm occurred in the differential spectrum when $CuCl_2$ was incubated with NAGE (line III), thus denoting chelation of Cu^{2+}.

The reducing activity of the NAGE, relative to L-ascorbic acid (Fig. 3 insert), over a wide range of concentrations is shown in Fig. 3. Linear relationships denoting reducing power were obtained for both NAGE ($y = 0.528x + 0.002$, $r^2 = 0.998$) and ascorbic acid ($y = 0.0027x + 0.0014$, $r^2 = 0.999$). Compared with ascorbic acid, NAGE exhibited a limited reducing power. From both standard curves, it was calculated that 1 mg of the NAGE was equivalent to 20.3 ± 0.3 μg ascorbic acid.

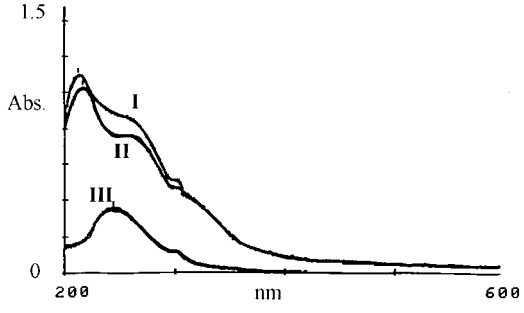

Fig. 2 Copper chelation assay. Briefly, 1 mg ml^{-1} of NAGE was incubated with 100 μM $CuCl_2$ at 37°C for 2 h. The spectra of NAGE and $CuCl_2$–NAGE complex were recorded against 10 mM phosphate buffer (pH 7.4) and corresponding concentration of $CuCl_2$ in phosphate buffer between 200 and 600 nm. Line 1 = NAGE spectrum; Line 2 = $CuCl_2$ spectrum; Line 3 = NAGE + $CuCl_2$ spectrum

The reaction of hydroxyl radical generated from Fenton reactants with DNA is shown in Fig. 4. A concentration-dependent DNA scission reaction was observed with Fe^{2+} (lanes 2–5), indicating a relationship between hydroxyl radical generation and concentration of Fe^{2+} according to the following scheme: $Fe^{2+} + H_2O \rightarrow {}^{\cdot}OH + {}^{-}OH + Fe^{3+}$. The site-specific genotoxic activity of Fenton-induced hydroxyl radicals was characterized by a loss of supercoiled DNA and generation of damaged or nicked circular DNA. The addition of NAGE to the Fenton reactant conditions, with increasing concentrations of Fe^{2+}, resulted in complete protection against DNA damage (Fig. 4, lanes 6–10), as reflected by the preservation of the supercoiled DNA and markedly reduced nicked circular DNA.

The ability of NAGE to suppress Cu^{2+}-induced human LDL oxidation was assessed by the reduction in the human LDL TBARS, conjugated diene and fluorescence products (Figs 5a and 5b, respectively). The presence of NAGE in this reaction mixture produced a significant ($P < 0.05$) concentration-dependent reduction in Cu^{2+}-induced LDL oxidation, as also evaluated by the decrease in electrophoretic mobility on agarose gel (Figs 5c and 5d). The noted inhibition of oxidized LDL on agarose gel corresponded to significant reductions in both TBARS and fluorescence products and, to a lesser extent, changes in conjugated dienes (Fig. 5a). The lower fluorescence readings signified protection of LDL apolipoprotein oxidation from NAGE and were significantly ($P < 0.05$) lower in oxidized LDL but higher ($P < 0.05$) than native LDL, indicating only partial protection against Cu^{2+}-induced oxidation. Use of the fluorescence LDL endpoint indicators of peroxidation produced a sensitive assessment of antioxidant activity attributed to NAGE, and corresponded directly with LDL mobility changes that reflected the extent of inhibition of LDL oxidation on the basis of migration on agarose gel (Fig. 5d).

Suppression of DPPH stable radicals by GSH alone and in combination with 0.5 mg ml^{-1} NAGE is shown in Fig. 6. A NAGE concentration of 0.5 mg ml^{-1} produced a significant reduction (15%) in DPHH radical activity. GSH alone produced a minimal (8%) reduction in DPPH over a concentration range of 5–100 μM. The combination of NAGE extract with GSH over a wide range of GSH concentrations produced a synergistic scavenging effect on quenching DPHH radicals. Maximal ability to quench DPHH radicals was obtained with 0.5 mg ml^{-1} NAGE and 25–100 μM GSH.

The observation that GSH was effective at scavenging DPPH radicals at a critical concentration confirms an antioxidant activity role to deactivate electrophilic free radicals. GSH is also an important component of the antioxidant enzymes, GSH-P_x and glutathione reductase[32], as well as being a critical component in redox cycling for

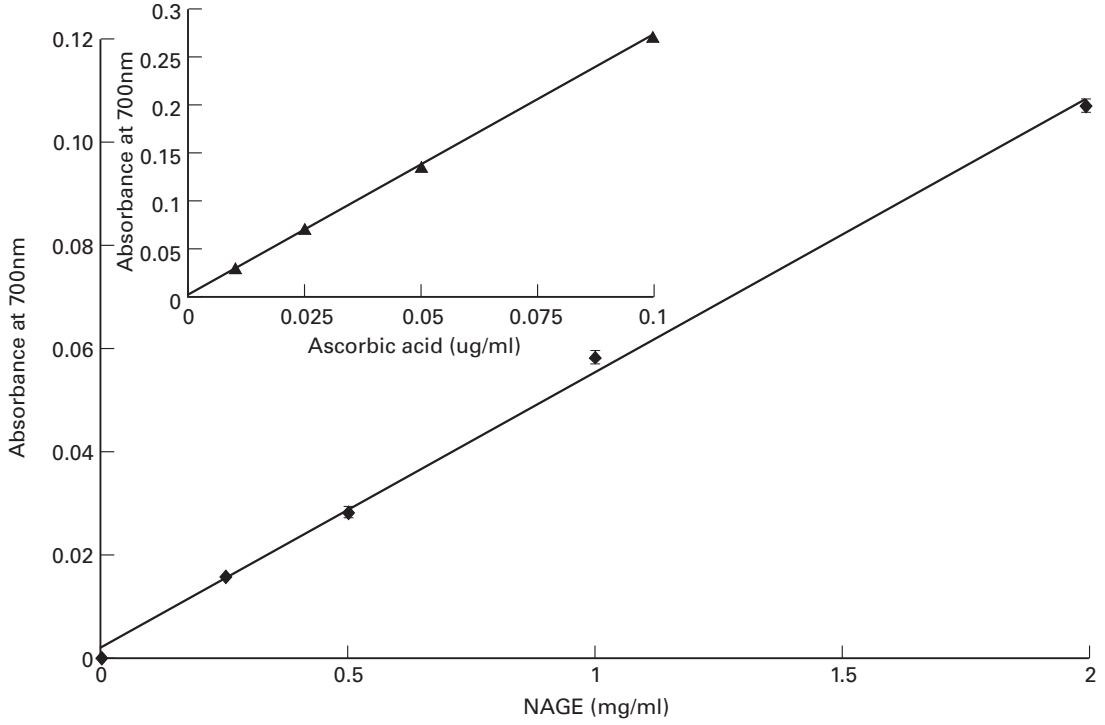

Fig. 3 Reducing power of NAGE: the method followed was that of Hu and Kitts[52] using L-ascorbic acid for comparison. Briefly, NAGE (0~2.0 mg ml^{-1} distilled deionized water) was mixed with 2.5 ml of phosphate buffer (0.2 M, pH 6.6) and 2.5 ml of 1.0% potassium ferricyanide and incubated for 20 min at 37°C, followed by the addition of 2.5 ml of 10% TCA solution. A 2.5 ml aliquot of the above solution was mixed with 2.5 ml of distilled deionized water and 0.5 ml of 0.1% ferric chloride, and absorbance measurements were taken at 700 nm. Equivalence of NAGE to ascorbic acid in terms of reducing power was calculated from the working curve of both substances. Insert graph represents reducing activity of ascorbic acid

regenerating oxidized ascorbic acid that occurs as a consequence of the regeneration of oxidized α-tocopherol[59]. In the present study, NAGE also effectively scavenged DPHH; however, when in the presence of GSH additional scavenging of DPPH radical was noted. This result denotes a potential degree of synergy between GSH and NAGE. It is noteworthy that GSH has previously been shown to reduce oxidized flavones[60], which supports our observation that potential important interactions could occur *in vivo* between these non-enzymatic antioxidant constituents in managing whole body oxidative stress. Similar results of potential synergy with GSH and cycoheterophyliin, a prenylflavonoid isolated from leaves of Formosan *Artocarpus heterophyllus*, have also been reported[61].

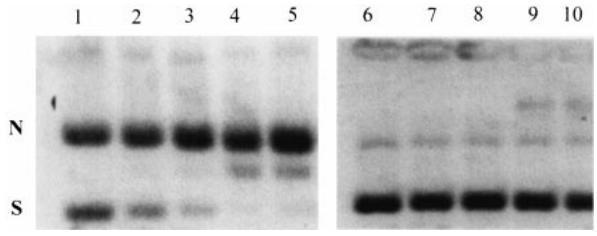

Fig. 4 DNA scission assay: Plasmid (pBR322) DNA from *Escherichia coli* was used to determine the efficacy of a NAGE to modulate metal ion-induced DNA strand scission. Experiments were conducted in phosphate buffer (50 mM, pH 7.4) under ambient oxygen pressure. NAGE (2 μl of 0.005% w/v) was incubated with ferrous sulphate (10, 50, 70 μM) and DNA (0.1 μg ml^{-1}) in a microcentrifuge tube for 60 min at 37°C. The reaction mixture was added to bromophenol blue loading dye (0.25%), containing 0.25% xylene cyanol FF, and 15% ficoll in water and loaded onto an agarose gel for electrophoresis [60 V in Tris acetate–EDTA buffer (0.04 M Tris acetate, 0.001 mM EDTA, pH 7.4)]. Agarose gels were stained with ethidium bromide for 20 min and DNA bands were visualized under UV illumination. Lane 1 = original supercoiled plasmid DNA; Lanes 2–5 = DNA + 5, 10, 50, 70 μM Fe^{2+}; Lanes 6–10 = Lanes 2–5 + NAGE (0.005%). S = supercoiled DNA; N = nicked circular DNA

Safety of ginseng

There are few reported cases of ginseng toxicity or descriptions of side effects attributed to either the quantity or quality of ginseng when taken at the recommended dosages. A careful evaluation of many of these reports has been published by Vogler *et al.*[4] Early animal studies, conducted in dogs, reported no adverse effect of ginseng on body weight or blood chemistry[62]. In mice, the LD_{50} for ginseng ranges from 10 to 30 g kg^{-1} (Ref. 17), with a lethal oral dose of purified ginseng as high as 5 g kg^{-1} body weight[62]. In a 2 year human study, 14 out of a total of 133 subjects were reported to experience side effects attributed

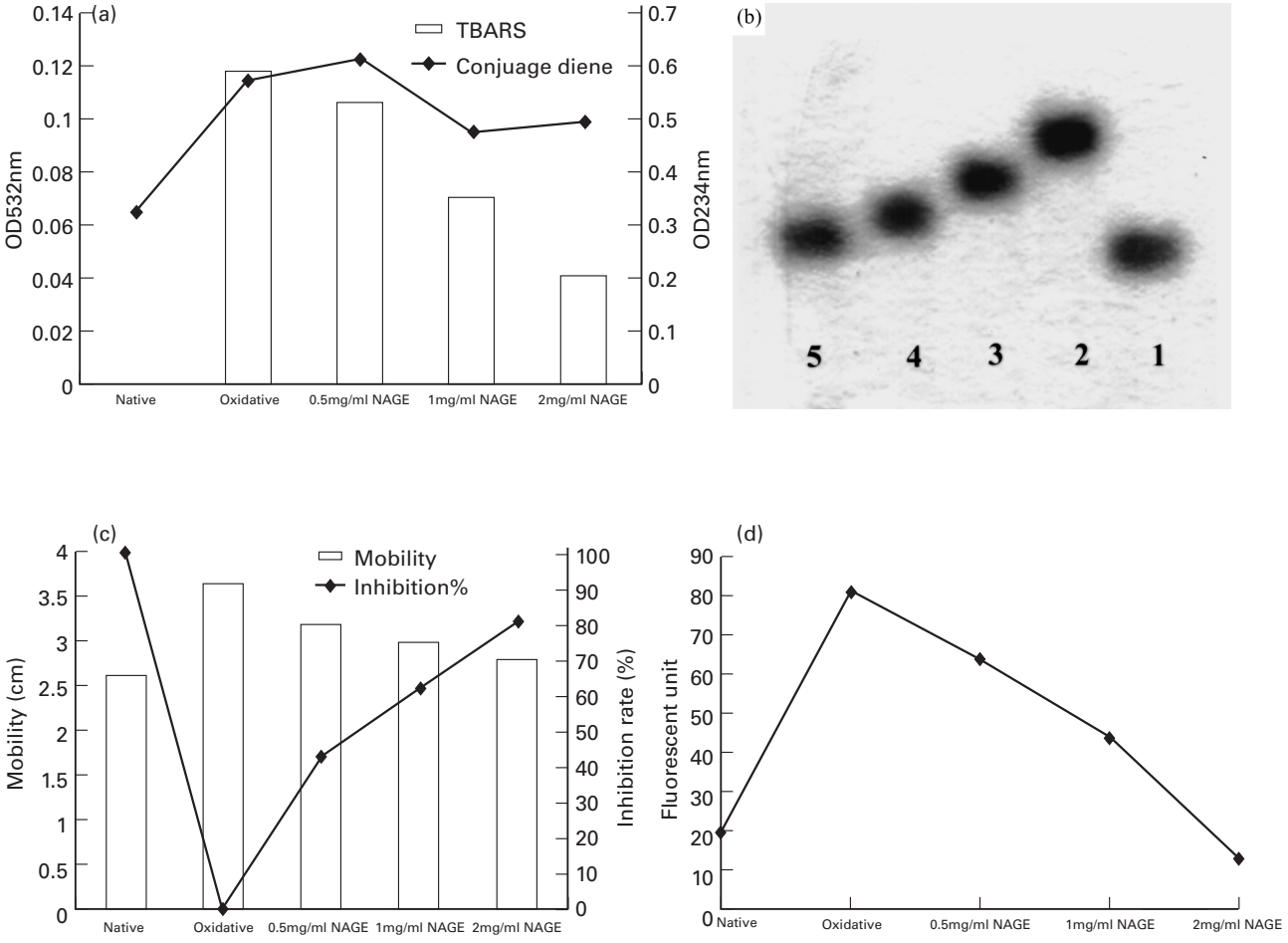

Fig. 5 Copper-mediated human low-density lipoprotein oxidation. Human LDL containing 0.01% EDTA was dialysed against 10 mM phosphate buffered saline (pH 7.4) at 4°C with nitrogen bubbled for 24 h, and the obtained LDL stock solution was stored under N_2 atmosphere in the dark (4°C) for less than 72 h upon expiration[52]. Copper mediated LDL oxidation was performed by incubating LDL (500 μg protein ml^{-1} in 10 mM phosphate buffer, pH 7.4) with freshly made $CuCl_2$ (10 μmol l^{-1}) at 37°C for 22 h. Adding 1 mM EDTA stopped the reaction. The extent of LDL oxidation was measured from the following parameters. (a) TBARS was according to the method of Kitts *et al.*[54] Conjugate dienes were measured at 234 nm with 10 times diluted incubated sample. □ = TBARS; ◆—◆—◆ = conjugate diene. (b) LDL oxidation was evaluated with agarose gel (0.6%) electrophoresis running in 50 mM barbital buffer, pH 8.6 and staining with Sudan Black B. (c) Mobility of native and oxidized LDL. Percentage inhibition on agarose gel electrophoresis was calculated as follows: Inhibition rate $(\%) = (C - S)/C \times 100\%$, where C = electrophoretic mobile distance (cm) of LDL treated with $CuCl_2$ and S = electrophoretic mobile distance (cm) of LDL treated with $CuCl_2$ as well as CNT-2000. □ = mobility, ◆—◆—◆ = % control mobility inhibition. (d) Fluorescence readings of native and oxidized LDL were taken with 30 times diluted sample in PBS (pH 7.4) at emission (430 nm) and excitation (360 nm) wavelengths

to long-term exposure of ginseng when consumed at levels up to 15 g day^{-1} (Ref. 63). Average intakes of ginseng were equivalent to consuming 6 × 500 mg ginseng capsules daily and produced side effects that included hypertension, gastrointestinal disturbances, insomnia and nervousness. The validity of these observations is difficult to evaluate because of the absence of a placebo treatment in the study and the fact that subjects were not controlled for other bioactive substance intake (e.g. caffeine). Moreover, as is the case with many studies with ginseng, the ginsenoside content of the ginseng consumed was not determined. Notwithstanding this, however, other subjects from this study who had consumed extremely high intakes of ginseng (e.g. greater than 15 g day^{-1}), showed symptoms of confusion and depression. This level of ginseng consumption far exceeds the German Commission E's[64] recommended daily intake of 1–2 g day^{-1} of Asian ginseng, containing 4–5% ginsenoside[65].

Ginseng may contain an endocrine-like active substance which can affect neonate development[66]. In one study, oestrogen-like activity attributed to chronic ginseng use was reported to cause swollen and painful breasts[67]. Ginseng–drug interactions have been observed in a few isolated situations, which include phenelzine, a monoamine oxidase inhibitor[68], and warfarin[69], an agent used to modulate blood viscosity factors. In both reports, there was little information that adequately characterized the prevalence and extent of risk attributed to this interaction. It is

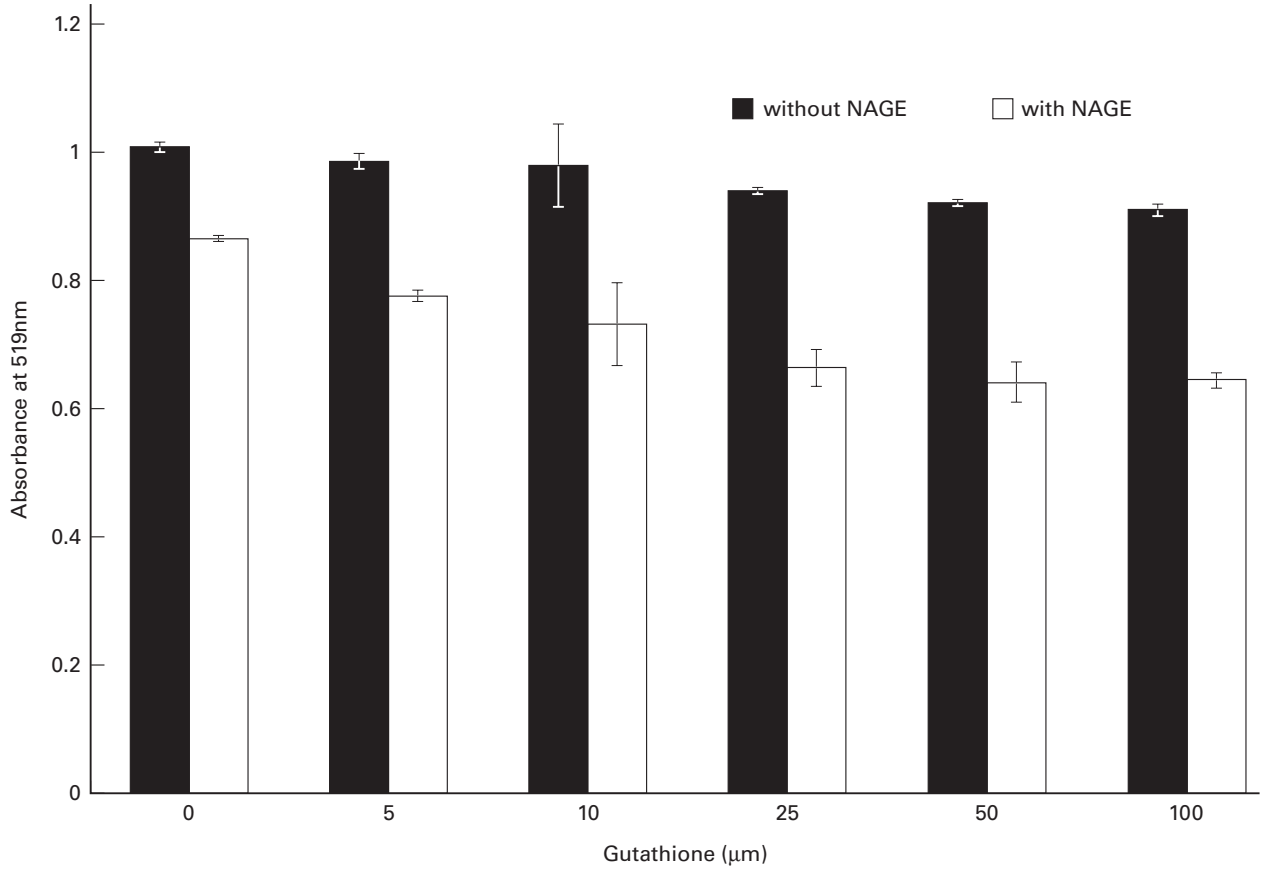

Fig. 6 Regeneration of CNT-2000 by glutathione in 1,1-diphenyl-2-picylhydrzyl analysis. A solution of DPPH stable radical (0.1 mM) was made to a final concentration in ethanol (95%) and was incubated with CNT-2000 (0.5 mg ml^{-1}), as well as with various amounts of glutathione. Absorbance readings were taken at 519 nm using a spectrophotometer. ■ = without NAGE; □ = with NAGE

noteworthy, however, that caution has been expressed concerning the use of Asian ginseng to combat acute stress, due to the fact that the hypertensive effect associated with these individuals is contra-indicated[70].

Finally, with the antioxidant characterization of ginseng reported herein, no prooxidant activity of the NAGE was observed. The copper chelating activity and low reducing potential of the NAGE represented an interesting property not present for all bioactive plant compounds possessing antioxidant activity. Compounds such as ascorbic acid[71–73] as well as polyphenolics, including myricetin[74,75] and the catechins in green tea[75], are effective metal chelators and thus possess antioxidant activity. Owing to their substantial reducing power, these compounds can also behave as prooxidant agents to carbohydrates, protein and DNA by reducing metal ions in redox reactions that are intrinsically involved in enhancing the rate of hydroxyl radical generation by the Haber–Weiss reaction[76], resulting in eventual breakdown of these macromolecules. The high redox potential of ascorbic acid has been shown to accelerate reduction of Cu^{2+} to its lower valency Cu^{1+} in aqueous medium and to promote accelerated DNA strand scission[77]. Our characterization of the NAGE in this study suggests that the formation of a Cu^{2+}–ginseng complex was sufficient to inhibit a further reaction with H_2O_2 and subsequent generation of a hydroxyl radical induced prooxidant activity. Further confirmation of this suggestion was obtained with the site-specific 2-deoxyribose assay results shown in Fig. 7. Owing to the limited reducing power of NAGE, very little prooxidant activity was observed over the concentration range 0.25–2 mg ml^{-1} NAGE in the presence of a Cu^{2+}-mediated Fenton reaction. The formation of TBARS, derived from 2-deoxyribose fragment production and resulting in hydroxyl radical generated from the Fenton reaction was greatest with L-ascorbic acid and only minimally increased with higher North American ginseng concentrations.

Conclusions

Ginseng has been shown in many studies to possess biological activity, but only recently have the specific mechanisms underlying these biological activities been shown at both the molecular and cellular levels. An inherent difficulty in many studies was the use of undefined ginseng extracts, which precluded confirmation of findings and absolute assessment of efficacy and safety by subsequent studies whereby different sources of ginseng

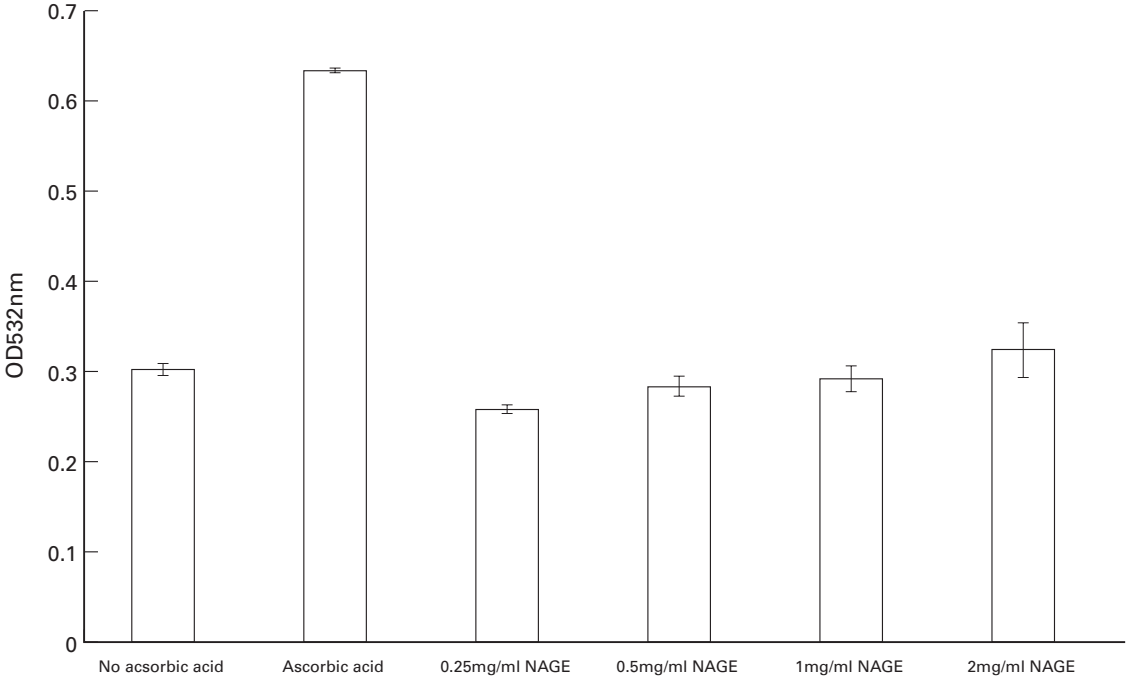

Fig. 7 Prooxidant assay. A modification of the method reported by Kitts *et al.*[54] was used. Briefly, the reaction system contained 3.6 mM 2-deoxyribose, 0.1 mM $CuCl_2$, 1 mM H_2O_2, 0.1 mM EDTA as well as the sample. The reaction mixture was incubated at 37°C for 1 h, followed by mixing with an equal volume of 10% (w/v) TCA and 0.5% TBA. OD_{532nm} measurements were taken after samples were incubated at 100°C for 15 min. If absorbance readings taken at 532 nm were higher than that of treatment without ascorbic acid, prooxidation was confirmed. Values represent mean ± SEM ($n = 8$)

were employed. This limitation is particularly true for ginseng, as evidenced by the apparent differences in pharmacological effects noted between *Panax ginseng* and *Panax quinquefolium*, and the fact that these prominent species of ginseng vary characteristically from the standpoint of the complex mixture of numerous potentially bioactive constituents (e.g. ginsenosides). The implications that this has for commercial products were pointed out in a survey of 50 commercial ginseng preparations, from 11 different countries, in which greater than 90% of the preparations varied between 2 and 9% in total ginsenoside content and some preparations contained no ginsenosides[78]. The availability of standardized extracts for both Asian and North American ginseng will assist greatly in advancing our knowledge on the role of this traditionally used herb as a dietary modulator of various physiological processes. The availability of standardized extracts of both Asian and North American ginseng will also complement the additional need for comparative, controlled double-blind human clinical studies that will assess the relative efficacy and safety of ginseng.

Acknowledgements

We wish to thank Dr. Micheal Li, Chai-Na-Ta Corp., Langley, British Columbia, for donation of the CNT-2000 North American ginseng extract. This work was supported by a NSERC strategic grant.

References

1 Liu CX, Xiao PG. Recent advances on ginseng research in China. *J. Ethnopharmacol.* 1992; **36**: 27–38.
2 Gillis CN. *Panax ginseng* pharmacology: A nitric acid link? *Biochem. Pharmacol.* 1997; **54**: 1–8.
3 Attele AS, Wu JA, Yuan CS. Ginseng pharmacology. *Biochem. Pharmacol.* 1999; **58**: 1685–93.
4 Vogler BK, Hittler MH, Ernst E. The efficacy of ginseng. A systematic review of randomised clinical trials. *Eur. J. Clin. Pharmacol.* 1999; **55**: 567–75.
5 Lee YS, Chung IS, Lee IR, Kim KH, Hong WS, Yun YS. Activation of multiple effector pathways of immune system by the antineoplastic immunostimulator acid polysaccharide ginsan, isolated from *Panax ginseng*. *Anticancer Res.* 1997; **17**: 323–31.
6 Kim K, Lee YS, Jung IS, *et al*. Acidic polysaccharide from *Panax ginseng*, ginsan, induces TH-1 cell and macrophage cytokines and generates LAK cells in synergy with IL-2. *Planta Med.* 1998; **64**: 110–15.
7 Ma YC, Zhu J, Luo L, *et al*. A comparative evaluation of ginsenosides in commercial ginseng products and tissue culture samples using HPLC. *J. Herb Spices Med. Plants* 1995; **3**: 41–50.
8 Li TSC, Mazza G, Cottrell AC, Gao L. Ginsenosides in roots and leaves of American ginseng. *J. Agric. Food Chem.* 1996; **44**: 717–20.
9 Yuan C-S, Wu JA, Lowell T, Gu M. Gut and brain effects of American ginseng root on brainstem neuronal activities in rats. *Am. J. Chin. Med.* 1998; **26**: 47–55.
10 Mizuno M, Yamada J, Terai H, Kozukue N, Lee YS, Tsuchida H. Differences in immunomodulating effects between wild and cultured *Panax ginseng*. *Biochem. Biophys. Res. Commun.* 1994; **200**: 1672–8.
11 Marasco C, Vargas Ruiz R, Salas Villagomez A, Begona Infante C. Double blind study of a multivitamin complex

supplemented with ginseng extract. *Drugs Exp. Clin. Res.* 1998; **22**: 323–9.
12 Zuin M, Attezzati ZM, Camisasca M, Riebenfield D, Podda M. Effects of a preparation containing a standardized ginseng extract combined with trace elements and multi-vitamins against hepatotoxin-induced chronic liver disease in the elderly. *J. Int. Med. Res.* 1987; **15**: 276–81.
13 Takino Y, Odani T, Hisayuki T, Hayashi T. Studies on the absorption, distribution, excretion and metabolism of ginseng saponins. I. Quantitative analysis of ginsenoside Rg1 in rats. *Chem. Pharm. Bull.* 1982; **30**: 2196–210.
14 Kajiwara H, Hewmming AM, Hirano H. Evidence of metal binding activities of pentadecapeptid from *Panax ginseng*. *J. Chromatogr.* 1996; **687**: 443–8.
15 Hasegawa H, Sung J-H, Benno Y. Role of human intestinal *prevotella oris* in hydrolyzing ginseng saponins. *Planta Med.* 1997; **63**: 436–40.
16 Lee BH, Lee SJ, Hui JH, Lee S, Huh JD, Moon CK. In vitro antigenotoxic activity of novel ginseng saponin metabolites formed by intestinal bacteria. *Planta Med.* 1998; **64**: 500–3.
17 Brekham II, Dardymov IV. New substances of plant origin, which increase non-specific resistance. *Annu. Rev. Pharmacol.* 1969; **9**: 419–30.
18 Pieralisi G, Ripari P, Vecchiet L. Effects of standardized ginseng extract combined with dimethylaminoethanol bitartrate, vitamins, minerals and trace elements on physical performance during exercise. *Clin. Ther.* 1991; **13**: 373–82.
19 Brahrke MS, Morgan WP. Evaluation of the ergogenic properties of ginseng. *Sports Med.* 1984; **18**: 229–48.
20 Hassan-Samira MM, Attia Attia M, Allam M, Elwan O. Effect of the standardized ginseng extract G115 on the metabolism and electrical activity of the rabbit's brain. *J. Int. Med. Res.* 1985; **13**: 342–8.
21 Kirchdorfer AM. Clinical trials with the standardized ginsenoside concentrate G115. In: Chang HN, Yeung HW, Tso WW, Koo A, eds. *Advances in Chinese Medicinal Materials Research*. Singapore: World Scientific Publishers, 1985: 529–42.
22 Wang L, Lee T. Effect of ginseng saponins on exercise performance in non-trained rats. *Planta Med.* 1998; **64**: 130–3.
23 Cherdrungsi P, Rungroeng D. The effects of standardized extract and exercise training on aerobic and anaerobic exercise capacities in humans. *Korean J. Ginseng Sci.* 1995; **19**: 93–100.
24 Engels HJ, Said JM, Wirth JC. Failure of chronic ginseng supplementation to affect work performance and energy metabolism in healthy adult females. *Nutr. Res.* 1996; **16**: 1295–1305.
25 Morris AC, Jacobs I, McLellan TM, Klugerman A, Wanga LCH, Zamecnik J. No ergogenic effect of ingestion. *Int. J. Sport Nutr.* 1996; **6**: 263–71.
26 Herman J, Engels HJ, Wirth J. No ergogenic effects of ginseng (*Panax ginseng* C.A. Meyer) during graded maximal exercise aerobic exercise. *J. Am. Diet. Assoc.* 1997; **97**: 1110–15.
27 Fulder SJ. Ginseng and the hypothalamic-pituitary control of stress. *Am. J. Chin. Med.* 1981; **9**: 112–18.
28 Hiai S, Yokoyama H, Oura H, Kawashima Y. Evaluation of corticosterone-secretion-inducing activities of ginsenosides and their prosapogenins and sapogenins. *Chem. Pharm. Bull.* 1983; **31**: 168–74.
29 Lee YJE, Chung K, Lee YH, Lee B, Huh B, Lee SK. Ginsenoside-Rg1, one of the major active molecules from *Panax ginseng*, is a functional ligand of glucocorticoid receptor. *Mol. Cell. Endocrinol.* 1997; **113**: 135–40.
30 Hiai S, Yokoyama H, Oura H. Features of ginseng saponin induced corticosterone release. *Endocrinol. Jpn.* 1979; **26**: 737–40.
31 Waki I, Kyo H, Yasuda M, Kimura M. Effects of hypoglycemic component of ginseng radix on insulin biosynthesis in normal and diabetic animals. *J. Pharmacobio-dynamics.* 1982; **5**: 547–9.
32 Sotaniemi EA, Haapakoski E, Rautio A. Ginseng therapy in non-insulin dependent diabetic patients. *Diabetes Care* 1995; **18**: 1373–5.
33 Yuan YV, Kitts DD. Endogenous antioxidants. Role of antioxidant enzymes in biological systems. In: Shahidi F, ed. *Natural Antioxidants: Chemistry, Health Effects and Applications*. Champaign, IL: AOCS Press, 1996: 258–70.
34 Deng HL, Zhang JT. Anti-lipid peroxidative effect of ginsenoside Rb1 and Rg1. *Chin. Med. J.* 1991; **104**: 395–8.
35 Rimar S, Lee-Mengel M, Gillis CN. Pulmonary protective vasodilating effects of a standardized *Panax ginseng* preparation following artificial gastric digestion. *Pulm. Pharmacol.* 1996; **9**: 205–9.
36 Voces J, Alvarez AI, Vila L, Ferrando A, Cabral de Oliveria C, Prieto JG. Effects of administration of the standardized *Panax ginseng* extract G-115 on hepatic antioxidant function after exhaustive exercise. *Comp. Biochem. Physiol.* 1999; **123**: 175–84.
37 Jeong TC, Kim HJ, Park JI, *et al.* Protective effects of red ginseng saponins against carbon tetrachloride-induced hepatotoxicity in Sprague-Dawley rats. *Planta Med.* 1997; **63**: 136–40.
38 Li Y, Zhao XJ. Effects of panaxadiol saponins on the contents of serum enzymes, lipid peroxides and SOD in hemorrhagic shock dogs. *Chin. J. Pathophysiol.* 1989; **5**: 539–44.
39 Hui-Ling D, Jun-Tian Z. Anti-lipid peroxidative effect of ginsenoside Rb1 and Rg2. *Chin. Med. J.* 1991; **104**: 395–8.
40 Kim YH, Park KH, Rho HM. Transcriptional activation of the Cu/Zn superoxide dismutase gene through the AP2 site by ginsenoside Rb2 extracted from a medicinal plant, *Panax ginseng*. *J. Biol. Chem.* 1996; **271**: 24539–43.
41 Zhang YG, Liu TP. Protective effects of total saponins of *Panax ginseng* on ischemia-reperfusion injury in rat brains. *Chin. J. Pharmacol. Toxicol.* 1994; **8**: 7–12.
42 Chen X, Gillis CN. Effect of free radicals on pulmonary vascular response to acetylcholine. *J. Appl. Physiol.* 1991; **71**: 821–5.
43 Kim HY, Chen X, Gillis CN. Ginsenosides protect pulmonary vascular endothelium against free radical-induced injury. *Biochem. Biophys. Res. Commun.* 1992; **189**: 670–6.
44 Yuan C-S, Attele AS, Wu JA, Lowell TK, Gu Z, Lin Y. *Panax quinquefolium* L. inhibits thrombin-induced endothelin release in vitro. *Am. J. Chin. Med.* 1999; **27**: 331–8.
45 Wink DA, Hanbauer I, Krishna MC, DeGraft W, Ganson J, Mitchell JB. Nitric oxide protects against cellular damage and cytotoxicity from reactive oxygen species. *Proc. Natl. Acad. Sci.* 1993; **90**: 9813–17.
46 Kang SY, Schini-Kerth VB, Kim ND. Ginsenosides of the protopanaxatriol group causes endothelium-dependent relaxation in the rat aorta. *Life Sci.* 1995; **56**: 1577–86.
47 Peng CF, Li YJ, Li YJ, Deng HW. Effects of ginsenosides on vasodilator nerve actions in the rat perfused mesentery are mediated by nitric oxide. *J. Pharm. Pharmacol.* 1995; **47**: 614–17.
48 Maffei-Facino R, Carini R, Aldini G, Berti F, Rossoni G. *Panax ginseng* administration in the rat prevents myocardial ischemia-reperfusion damage induced by hyperbaric oxygen: evidence for an antioxidant intervention. *Planta Med.* 1999; **65**: 614–19.
49 Abuja PM, Murkovic M, Pfannhauser W. Antioxidant and prooxidant activities of elderberry (*Sambucus nigra*) extract in low-density lipoprotein oxidation. *J. Agric. Food Chem.* 1998; **46**: 4091–6.
50 Wang H, Cao G, Prior RL. Oxygen radical absorbing capacity of anthocyanins. *J. Agric. Food Chem.* 1997; **45**: 304–9.
51 Kitts DD, Yuan YV, Wijewickreme AN, Thompson LU. Antioxidant activity of the flaxseed lignan secoisolaiciresinol

diglycoside and its mammalian lignan metabolites enterodiol and enterolactone. *Mol. Cell. Biochem.* 1999; **202**: 91–100.
52 Hu C, Kitts DD. Studies on the antioxidant activity of echinacea root extract. *J. Agric. Food Chem.* 2000; **48**: 466–72.
53 Zhang D, Yasuda Y, Yu P, *et al.* Ginseng extract scavenges hydroxyl radical and protects unsaturated fatty acids from decomposition caused by iron-mediated lipid peroxidation. *Free Radical Biol. Med.* 1996; **20**: 145–50.
54 Kitts DD, Wijewickreme AN, Hu C. Antioxidant properties of a North American ginseng extract. *Mol. Cell. Biochem.* 2000; **203**: 1–10.
55 Huong NTT, Matsumoto K, Kasai R, Yamosaka K, Watanabe H. In vitro antioxidant activity of vietnamese ginseng saponin and its components. *Biol. Pharm. Bull.* 1998; **21**: 978–81.
56 Zhong GG, Qi H, Zhao CY, Jiang Y. Comparative observation on the influence of 11 ginsenoside monomers on the free radical contents of myocardiocytes with the electron spin resonance method. *Acta Biochem. Biophys. Sin.* 1993; **25**: 667–71.
57 Mei B, Wang YF, Wu JX, Chen WZ. Protective effects of ginsenosides on oxygen free radical induced damages of cultured vascular endothelial cells in vitro. *Acta Pharm. Sin.* 1994; **29**: 801–8.
58 Suh DY, Han YN, Han BH. Malitol, an antioxidant component of Korean red ginseng shows little prooxidant activity. *Arch. Pharm. Res.* 1996; **19**: 112–15.
59 Juurlink BNJ. Management of oxidative stress in the CNS: The many roles of glutathione. *Neurotoxicity Res.* 1999; **1**: 119–40.
60 Coteller N, Bernier JL, Henichart JP, Catteau JP, Gaydou E, Wallet JC. Scavenger and antioxidant properties of ten synthetic flavones. *Free Radical Biol. Med.* 1992; **13**: 211–19.
61 Ko FN, Cheng ZJ, Lin CN, Teng CM. Scavenger and antioxidant properties of prenylflavones isolated from *Artocarpus heterophyllus*. *Free Radical Biol. Med.* 1998; **25**: 166–8.
62 Hess FG, Parent RA, Stevens KR, Cox GE, Becci PJ. Effect of subchronic feeding of ginseng extract G-115 in beagle dogs. *Food Chem. Toxicol.* 1983; **21**: 95–7.
63 Siegel RK. Ginseng abuse syndrome: Problems with the panacea. *JAMA* 1979; **241**: 1614–15.
64 *The German Commission E Monographs*. Translated into English: Blumenthal M, Hall T, Rister R, eds. Klein S, Rister R, transl. Austin, TX: American Botanical Council, 1996.
65 Klepser TB, Klepser ME. Unsafe herbal therapies. *Am. J. Health System Pharm.* 1999; **56**: 125.
66 Awang DVC. Maternal use of ginseng and neonatal androgenization. [Letter, comment]. *JAMA* 1991; **265**: 1828.
67 Palmer BV, Montgomery ACV, Monteiro JCMP. Ginseng and mastalgia. *BMJ* 1978; **6122**: 1284.
68 Jones BD, Runkis AM. Interaction of ginseng with phenelzine. *J. Clin. Psychopharmacol.* 1987; **7**: 201–2.
69 Janetzky K, Morreale AP. Probable interactions between warfarin and ginseng. *Am. J. Health Syst. Pharm.* 1997; **54**: 692–3.
70 Awang DVC. The anti-stress potential of North American ginseng (*Panax quinquefolius* L.). *J. Herbs, Spices Med. Plants* 1998; **62**: 87–91.
71 Mahoney JR, Graft E. Role of alpha-tocopherol, ascorbic acid, citric acid and EDTA as oxidants in model systems. *J. Food Sci.* 1986; **51**: 1293–6.
72 Aruoma OI, Evans PJ, Kaur H, Sutcliffe L, Halliwell B. An evaluation of the antioxidant and potential prooxidant properties of food additives and trolox C, vitamin E and probucol. *Free Radical Res. Commun.* 1990; **10**: 143–57.
73 Biaglow JE, Manevich Y, Uckin F, Held KD. Quantitation of hydroxyl radicals produced by radiation and copper-linked oxidation of ascorbate by 2-deoxy-D-ribose method. *Free Radical Biol. Med.* 1997; **22**: 1129–38.
74 Laughton ML, Halliwell PL, Evans PL, Hoult JRS. Antioxidant and prooxidant actions of the plant phenolics, quercetin, gossypol and myricetin. *Biochem. Pharmacol.* 1989; **38**: 2859–65.
75 Roedig-Penman A, Gordon MH. Antioxidant properties of catechins and green tea extracts in model food emulsions. *J. Agric. Food Chem.* 1997; **45**: 4267–70.
76 Halliwell B, Grootveld M, Gutteridge B. Methods for the measurement of hydroxyl radicals in biochemical systems. Deoxyribose degradation and aromatic hydroxylation. *Methods Biochem. Anal.* 1987; **33**: 59–87.
77 Ueda J, Saito N, Shimazu Y, Ozawa T. A comparison of scavenging abilities of antioxidants against hydroxyl radicals. *Arch. Biochem. Biophys.* 1996; **333**: 377–84.
78 Cui J, Garle M, Bjorkhem I, Eneroth P. What do commercial ginseng preparations contain? *Lancet* 1994; **344**: 134.
79 Choi SW, Cho EH, Chi SY. Ginsenosides activate DNA polymerase from bovine placenta. *Life Sci.* 1995; **57**: 1359–65.
80 Tachikawa E, Kudo K, Kashimoto T. Ginseng saponins reduce acetylcholine-evoked Na^+ influx and catecholamine secretion in bovine adrenal chromaffin cells. *J. Pharmacol. Exp. Ther.* 1995; **273**: 629–36.
81 See DM, Broumand N, Sahl L, Tills JG. In vitro effects of echinacea and ginseng on natural killer and antibody-dependent cell cytotoxicity in healthy subjects and chronic fatigue syndrome or acquired immunodeficient syndrome patients. *Immunopharmacology* 1997; **35**: 229–35.
82 Kenarova B, Neychev H, Hadijiivariova C, Petkov VD. Immunomodulatory activity of ginsenoside Rg1 from *Panax ginseng*. *Jpn. J. Pharmacol.* 1990; **54**: 447–54.
83 Ro JY, Ahn YS, Kim KH. Inhibitory effect of ginsenoside on the mediator release of the guinea pig mast cells activated by specific antigen-antibody reactions. *Int. J. Immunopharmacol.* 1998; **20**: 625–41.
84 Lui J, Zhang JT. Immunoregulatory effects of ginsenoside Rg1 in aged rats. *Yao Hsueh Hsueh Pao-Acta Pharm. Sin.* 1995; **30**: 818–23.
85 Lui J, Wang S, Liu H, Yang L, Nan G. Stimulatory effect of saponin from *Panax ginseng* on immune function of lymphocytes in the elderly. *Mech. Ageing Dev.* 1995; **83**: 43–53.
86 Matsunaga H, Katano M, Yamamoto H, Mori M, Takata K. Studies on the panaxatriol of *Panax ginseng* C.A. Meyer. Isolation, determination and antitumour activity. *Chem. Pharm. Bull.* 1989; **37**: 1281–91.
87 Duda RB, Zhong Y, Navas V, Li MZC, Toy BR, Alavarez JG. American ginseng and breast cancer therapeutic agents synergistically inhibit MCF-7 breast cancer cell growth. *J. Surg. Oncol.* 1999; **72**: 230–9.
88 Tode T, Kikuchi Y, Kita T, Hirata J, Imaizumi I, Nagata I. Inhibitory effects by oral administration of ginsenoside Rh2 on the growth of human ovarian cancer cells in nude mice. *J. Cancer Res. Clin. Oncol.* 1993; **120**: 24–6.
89 Zhu JH, Takeshita T, Kitagawa I, Morimorto K. Suppression of the formation of sister chromatid exchanges by low concentrations of ginsenoside Rh2 in human blood lymphocytes. *Cancer Res.* 1995; **55**: 1221–3.
90 Wakabaayashi C, Murakami K, Hasegawa H, Murata J, Saiki I. An intestinal bacterial metabolite of ginseng protopanaxadiol saponins has the ability to induce apoptosis in tumour cells. *Biochem. Biophys. Res. Commun.* 1998; **246**: 725–30.
91 Oh M, Choi YH, Choi SH, *et al.* Antiproliferating effects of ginsenoside Rh2 on MCF-& human breast cancer cells. *Int. J. Oncol.* 1999; **14**: 869–75.
92 Kim T-H, Lee Y-S, Cho C-K, Park S, Choi S-Y, Yoo S-Y. Protective effect of ginseng on radiation-induced DNA double strand breaks and repair in murine lymphocytes. *Cancer Biother. Radiopharm.* 1996; **11**: 267–72.
93 Mochizuki M, Yoo YC, Maatsuzawa K, *et al.* Inhibitory effect of tumor metastasis in mice by saponins, ginsenoside-Rb2,

20(R)- and 20 (S)-ginsenoside-Rg3, of red ginseng. *Biol. Pharm. Bull.* 1995; **18**: 1197–1202.
94 Yun TK. Experimental and epidemiological evidence of the cancer-preventative effects of *Panax ginseng* C.A. Meyer. *Nutr. Rev.* 1996; **54**: S71–81.
95 Yun T-K, Choi SY. Non-organ specific cancer prevention of ginseng: a prospective study in Korea. *Int. J. Epidemiol.* 1998; **27**: 359–64.
96 Zhang Y, Xu XU, Jiang YP. Effects of ginsenosides on myocardial ischemia/re-perfusion damage in open heart surgery patients. *Med. J. China* 1994; **74**: 626–8.
97 Lee J-H, Park H-J. Effects of lipophilic fraction from Korean red ginseng on platelet aggregation and blood coagulation in rats fed with corn oil and beef tallow diet. *Korean J. Ginseng Sci.* 1995; **19**: 206–11.
98 Chen X. Cardiovascular protection by ginsenosides and their nitric oxide releasing action. *Clin. Exp. Pharmacol. Physiol.* 1996; **23**: 728–32.
99 Huang YS. Effect of ginsenosides Rb1 and Rg1 on lipid peroxidation of rat in vitro. *Chung Kuo I Shueh Ko Hsueh Yuan Hsueh Pao* 1989; **11**: 460–2.
100 Kim J-S, Kim K-W, Choi K-J, *et al.* Screening of active components of red ginseng saponin. *Korean J. Ginseng Sci.* 1996; **20**: 173–8.
101 Chang MS, Choi KJ, Rho HM. Effect of the contents of ratio of panaxadiol ginsenosides extracted from compartments of ginseng on the transcription of Cu/Zn superoxide dismutase gene. *J. Ginseng Res.* 1999; **23**: 44–9.