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## Efficacy and safety of ginseng

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### 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<sup>1–3</sup>. 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.*<sup>4</sup> 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

proportions among different species of ginseng<sup>1,3</sup>. 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<sup>5,6</sup>. 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<sup>7</sup>.

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<sup>8</sup>. 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*<sup>9</sup>. 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<sup>10</sup>, 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<sup>11,12</sup>.

### Ginseng metabolism

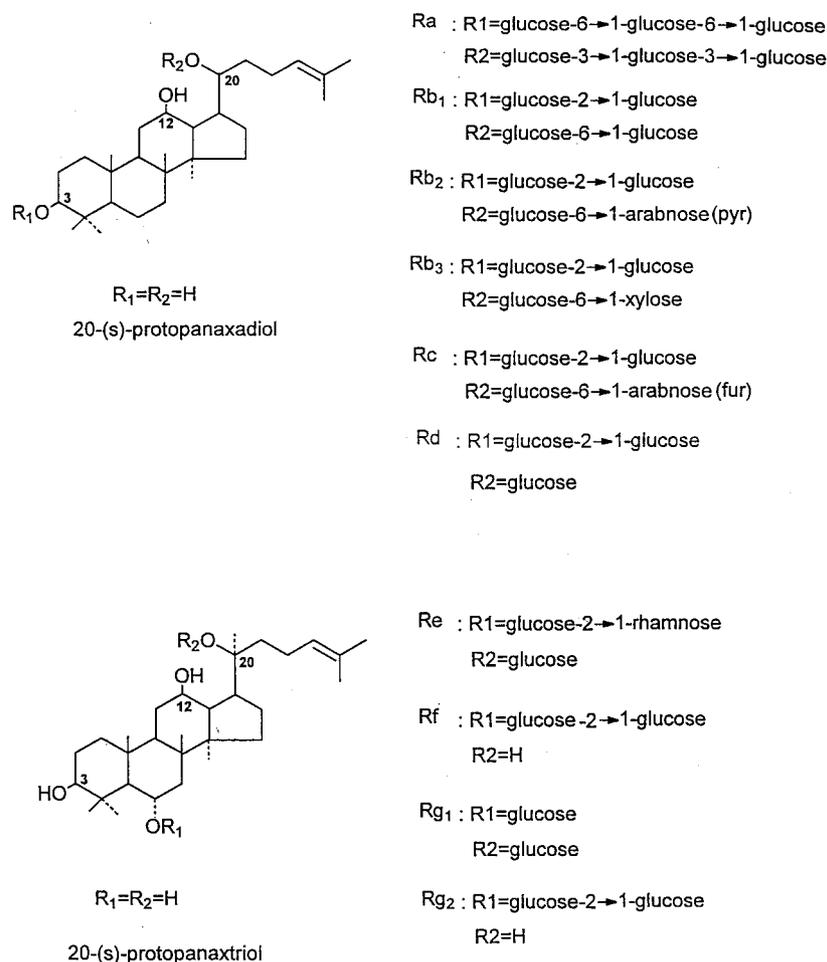
Central to the difficulties in explaining, in scientific terms, the underlying biochemical mechanisms for various

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<sup>13</sup>. Very small recoveries of this ginsenoside 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)<sup>14</sup>. 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<sup>15</sup>, and which in turn are the likely forms absorbed from the intestine. Bacterial metabolites of ginseng have also been reported to have antigenotoxic properties<sup>16</sup>. 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<sup>17</sup> are listed in Table 1, and probably involve changes in tissue oxygen uptake and transitory alterations in carbohydrate and lipid utilization. Peralisi *et al.*<sup>18</sup> 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<sup>19</sup>. 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<sup>20</sup>. Reduced lactic acid metabolism by ginseng has also been reported in athletes undergoing intense exercise after consumption of 4% ginsenoside<sup>21</sup>. 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<sup>22</sup>. 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<sup>22–28</sup> and incorporated in sports drinks to enhance athletic performance<sup>23</sup>. Many of these studies, however, have questioned the proposed ergogenic effect of ginseng<sup>24–26</sup>. In a clinical study comprising two dosage treatment groups receiving 8 and 16 mg kg<sup>-1</sup> 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<sup>25</sup>. A randomized, double-blinded, placebo controlled study of healthy men receiving 200 and 400 mg day<sup>-1</sup> 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<sup>26</sup>. Similar

results have been reported in healthy adult females using a randomized, double-blind placebo-controlled experimental design<sup>25</sup>.

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<sup>27</sup> and corticosterone concentrations<sup>28</sup>. 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<sup>29</sup>. 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<sup>30</sup>. 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 leukotriene 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<sup>31</sup>. 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<sup>32</sup>. 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<sup>33</sup>, 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,  $\beta$ -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<sup>33</sup>.

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<sup>34–36</sup>. Common to these studies has been the demonstration of a dose-dependent, ginseng-induced increase in GSH-P<sub>x</sub> 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<sub>x</sub> and SOD activities<sup>36</sup>. Marked protective effects of ginseng against chemical induced hepatotoxicity has also been reported<sup>37</sup>, 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<sup>38</sup>, and characterized in a ferrous/cysteine peroxide induction model system using hepatic microsomes<sup>39</sup>. The

**Table 2** Standards of evidence for ginseng antioxidant activity

Evidence	Reference
<i>In vitro model mechanisms</i>	
Inhibition of Fenton reaction induced radicals	
(a) Fe <sup>2+</sup> -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
<i>In vivo mechanisms</i>	
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<sup>39</sup>. These same workers observed significant reductions (28%) in malonaldehyde and associated increases in GSH-P<sub>x</sub> (96.4%) and CAT (47%) after administering 50 mg kg<sup>-1</sup> day<sup>-1</sup> 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<sup>40</sup> 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<sup>1</sup> 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 ischaemic re-perfusion injury in the rat brain<sup>41</sup>. 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<sup>2,42</sup>. 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<sup>42-44</sup>. Collectively, the findings that nitric oxide protects against Chinese hamster fibroblast V79 cytotoxicity induced by generation of reactive oxygen species<sup>45</sup>, and the fact that ginseng increases both nitrite and cGMP levels in rat serum and urine<sup>46</sup>, as well as facilitating endogenous nitric oxide release<sup>47</sup>, 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<sup>44</sup>. Ginseng extracts derived from *Panax ginseng* have also been shown to exhibit a dose-dependent protection against free radical induced injury in pulmonary<sup>35</sup> and myocardial<sup>48</sup> 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<sup>35</sup>.

In recent years, numerous components derived from fruit<sup>49</sup>, vegetable<sup>50</sup>, oilseed<sup>51</sup> and herb<sup>52</sup> 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<sup>53</sup>. 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<sup>54</sup>. 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 vietnamsis* root extract showed inhibition of TBARS formation in mouse tissue homogenate<sup>55</sup>, 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

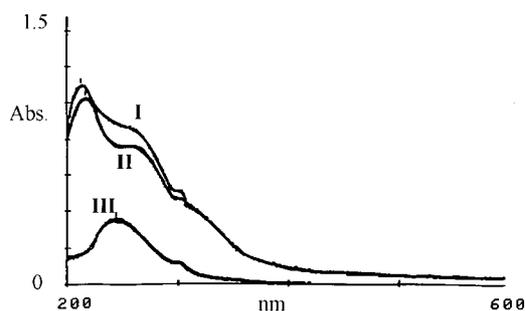
conclusion that this ginsenoside inhibits peroxy radical induced DNA breakage (Hu and Kitts, unpublished data). Ginsenosides Rb1, Rb2, Rb3 and Rc, but not Rd, have also been shown to protect against superoxide radical induced damage of cultured cardiac myocytes<sup>56</sup>. Similar *in vitro* studies conducted with individual ginsenosides have indicated some degree of protection against free radical induced endothelial cell damage<sup>57</sup>. 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<sup>58</sup>. 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<sup>-1</sup> NAGE ginseng (line I) and 100 μM CuCl<sub>2</sub> (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<sub>2</sub> solutions. A shift in absorption maxima from 217 nm to 245 nm occurred in the differential spectrum when CuCl<sub>2</sub> was incubated with NAGE (line III), thus denoting chelation of Cu<sup>2+</sup>.

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



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

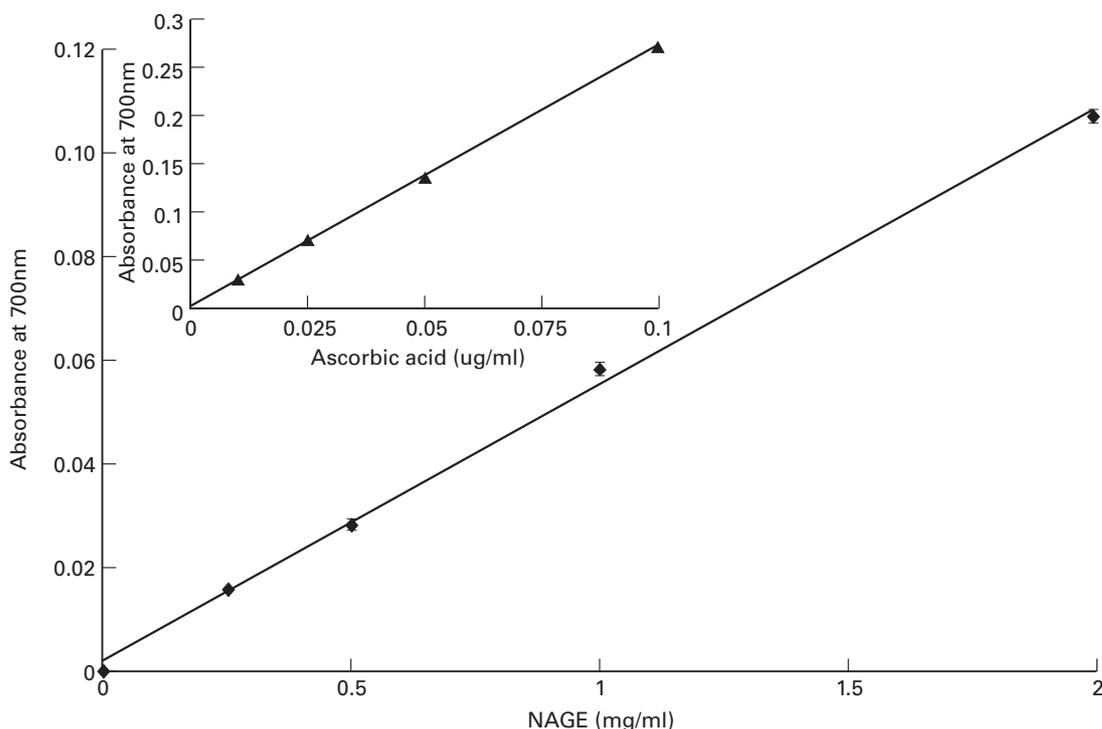
limited reducing power. From both standard curves, it was calculated that 1 mg of the NAGE was equivalent to 20.3 ± 0.3 μg ascorbic acid.

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<sup>2+</sup> (lanes 2–5), indicating a relationship between hydroxyl radical generation and concentration of Fe<sup>2+</sup> according to the following scheme: Fe<sup>2+</sup> + H<sub>2</sub>O → ·OH + <sup>-</sup>OH + Fe<sup>3+</sup>. 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<sup>2+</sup>, 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<sup>2+</sup>-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<sup>2+</sup>-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<sup>2+</sup>-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<sup>-1</sup> NAGE is shown in Fig. 6. A NAGE concentration of 0.5 mg ml<sup>-1</sup> produced a significant reduction (15%) in DPPH 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 DPPH radicals. Maximal ability to quench DPPH radicals was obtained with 0.5 mg ml<sup>-1</sup> NAGE and 25–100 μM GSH.

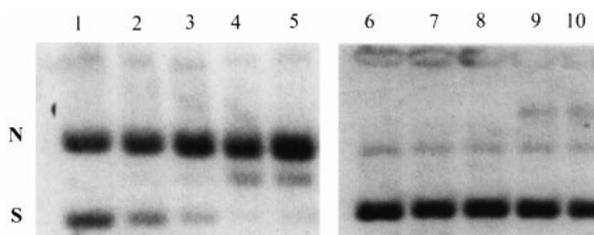
The observation that GSH was effective at scavenging DPPH radicals at a critical concentration confirms an antioxidant activity role to deactivate electrophilic free radicals. GSH is also an important component of the antioxidant enzymes, GSH-P<sub>x</sub> and glutathione reductase<sup>32</sup>, as well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



**Fig. 3** Reducing power of NAGE: the method followed was that of Hu and Kitts<sup>52</sup> using L-ascorbic acid for comparison. Briefly, NAGE (0~2.0 mg ml<sup>-1</sup> 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  $\alpha$ -tocopherol<sup>59</sup>. In the present study, NAGE also effectively scavenged DPPH; however, when in the presence of

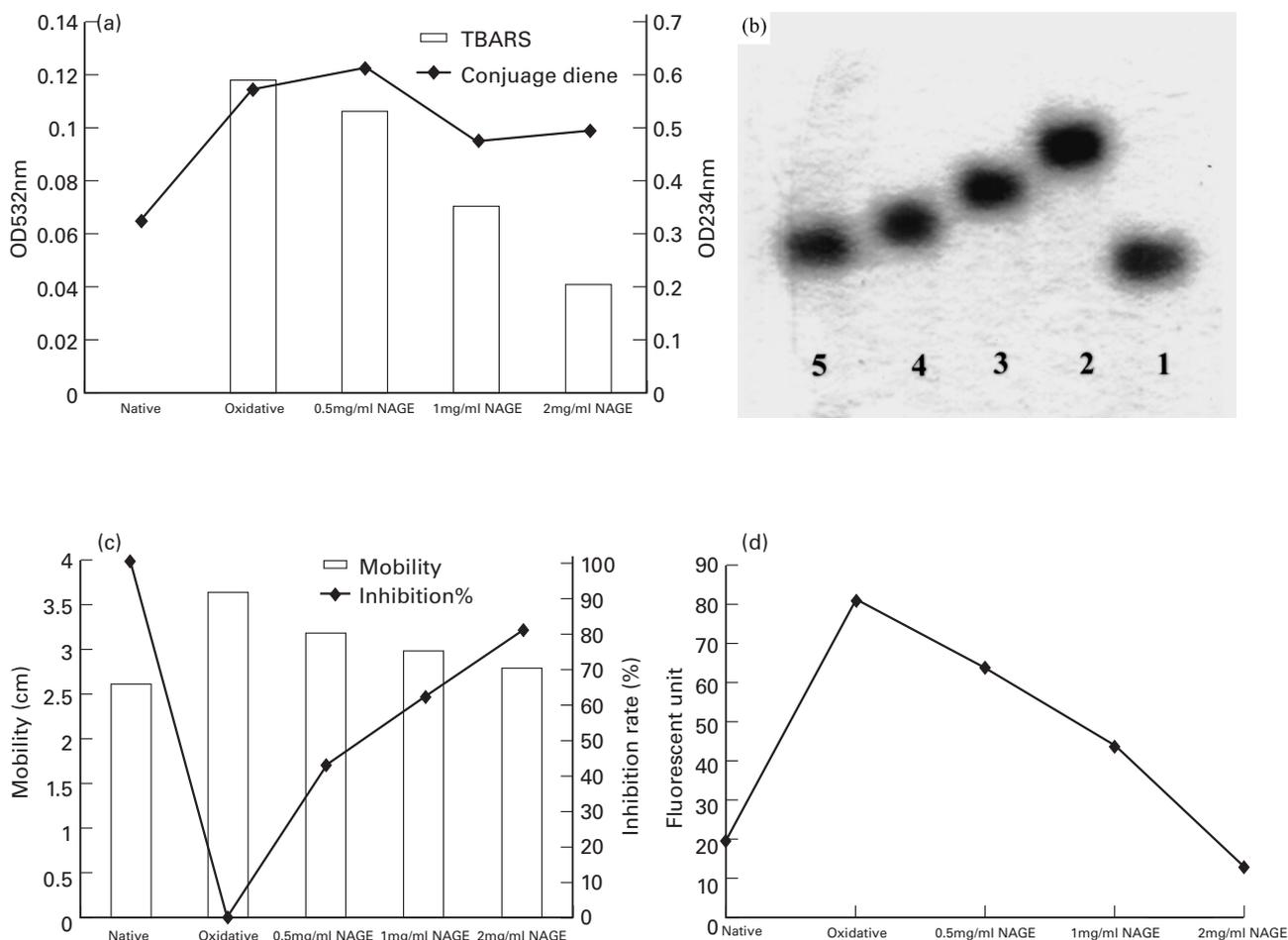
GSH additional scavenging of DPPH radical was noted. This result denotes a potential degree of synergy between GSH and NAGE. It is noteworthy that GSH has previously been shown to reduce oxidized flavones<sup>60</sup>, which supports our observation that potential important interactions could occur *in vivo* between these non-enzymatic antioxidant constituents in managing whole body oxidative stress. Similar results of potential synergy with GSH and cycoheterophyllin, a prenylflavonoid isolated from leaves of Formosan *Artocarpus heterophyllus*, have also been reported<sup>61</sup>.



**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  $\mu$ l of 0.005% w/v) was incubated with ferrous sulphate (10, 50, 70  $\mu$ M) and DNA (0.1  $\mu$ g ml<sup>-1</sup>) 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  $\mu$ M Fe<sup>2+</sup>; 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.*<sup>4</sup> Early animal studies, conducted in dogs, reported no adverse effect of ginseng on body weight or blood chemistry<sup>62</sup>. In mice, the LD<sub>50</sub> for ginseng ranges from 10 to 30 g kg<sup>-1</sup> (Ref. 17), with a lethal oral dose of purified ginseng as high as 5 g kg<sup>-1</sup> body weight<sup>62</sup>. In a 2 year human study, 14 out of a total of 133 subjects were reported to experience side effects attributed

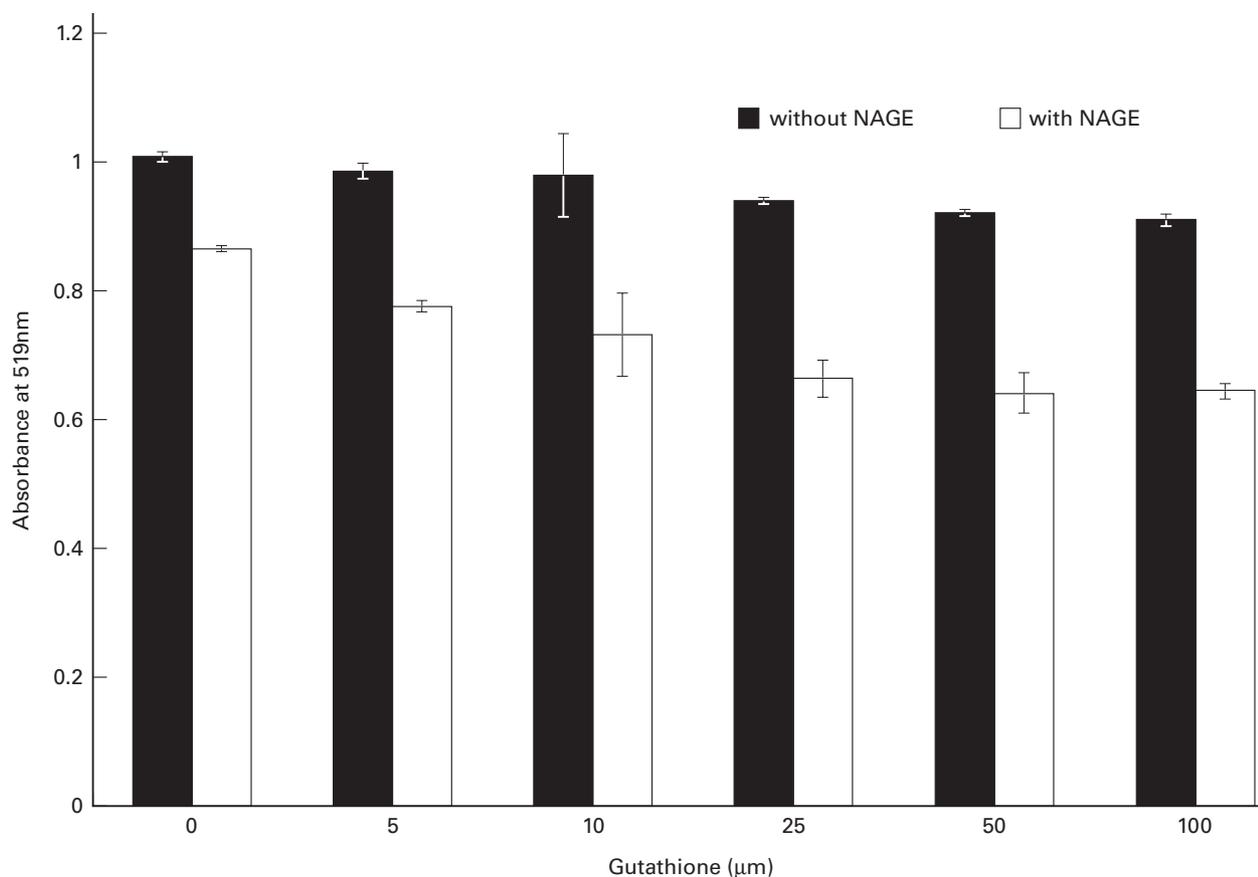


**Fig. 5** Copper-mediated human low-density lipoprotein oxidation. Human LDL containing 0.01% EDTA was dialysed against 10 mM phosphate buffered saline (pH 7.4) at 4°C with nitrogen bubbled for 24 h, and the obtained LDL stock solution was stored under N<sub>2</sub> atmosphere in the dark (4°C) for less than 72 h upon expiration<sup>52</sup>. Copper mediated LDL oxidation was performed by incubating LDL (500 µg protein ml<sup>-1</sup> in 10 mM phosphate buffer, pH 7.4) with freshly made CuCl<sub>2</sub> (10 µmol l<sup>-1</sup>) 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.*<sup>54</sup> 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<sub>2</sub> and  $S$  = electrophoretic mobile distance (cm) of LDL treated with CuCl<sub>2</sub> 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<sup>-1</sup> (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<sup>-1</sup>), showed symptoms

of confusion and depression. This level of ginseng consumption far exceeds the German Commission E's<sup>64</sup> recommended daily intake of 1–2 g day<sup>-1</sup> of Asian ginseng, containing 4–5% ginsenoside<sup>65</sup>.

Ginseng may contain an endocrine-like active substance which can affect neonate development<sup>66</sup>. In one study, oestrogen-like activity attributed to chronic ginseng use was reported to cause swollen and painful breasts<sup>67</sup>. Ginseng–drug interactions have been observed in a few isolated situations, which include phenelzine, a monoamine oxidase inhibitor<sup>68</sup>, and warfarin<sup>69</sup>, 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-picrylhydrazyl 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<sup>-1</sup>), as well as with various amounts of glutathione. Absorbance readings were taken at 519 nm using a spectrophotometer. ■ = without NAGE; □ = with NAGE

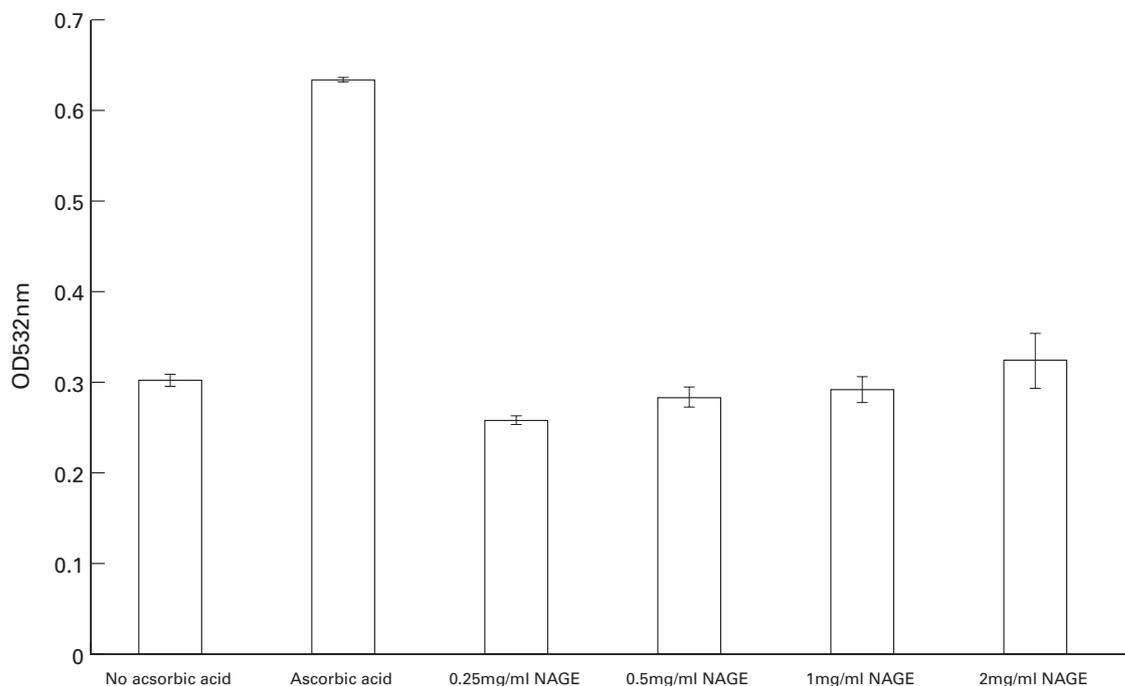
noteworthy, however, that caution has been expressed concerning the use of Asian ginseng to combat acute stress, due to the fact that the hypertensive effect associated with these individuals is contra-indicated<sup>70</sup>.

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<sup>71–73</sup> as well as polyphenolics, including myricetin<sup>74,75</sup> and the catechins in green tea<sup>75</sup>, 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<sup>76</sup>, resulting in eventual breakdown of these macromolecules. The high redox potential of ascorbic acid has been shown to accelerate reduction of Cu<sup>2+</sup> to its lower valency Cu<sup>1+</sup> in aqueous medium and to promote accelerated DNA strand scission<sup>77</sup>. Our characterization of the NAGE in this study suggests that the formation of a Cu<sup>2+</sup>–ginseng complex was

sufficient to inhibit a further reaction with H<sub>2</sub>O<sub>2</sub> 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<sup>-1</sup> NAGE in the presence of a Cu<sup>2+</sup>-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.*<sup>54</sup> was used. Briefly, the reaction system contained 3.6 mM 2-deoxyribose, 0.1 mM CuCl<sub>2</sub>, 1 mM H<sub>2</sub>O<sub>2</sub>, 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<sub>532nm</sub> 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<sup>78</sup>. 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.

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