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A Systematic Review: Antioxidant Activity of *Panax ginseng* C.A. Meyer and Its Major Components, Ginsenosides

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ABSTRACT

Ginseng is actually a collection of 11 distinct species of slow-growing perennial plants with fleshy roots, but *Panax ginseng* C.A. Meyer (*Araliaceae*) or Korean ginseng is the main one. The root of *P. ginseng* is a traditional medicine in Korea, China and Japan that has been shown to produce a variety of medicinal effects. The reported pharmacological activities of ginseng and its constituents are related to possess antistress and antioxidant effects. The excess of free radicals may lead to peroxidative impairment of membrane lipids and consequently disrupt cellular functions and cause their death. This review details the bibliography supporting the medicinal efficacy of ginseng and evidence has been closely linked to its protective properties against free radicals.

Keywords: ginseng, oxidative stress, reactive oxygen species, drug, neuroprotection, adaptogen

Abbreviations: ATP, adenosine-5'-triphosphate; **CAT**, catalase; **CNS**, central nervous system; **DCF-DA**, 2′,7′-Diclorofluorescein diacetate; **DPPH**, 1,1-diphenyl-2-picrylhydrazyl; **GPx**, glutathione peroxidase; **GR**, glutathione reductase; **GSH**, glutathione; **GSSG**, oxidized glutathione; **MDA**, malondialdehyde; **MPP+**, 1-methyl-4-phenylpyridinium; **MPTP**, 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine; **NADPH**, nicotinamide adenine dinucleotide phosphate; **OGD**, oxygen-glucose deprivation; **ROS**, reactive oxygen species; **RT-PCR**, reverse transcriptase PCR

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INTRODUCTION

Ginseng root is a traditional medicine in Korea, China and Japan that has been shown to produce a variety of medicinal effects. This crude drug has been empirically used as a psychic energizer and a general tonic in traditional medicine to increase vitality, health and longevity, especially in older persons, and for its cancer-preventing potential (Wang and Joseph 1999; Shin *et al*. 2000). Much interest has been focused on the effects of ginseng as an adaptogen, a substance which helps the body to resist the adverse influences of harmful factors and improves the restoration of homeostasis irrespective of the direction of the altered physiological function.

The pharmacological effects of ginseng species have been demonstrated on the central nervous system (CNS) and on the cardiovascular, endocrine and immune systems (Kitts *et al.* 2000; Atelle *et al*. 2009). The drug and its constituents are thought to possess antineoplastic, antistress and antioxidant effects (Tang and Eisenbrand 1992; Seong *et al.* 1995). Ginseng saponins, also called ginsenosides, are the main active compounds responsible for the effects of ginseng. Ginsenosides are derived from triterpene dammarane and can be classified into two classes: protopanaxadiol derivatives, mainly consisting of Rb_1, Rb_2, Rb_3, Rc, Rd and Rh_2, and protopanaxatriol derivatives, mainly involving Re, Rf, Rg_1 and Rg_2 (**Fig. 1**).

Reactive oxygen species (ROS) are reactive chemical entities which are classified into two categories: free radicals and non-radical derivatives (**Table 1**). Free radicals are species characterized by having one or more unpaired electrons which make them more reactive than the corresponding non-radicals. These agents in low concentrations serve as signalling molecules; however, ROS elicit harmful effects when produced in excess. The toxicity associated with the excessive production of these compounds is prevented by antioxidant defence systems (**Table 2**) (Dhalla *et al.* 2000). Oxidative stress results from an imbalance between ROS and antioxidant defence systems with deleterious effects on cells, e.g. lipid peroxidation, protein oxidation and DNA mutagenesis, resulting in cellular dysfunction. Oxidative stress has been linked to cardiovascular disease, diabetes, pulmonary disease, cancer, and other degenerative diseases (Stohs 1995). Oxidative stress-induced cell damage has long been implicated both in the physiologic process of aging and in a variety of neurodegenerative diseases such as Alzheimer's disease, Parkinson's disease, and amyotrophic lateral sclerosis (Finkel and Holbrook 2000; Barnham *et al.* 2004; Loh *et al.* 2006; Castellani *et al.* 2007). Oxidative stress is mediated by ROS, including free radicals such as

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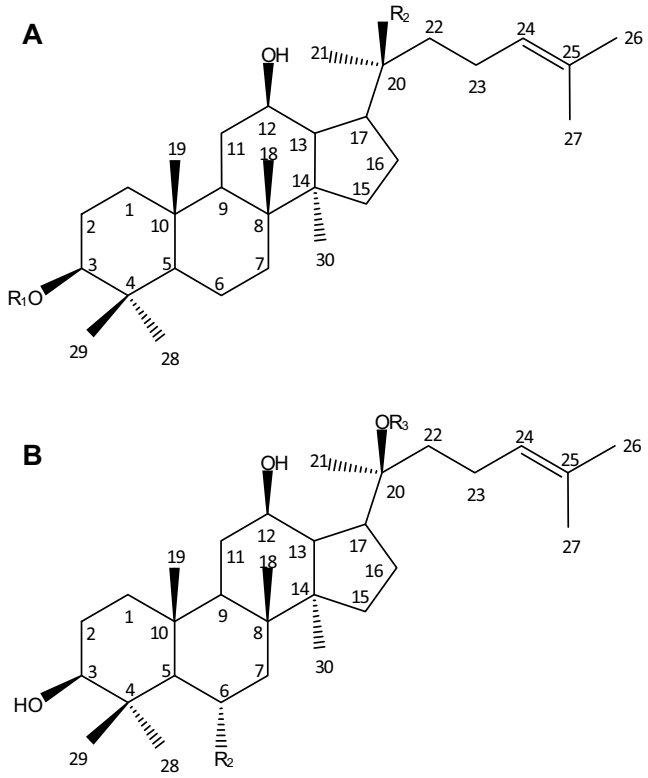

Fig. 1 Chemical structure of ginsenosides. (**A**) 20 (S)-protopanaxadiol ginsenosides, (**B**) 20 (S)-protopanaxatriol ginsenosides.

20 (S)-protopanaxadiol ginsenosides

Ginsenosides	R_1	R_2
Rb_1	-Glc(2-1)Glc	-Glc(6-1)Glc
Rb_2	-Glc(2-1)Glc	-Glc(6-1)Ara(p)
Rc	-Glc(2-1)Glc	-Glc
Rd	-Glc(2-1)Glc	-Glc
Rg_3	-Glc(2-1)Glc	-H
Rh_2	-Glc	-H

20 (S)-protopanaxatriol ginsenosides

Ginsenosides	R_1	R_2
Re	-Glc(2-1)Rha	-Glc
Rf	-Glc(2-1)Glc	-H
Rg_1	-Glc	-Glc
Rg_2	-Glc(2-1)Rha	-H
Rh_1	-Glc	-H

Ara(p): arabinopyranose, Ara(f): arabinofuranose, Glc: glucose, Rha: rhamnose

superoxide ions (O^{2-}) and hydroxyl radical ($^{\cdot}OH$) as well as non-free radical species such as hydrogen peroxide (H_2O_2) which are generated as by-products of normal and aberrant metabolic processes that utilize molecular oxygen. ROS cause oxidative damage to various biological macromolecules including DNA, lipids, and proteins, thereby altering several signaling pathways that ultimately promote cellular damage and death (Chan *et al.* 2001; Loh *et al.* 2006).

There is growing interest in therapeutic strategies with neuroprotectants aimed at counteracting oxidative stress-induced damage associated with neurodegenerative diseases (Moosmann and Behl 2002; Barnham *et al.* 2004).

The sequential activities of superoxide dismutase (SOD) and glutathione peroxidase are the principal mechanisms for removal of ROS from cells. In addition to glutathione peroxidase, catalase activity is an important antioxidant pathway in the removal of hydro, but not organic, peroxides. Catalase is a more efficient scavenger of H_2O_2 at higher concentrations, whereas, glutathione peroxidase activity is favoured at lower H_2O_2 concentrations (Ehrhart and Zeevalk 2001). Although no treatments after H_2O_2 exposure showed differences with H_2O_2-treated cells, ginseng pretreatment showed protection in antioxidant enzymes activities.

Table 1 The reactive oxygen and nitrogen species. The superscripted dot indicates an unpaired electron and the negative charge indicates a gained electron. R, lipid chain. Singlet oxygen is an unstable molecule due to the two electrons present in its outer orbit spinning in opposite directions.

Free radicals		Non-radicals	
$O_2^{\cdot-}$	Superoxide anion radical	H_2O_2	Hydrogen peroxide
$OH^{\cdot}$	Hydroxyl radical	HOCl	Hypocholours acid
$O_2H^{\cdot}$	Perhydroxyl radical	$ONOO^-$	Peroxynitrite
$ROO^{\cdot}$	Lipid peroxide (peroxyl)	$^1O_2$	Singlet oxygen
$RO^{\cdot}$	Alkoxyl		
$NO^{\cdot}$	Nitrit oxide		
$NO_2^{\cdot}$	Nitrogen dioxide		

Table 2 Antioxidant defence mechanisms. GSH, reduced glutathione; GSSG, oxidized glutathione; R, lipid chain.

	Enzymatic scavengers	Non-enzymatic scavengers
SOD	Superoxide dismutase	Vitamin A
	$2O_2^{\cdot} + 2H^+ \rightarrow H_2O_2 + O_2$	Vitamin C (ascorbic acid)
		Vitamin E (α-tocopherol)
CAT	Catalase	β-carotene
	$2H_2O_2 \rightarrow O_2 + H_2O$	Cysteine
		Coenzyme Q
GPX	Glutathione peroxidase	Uric acid
	$2GSH + H_2O_2 \rightarrow GSSG + 2H_2O$	Flavonoids
	$2GSH + ROOH \rightarrow GSSG + ROH + 2H_2O$	Glutathione
		Thioether compounds
GST	Glutathione-*S*-tranferase	Lipoic acid
	$RX + GSH \rightarrow RSG + HX$	
GR	Glutathione reductase	
	$GSSG + NADPH + H^+ \rightarrow 2GSH + NADP^+$	

Glutathione (GSH) is involved in the removal of hydro- and organic-peroxides that are formed as products of normal cellular processes or toxic insults. During normal functioning of the respiratory chain <2% of mitochondrial O_2 is reduced and released as superoxide anion, which is converted to H_2O_2 by SOD and then further reduced by glutathione peroxidase. The reduction of peroxide by glutathione peroxidase results in the oxidation of GSH to GSSG. Oxidized GSSG is reduced back to GSH by the NADPH-dependent activity of glutathione reductase, thereby recycling GSH and limiting the accumulation of GSSG in cells (Ehrhart and Zeevalk 2001). The recovery of GSH level may be explained by the up-regulation of GR activity upon H_2O_2 treatment. Increased GR activity can increase GSH availability and, as a result, promote the elimination of H_2O_2 by GPx, which makes the cells more resistant to H_2O_2. The conversion of GSSG to GSH by GR is dependent on the amount of NADPH, which also plays a pivotal role in cellular antioxidant capacity. In a previous study, Yang *et al.* suggested that the reducing power of cells can be estimated by evaluating the value of [NADPH]/[$NADP^+$], by its correlation with glutathione reduction reaction (Yang *et al.* 2004).

Oxidative stress causes cell death when intracellular levels of metabolic and antioxidant enzymes (especially glutathione related enzymes) and substrates (glutathione, glucose and ATP) are exhausted. Evidence supporting the medicinal efficacy of ginseng has been closely linked to its protective properties against free radicals. In this article we will review the evidence of the different aspects that involve the antioxidant activity of the ginseng extract and the main isolated ginsenosides.

GINSENG

P. ginseng and its related species have already had thousands of years of human exposure with little reported toxicity. Recent surveys indicate that ginseng remains one of the most commonly used natural products in the United States (Harnack *et al.* 2001).

The pharmacological effects of ginseng have been demonstrated in the CNS and in the cardiovascular, endocrine and immune systems (Tang and Eisenbrand 1992; Attele *et al.* 1999; Shah *et al.* 2005; Wang *et al.* 2006). Ginseng appears to act mainly on the hypothalamus and has a sparing action on the adrenal cortex, mediated through anterior pituitary and ACTH release. In addition to anti-neoplastic and immunomodulatory effects, ginseng has neuroprotective action. Some of ginseng's active compounds exert beneficial effects on aging, central nervous system disorders (CNS) and neurodegeneration (Lian *et al.* 2005; Radad *et al.* 2006). Bastianetto and Quirion (2002) screened natural extracts as possible protective agents of brain aging. *P. ginseng* berry extract had an antidiabetic effect in *ob*/*ob* mice with a loss of body weight (Attele *et al.* 1999). Calorie restriction ameliorated neurodegenerative phenotypes, as well as in age-related behavioral deficits in the triple-transgenic mouse model of AD (Halagappa *et al.* 2007; Wu *et al.* 2007).

Ginseng root has been studied for its antioxidant potential, and is known to scavenge ROS, to chelate metal ions and to prevent LDL peroxidation via a distinct concentration-dependent mechanism. Many reports refer to Korean and American ginseng´s capacity to scavenge free radicals (hydroxyl radicals or DPPH), to chelate metal ions and to protect against lipoprotein oxidation (Seong *et al.* 1995; Kitts *et al.* 2000). The antioxidant activity of ginseng extracts and their components in other experimental models has also been studied (Chen *et al.* 1987; Facino *et al.* 1999; Voces *et al.* 1999). Park *et al*. (2010) investigated the effect of red ginseng extract (RGE) on polychlorinated biphenyls (PCB) – ubiquitous environmental contaminants – because there has been compelling evidence supporting that PCB-induced cytotoxicity is mediated through generation of reactive oxygen species (ROS). PC12 cells treated with PCB126 exhibited increased accumulation of intracellular ROS and underwent apoptosis as determined by positive in situ terminal end-labeling (TUNEL staining) and the perturbation of the mitochondrial membrane potential. RGE treatment attenuated PCB126-induced cytotoxicity, apoptotic features and intracellular ROS accumulation and upregulated heme oxygenase-1 (HO-1) and glutamate cysteine ligase (GCLC) that are key antioxidant enzymes essential for cellular defense against oxidative stress. These findings, taken together, suggest that HO-1 and GCLC induction via Nrf2 activation may contribute to cytoprotection exerted by RGE against PCB126-induced oxidative stress.

Ginseng root extract inhibits calcium channels in rat sensory neurons (Nah and McCleskey 1994). Facino *et al.* (1999) evidenced antioxidant properties of *Panax ginseng* administration in rats against myocardial ischemia–reperfusion damage.

The root of Korean ginseng is endowed with significant antioxidant properties and this is the base for its protection against acute oxidant stress. Kim and Packer (2002) have shown the free radical-scavenging activity of red ginseng aqueous extracts. Ginseng also has protective effects on endothelial cells against damage by lipid peroxidation (Mei *et al.* 1994) and hepatoprotective effects against oxidative stress induced by exhaustive exercise (Voces *et al.* 1999). Ginseng root extract is effective in reducing cellular death induced by H_2O_2 in astrocytes (Naval *et al.* 2007) and in cardiomyocites exposed to acute oxidant stress (Shao *et al.* 2004).

Most pharmacological actions of ginseng are attributed to ginsenosides, which can act in a wide range of tissues. Wang *et al.* (2007) found that the glycosidic fraction from dried roots of ginseng showed protective effects on liver induced by D-galactosamine and lipopolysaccharide. Findings of Marie *et al.* (2008) showed the effects of glycosidic fraction from the dried roots of ginseng and proved that this extract has hepatoprotective effects. Thus, in the present studies the hepatotoxicity seems to be a consequence of the formation of haloalkane free radicals. Ginseng extract inhibited lipid peroxidation significantly (Gum *et al.* 2007). Ginseng extract has been reported to have antioxidant potential and scavenge superoxide radicals (Keum *et al.* 2000; Kitts *et al.* 2000). Ginseng extract is known to inhibit the lipid peroxidation in hepatocytes in restraint stress (Salem 2001). Ginseng maintains the GSH which executes its metalloprotective function through free radical scavenging, restoration of damaged molecules by hydrogen donation, and reduction of peroxides and maintenance of protein thiols in the reduced state (Agarwal *et al.* 1997; Kemble *et al.* 1997).

The "Ginsen", a polysaccharide from ginseng cured hepatotoxicity by normalizing SGOT and SGPT level against chemical induced injury (Song *et al.* 2004). Lin *et al.* (2003) reported that *P. ginseng* ameliorated rise in SGOT and SGPT levels as a result of ethanol-induced hepatotoxicity in mice and also inhibited production of free radicals that caused lipid peroxidation.

Therefore, in the study of Shukla and Kumar (2009) ginseng extract was found to be effective in protecting hepatic toxicity caused by elevated lipid peroxidation after cadmium intoxication in group IV. Furthermore, a highly significant increase in GSH level was observed in group IV. It was observed that the level of SGOT and SGPT showed a significant decline in group IV. $CdCl_2$-induced toxicity may be alleviated by ginseng root extract, which is reflected in the decline of LPO, SGOT, SGPT and the elevation in GSH and alkaline phosphatase activities in Swiss albino mice. In several cases, a lack of concentration/response relationship was found as we found sporadic positives at intermediate concentration; also biphasic relationships were observed between effect and concentration. These results agree with previously reported results about ginseng. As a matter of fact, biphasic actions depending on the concentration or assayed time have been reported: *P. ginseng* and *Eleutherococcus senticosus* may exaggerate an already existing biphasic response to stress via inhibition of enzymes which limit the binding of stress hormones to their receptors (Gaffney *et al.* 2001). Ginseng has been mentioned to show estrogenic activity directly or indirectly (Amato *et al.* 2002; Naval *et al.* 2002).

Ginseng is widely recognized by the scientific community as an agent able to regulate different organs and systems of the body to recover a homeostatic status. *P. ginseng* is able to prolong lifespan and survival rate against physical injuries such as hypoxia (Wang and Lee 1998) or prolonged irradiation (Brekhman and Dardymov 1969; Takeda *et al.* 1982; Zang 1987). Several effects of ginseng could be considered as opposite activities as they normalize the corporal status when exposed to contrary stimuli such as high and low temperature (Chang and But 1986).

Ginsenosides

Purified ginsenosides have similar effects to ginseng root extract, suggesting that these compounds are likely responsible for the protective activity of ginseng extract. Ginseng saponins may modulate the activity of the root in its proliferative and antioxidant effects and also exhibit protection against free radical-induced damage (Huong *et al.* 1998). *P. ginseng* saponins have shown a suppressive action on the lipid peroxidation caused by radical generating systems in tissue preparations; they also attenuate lipid peroxidation in the rat liver homogenate (Li *et al.* 1999).

Ginsenosides have proved to exert protective effects that are attributed to their antioxidant ability that prevents the decrease of antioxidant enzymes and act as a free-radicals scavenger. Ginsenosides alleviated oxidative stress by scavenging of free radicals, inhibiting of NO production which usually accompanies glutamate excitotoxicity, inducing superoxide dismutase (SOD1) and catalase genes and reducing lipid peroxidation (Braughler *et al.* 1988; Chang *et al*. 1999).

Central nervous system

Ginseng and ginsenosides have been studied in different cellular types of the CNS, especially in neurons, to test their effect on calcium channels, neurotransmitters release and apoptotic-related enzymes such as Rg_1 in MPP^+-induced toxicity, or dopaminergic cells against glutamate (Radad *et al.* 2004); the effect of ginsenoside Rb_1 on central cholinergic metabolism (Benishin *et al.* 1991); the effects of Rg_1 or Rb_1 on Aβ-induced memory impairment (Tohda *et al.* 2004) and ginseng total saponin and ginsenosides Rb_1 and Rg_1 on spinal cord neurons *in vitro* (Liao *et al.* 2002).

Ginseng saponins are endowed with significant antioxidant properties that justify their glioprotection against acute oxidative stress. Ginsenoside Rb_1 protects ischemic hippocampal neurons (Lim *et al.* 1997) and ginsenosides Rb_1 and Rg_1 reduce lipid peroxidation of brain microsomes (Deng and Zhang 1991); Ginsenoside Rb_1 has radical scavenging activity and ameliorates ischemic damage in hippocampal CA1 neurons *in vivo* (Lim *et al*. 1997). Ginsenoside Rd enhanced astrocyte differentiation from neural stem cells (Shi *et al.* 2005). Experiments using an *in vitro* model of cellular injury induced by amyloid Aβ demonstrated the neuroprotective effect of ginsenoside Re and also found that it is capable of protecting PC12 cells from the damage induced by serum-free medium (Ji *et al.* 2006), which is consistent with previous reports in other cellular models such as cerebral cortex neurons in cell cultures (Himi *et al.* 1989). Ginsenoside Rg_1 attenuates dopamine-induced apoptosis in PC12 cells and reduces MPTP-induced substantia nigra neuron loss by suppressing oxidative stress (Chen XC *et al.* 2003, 2005) and increases ischemia-induced cell proliferation and survival in the dentate gyrus of adult gerbils (Shen *et al.* 2003). Ginsenoside Rg_3 helps to prevent decreases in antioxidant enzymes such as superoxide dismutase and glutathione peroxidase in rat brain (Tian *et al.* 2005).

Naval *et al.* suggest that ginsenosides could protect astrocytes from oxidative stress generated by H_2O_2. There is protective effect of the main ginsenosides on hydrogen peroxide-induced oxidative damage in astrocytic primary culture: the isolated ginsenosides Rb_1, Rb_2, Re and Rg_1 are effective in reducing astrocytic death induced by H_2O_2. Rb_1, Re, and Rg_1 could activate antioxidant enzymes, including SOD, GPx, and GR, and protect astrocytes from H_2O_2-induced cell death (Naval *et al.* 2007). All the tested ginsenosides reduced the ROS formation percentage, ginsenoside Re being the most active (46.3% of ROS reduction).

Rudakewich *et al.* (2001) concluded that ginsenosides Rb_1 and Rg_1 potentiate NGF-induced neurite outgrowth in cell culture. Ginsenoside Rg_1 was shown to interrupt dopamine-induced elevation of ROS or NO generation in pheochromocytoma cells (PC12) (Chen *et al.* 2003). Moreover, ginseng radix attenuated MPP^+-induced apoptosis as it decreased the intensity of MPP^+-induced DNA laddering in PC12 cells and ginsenoside Rg_1 had protective effects against MPTP-induced apoptosis in the mouse substantia nigra (Chen *et al.* 2002; Kim *et al.* 2003). It has been reported that ginsenosides Rb_1, Rg_1, Rc, and Re inhibited tyrosine hydroxylase activity and exhibited anti-dopaminergic action since they reduced the availability of dopamine at presynaptic dopamine receptors (Kim *et al.* 1999).

Ginsenosides also exhibit protection against free radical-induced damage (Li *et al.* 1999). Rg_1 could substantially attenuate iron accumulation in the substantia nigra in 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP)-treated PD mice (Wang *et al.* 2009). Since up-regulation of DMT1-IRE was shown to account for the iron accumulation in 1-methyl-4-phenylpyridinium (MPPþ)-treated dopaminergic cell line MES23.5 (Zhang *et al.* 2009), Xu *et al.* hypothesized that Rg_1 might attenuate iron accumulation via regulating divalent metal transporter 1 without iron responsive element (DMT1-IRE) expression and showed that Rg_1 could attenuate MPPþ-induced up-regulation of DMT1-IRE probably through inhibiting ROS-nuclear factor-kappaB (NF-kB) pathway, which decreased the iron influx and iron-induced oxidative stress.

Some reports showed that neuroprotection by ginseng may be, in part, due to its effect on glial cell populations. In this respect, it has been reported that ginseng total saponins prevented astrocytic swelling induced by glutamate (Seong *et al.* 1995) and ginsenoside Rg_1 inhibited microglial respiratory burst activity and decreased the accumulation of NO produced by activated microglia (Gong and Zhang 1999).

As oxidative stress has been suggested to be crucially involved in the pathophysiologic process of ischemia (Doyle *et al.* 2008), Ye *et al.* (2009) postulated that ginsenoside Rd probably possess an ability to protect neurons from ischemic damage. The chemical structure of Rd (sugar moiety attached to the 20-position of the triterpene dammarane) may possibly contribute to its direct antioxidant property (Liu *et al.* 2003). H_2O_2 led to mitochondrial membrane depolarization (MMP). Ginsenoside Rd prevented the loss of the MMP, suggesting that the electron transport chain was maintained, which may be associated with the inhibition of the intracellular accumulation of ROS (Ye *et al.* 2008).

Previous studies on the antioxidative effects of ginsenosides focused mainly on their direct ROS-scavenger activity (Kang *et al.* 2006). A study by Ng and Yang (2008) showed that treatment with the protopanaxatriol-type ginsenoside Re would increase the GSH/GSSG ratio and modulate cell proliferation in the C6 glioma cell. Other than Re, there is a lack of evidence to support the modulating ability of other ginsenosides on the intracellular redox status. This study was conducted to investigate the protective mechanism of protopanaxatriol ginsenosides against H_2O_2-induced oxidative injury on human endothelial cells using metabolic indicators that reflect cellular energy and redox states.

The cellular redox status indicates the ability of cells to maintain a low level of ROS. Ginsenoside Rd can protect PC12 cells from oxygen–glucose deprivation-induced oxidative stress (Colognato *et al.* 2006). Ginsenoside Rd, is one of the main active components of ginsenosides. It has been shown to have a number of pharmacologic actions such as inhibiting calcium influx through receptor- and store-operated calcium channels (Guan *et al.* 2006) enhancing astrocyte differentiation from neural stem cells (Shi *et al.* 2005) and significantly reducing the 3-nitropropionic acid-induced motor impairment and cell loss in the striatum (Lian *et al.* 2005). In the CNS, ginsenoside Rd was reported to be effective in decreasing ROS formation in cultured astrocytes (Tang and Eisenbrand 1992). Its antioxidant properties in neuron-like cells explain the protective role and mechanism of ginsenoside Rd against oxidative stress induced by H_2O_2 in cultured PC12 cells.

Ginsenoside Rd can exert neuroprotective effects against H_2O_2-induced oxidative stress in PC12 cells (Ye *et al.* 2008). Concurrent treatment with ginsenoside Rd inhibits intracellular ROS formation, reduces the level of the lipid peroxidation product (malondialdehyde, MDA), and maintains cellular antioxidant activity (SOD and GPx). When cells were only exposed to exogenous H_2O_2, the DCF fluorescence significantly increased. Although a small part of H_2O_2 may be scavenged by cellular antioxidant enzymes, it can directly cause oxidation of various intracellular targets including the fluorescence probe DCFH-DA. The formation of hydroxyl radicals mediated by intracellular heavy metal ions could also contribute to the increased DCF fluorescence in response to H_2O_2. These results suggest that ginsenoside Rd exerts its antioxidant effects in the intracellular compartment.

The antioxidant activity of ginsenoside Rd was observed at doses of 1-10 mM, whereas ginsenoside Rd 50 mM did not show protective effects (Ye *et al.* 2008). Scavenging of ROS may also occur *via* recruitment of the endogenous antioxidative system, such as induction of SOD and GPX activities by ginsenoside Rd. Alternatively, a possible direct scavenging of H_2O_2 by ginsenoside Rd during the incubation period cannot be ruled out. However,

the antioxidant action was also found in other cellular models and the concentrations of ginsenoside Rd required for neuroprotection are far lower than those of H_2O_2 used in the assay, suggesting that it may not be a simple stoichiometric reaction. Naval *et al.* (Naval *et al.* 2007) previously evaluated individual ginsenosides in primary astrocyte cultures using an oxidative stress model with H_2O_2 and found that ginsenoside Rd decreased ROS formation at the dose of 5-100 mM. The reason for this discrepancy may be the different cell cultures used. Different cell types have different functions that are determined by their genetic codes and enzyme content. Because of that, the responses to different stimuli depend on the function for which they are naturally prepared. The PC12 cells used in this research are clonal cells derived from rat pheochromocytoma. Treatment with nerve growth factor induces the differentiation of PC12 cells into a sympathetic neuron-like phenotype (Greene and Tischler 1976). It has been widely used as a model for neurobiologic, neuropharmacologic, and neurotoxicologic studies. The response of PC12 cells to ginsenoside Rd may not be exactly the same as that observed in other cells.

In addition to producing an increase in ROS and consequent lipid peroxidation, H_2O_2 exposure can cause an elevation of intracellular calcium levels. The occurrence of large increases in intracellular calcium represents a detrimental insult from oxidative stress imposed by ROS in the cells. Sustained elevated calcium levels in cells may impair mitochondrial function and activate phospholipase, protease, and endonucleases leading to irreversible membrane, organelle, and chromatin damage and eventually to cell death. Therefore, Ca^{2+} plays an important role in the development of oxidative injury.It has been shown that ginsenoside Rd inhibits Ca^{2+} entry through receptor-operated and store-operated calcium channels (Guan *et al.* 2006). This may possibly provide an explanation for the neuroprotection of ginsenoside Rd against H_2O_2.

Additionally, ginsenoside Rd is highly lipophilic and can easily diffuse across biological membranes and the blood-brain barrier. In conclusion, ginsenoside Rd not only decreases oxidative stress-induced ROS overproduction and lipid peroxidation, but also maintains endogenous antioxidant enzymatic activities, stabilizes mitochondrial function, and subsequently attenuates PC12 cell injury (Ye *et al.* 2008).

Ye *et al.* (2009) explained that exposure to oxygen-glucose deprivation (OGD) resulted in the cell viability loss of hippocampal neurons in a time-dependent manner. However, ginsenoside Rd presented neuroprotective effects against OGD-induced cytotoxicity in cultured hippocampal neurons. Concurrent treatment of ginsenoside Rd decreased the cell viability loss and LDH release induced by OGD, which was in parallel with the morphological analyses of apoptosis. One possible mechanism of the neuroprotection against OGD is the antioxidant activity of ginsenoside Rd. The participation of free radicals in the production of ischemia or reperfusion injury was suggested by the effectiveness of free radical scavenging drugs and SOD (Lipton 1999; Warner *et al.* 2004). Ginsenoside Rd markedly decreased ROS accumulation and suppressed lipid peroxidation (lower level of MDA). Moreover, ginsenoside Rd did not significantly affect antioxidant enzyme activities (CAT, SOD, and GPx) in hippocampal neuronal cultures under normal conditions, which indicate that the antioxidative ability of ginsenoside Rd may be due to its direct scavenging of ROS rather than the recruitment of the endogenous antioxidative system. Additionally, ginsenoside Rd is highly lipophilic and can easily diffuse across biological membranes and the blood–brain barrier in an energy-deficient environment. The neuroprotective efficacy of ginsenoside Rd *in vitro* is probably associated with inhibition of oxidative stress impairment and preservation of MMP (Ye *et al.* 2009).

There are studies about gene expression patterns of antioxidant enzymes. Three types of glutathione peroxidases [*GPx*; cytosolic (*cGPx*), plasma (*pGPx*) and phospholipid hydroperoxide (*phGPx*) forms], in cultured rat embryos (embryonic days 9.5-11.5) were exposed to ginsenosides Rb_1, Rg_1, Re and Rc at levels of 5, 50 and 100 μg/ml. With regard to total morphological scores, no significant differences were noted in the embryos exposed to all doses of ginsenosides, with the exception of 50 μg/ml of Rc. In the cultured embryos exposed to Rg_1, a majority of the developmental parameters were normal, but growth of the hind- and mid- brains and the caudal neural tube was significantly increased compared with that observed in the control group ($P<0.05$). Furthermore, ginsenoside Rc significantly enhanced the growth of a variety of developmental parameters in the cultured embryos, with the exception of the hindlimbs. According to the results of our semiquantitative RT-PCR analysis, the levels of *cGPx* and *phGPx* mRNA in the cultured embryos were unaffected by treatment with the ginsenosides. However, the levels of *pGPx* mRNA increased significantly in the embryos treated with ginsenosides Re, Rc and Rb_1 compared with the control group ($P<0.05$). These findings indicate that ginsenosides may exert a stimulatory effect on the growth of embryos via differential expression of *GPx* genes (Lee *et al.* 2008).

Pituitary adenylate cyclase-activating polypeptide (PACAP) exhibits a neuroprotective effect in many neuronal cells and is capable of neuron prevention from apoptosis induced by Aβ *in vitro*. The neuroprotective effect of PACAP is also introduced by an activation of the α-secretase pathway to further produce secretion of APP that possessed neuroprotective, anti-apoptotic and growth-promoting properties. PACAP has also been introduced as a neuron protector against oxidative stress. It has been demonstrated that reactive astrocytes induced by Aβ contributes to disease progression in AD. PACAP is introduced to regulate the activities of glial cells in cell proliferation, glycogen metabolism and cell plasticity, and stimulates the release of neuroprotective factors as well as gliotransmitters/gliopeptides (Shieh *et al.* 2008).

Ginsenoside Rh_2 increased the cell proliferation of RBA1. It has been mentioned that Rh_2 inhibits cell growth in cancer cells at doses higher than 12 μM (Lee *et al.* 1996; Park *et al.* 1997; Ham *et al.* 2006). In addition to the difference of cell types, actions of Rh_2 can also be various due to the dose used. Moreover, in the co-incubation of Aβ-treated cells with Rh_2, the inhibited cell proliferation was reversed by Rh_2 in a concentration-dependent manner. This result is similar to previous reports using other kinds of ginsenoside (Rg_1, Rb_1 and Re) to abate Aβ-induced neurotoxincity (Tohda *et al.* 2004; Ji *et al.* 2006). Activation of antioxidant enzymes involve ginsenosides-induced neuroprotection (Chen *et al.* 2002; Zhou *et al.* 2006; Sanakana *et al.* 2007). The neuroprotective effect of Rh_2 depends on the increase of PACAP (Shieh *et al.* 2008).

Ginsenosides (except Ro) belong to a family of steroid-like molecules. The hydrophobic properties of ginsenosides favour their binding to the intracellular steroid hormone receptors such as estrogen receptors (ERs) (Attele *et al.* 1999; Lee *et al.* 2003). ER is expressed in astrocytes (Hosli *et al.* 2000) to play an important role in estrogen-induced development, including synapse formation, plasticity, neuronal morphology, and neuroprotection (Maccioni *et al.* 2001). Rg_1 stimulates cell proliferation in ER-positive human breast cancer cell line MCF-7 and this effect is inhibited by ICI. However, Rg_1 failed to displace the binding of radioactive 17β-estrodiol (E2) in MCF-7 cells, suggesting that the direct effect of Rg_1 on ER is not needed for its estrogenic action (Chan *et al.* 2002). Also, Rh_2 had estrogenic activity and competed with estrogen binding to ER whereas Rb_1 activated ER independent of receptor binding (Lee *et al.* 2003; Cho *et al.* 2004). Rb_1 augments the cellular antioxidant defense capacity through ERdependent HO-1 induction via the PI3K-Nrf2 signaling pathway, thereby protecting cells from oxidative stress. Rb_1 protects neurons against catecholaminergic neurotoxicity, most likely through an antioxidant pathway. Rb_1 has a partial cytoprotective role in dopaminergic cell culture systems and for this reason may serve as a useful agent in Parkinson's

Disease (Hwang and Jeong 2010). Shieh *et al.* (2008) found that Rh_2 caused an increase of PACAP expression and cell growth of RBA1. Both actions of Rh_2 mediated PAC1, but not ER, to reverse the Aβ-induced inhibition and/or toxicity. Otherwise, sustained intracerebroventricular infusion of Rg_1 may modulate the effects of interleukin-1β on an increase in water intake and sustained decrease in food intake, resulting in a lowering of body temperature (Kang *et al.* 1995). Rb_1 was also found to show a suppressive effect (Etou *et al.* 1988). Moreover, an epidemiological study showed that individuals with a low calorie intake have a reduced risk of developing AD (Luchsinger *et al.* 2002; Kivipelto *et al.* 2005). Calorie restriction ameliorated neurodegenerative phenotypes in forebrain-specific presenilin-1 and presenilin-2 double knockout mice, as well as in age-related behavioral deficits in the triple-transgenic mouse model of AD (Halagappa *et al.* 2007; Wu *et al.* 2007). Lee *et al.* (2006, 2007) demonstrated that Rh_2 increased insulin secretion in Wistar rats and improved insulin sensitivity in fructose-rich chow-fed rats. Therefore, reduced food intake might also protect against AD.

However, not all ginsenosides possess antioxidative properties; Liu *et al.* showed that some ginsenosides such as Rg_3 may act as pro-oxidants to accelerate 2,2′-azobis(2-amidinopropane hydrochloride)-induced hemolysis in human erythrocytes (Liu *et al.* 2003). Ginsenoside Rg_3 was also found to possess antiangiogenic and anticancer properties by inducing apoptosis. The pretreatment with protopanaxatriol (PPT), one of the major ginsenosides metabolites, was able to prevent the metabolic changes observed in H_2O_2-treated cells (Yue *et al.* 2006).This may be consistent with the notion that the action of ginsenosides becomes obvious when cells are stressed. However, it will be of great importance to find out what changes might occur that could affect the subsequent cellular response to H_2O_2.

Liao *et al.* (2002) identified ginsenosides Rb_1 and Rg_1 as efficient neuroprotective agents for spinal cord neurons, namely against oxidative stress induced by H_2O_2. Shen *et al.* (2007) showed that ginsenoside Rg_1 could attenuate glutamate-induced lung injury by interrupting the generation of ROS. Likewise, ginsenoside Rg_2 can efficiently protect PC12 cells against glutamate-induced neuronal injury (Li *et al.* 2006). In the central nervous system, ginsenoside Rd was reported to be effective in a protective role against oxidative stress induced by H_2O_2 in cultured PC12 cells (Ye *et al.* 2008). The protective effects of ginsenoside Rd on H_2O_2-induced cytotoxicity may be ascribed to its antioxidative properties by reducing the intracellular ROS level, decreasing malondialdehyde production (an index of lipid peroxidation) and enhancing the antioxidant enzymatic activities of superoxide dismutase and glutathione peroxidase (Ye *et al.* 2008). It has also been shown that ginseng root extract and individual ginsenosides protect astrocytes from H_2O_2-induced oxidative damage (Naval *et al.* 2007).

Cardiovascular system

Extensive studies have been conducted on the protective effects of ginseng against free radical damage on the vascular endothelium. Zhong and Jiang (1997) examined cellular structures of free radical damage on myocardial cells induced by xanthine. They measured free radicals with an electron spin resonance technique and discovered certain ginsenosides (Rb_1, Rb_2, Rb_3, Rc, Re, Rg_1, Rg_2, and Rh_1) which were counteracting the action of free radicals induced by xanthine. In an animal model, Chen (1996) showed that ginsenosides protected against myocardial reperfusion injury with a concomitant increase in 6-keto-Prostaglandin F1a and a decrease in lipid peroxidation, and also protected the rabbit pulmonary and aortic endothelium against electrolysis-induced free radical damage. Xie *et al.* (2006) showed that ginsenoside Re has antioxidant properties and this protection is, at least in part, mediated by its radical scavenging properties, especially for H_2O_2 and hydroxyl radicals. Wang *et al.* (2010) demonstrated that ginsenoside Rb_3 attenuated isoproterenol-induced myocardial injury and heart function impairment in rats, which may be, in part, by virtue of increasing the activities of myocardial antioxidant enzymes (CAT and SOD) and inhibiting myocardial lipid peroxidation in myocardial ischemia.

Rg_2 and Rh_1 appeared to inhibit the oxidation of the SH-group in the cysteine residue of the erythrocyte membrane protein. They prevented the oxidative stress-induced elevation of erythrocyte suspension viscosity and the impairment of erythrocyte elongation in response to shear stress (Samukawa *et al.* 2008).

In an early pilot clinical trial, ginsenosides prevented acute oxidant injury following reperfusion (Zhan *et al.* 1994). Increasing evidence suggests that intestinal microflora can modify ginsenosides into various metabolites that are absorbed through the intestine (Hasegawa 2004). It is suggested that the some protective effects of ginsenosides are due to the metabolites, particularly in cardiovascular system in which these metabolites interact with the vascular endothelium (Sengupta *et al.* 2004).

Ginsenosides can change the intracellular redox state and affect the ability of cells to handle oxidative stress. The GSH redox cycle represents the most important H_2O_2 elimination pathway in endothelial cells. PPT can prevent H_2O_2-induced cell death. It has been demonstrated that H_2O_2 can cause DNA damage, affect mitochondrial function, and alter redox state. The ginsenoside PPT could provide protection against redox change and energy depletion, but only partially against DNA damage. Although it is still not clear what the basic mechanism of action of PPT is, it was demonstrated that pretreatment with PPT could improve the GSH/GSSG ratio by up-regulating GPx and GR activities. This clearly demonstrated the antioxidative effects of ginsenoside in endothelial cells and supports the notion that ginsenoside metabolites circulating in our body after the consumption of ginseng may provide cardiovascular-protective effects against oxidative stress by modulating the intracellular redox status.PPT can prevent the H_2O_2-induced depletion of GSH/GSSG ratio, indicating that PPT-pretreated cells were less oxidized during oxidative stress (Kwok *et al.* 2010). The early shift of GSH/GSSG in the first 30 minute post-oxidative challenge can induce an irreversible death signal, which cannot be reversed by the recovery of GSH/GSSG ratio later (Pias and Aw 2002). PPT may inhibit the triggering of the death signal to prevent cell death. It was suggested that this may also correlate with the nuclear factor-erythroid 2 (Nrf2) (Harvey *et al.* 2009), which is known to regulate GR activity via transcriptional regulation and maintain cellular redox state. PPT may enhance GR activity by regulating Nrf2 transcriptional activity.

PPT may act as a metal chelator to prevent the action of H_2O_2. H_2O_2 can generate toxic hydroxyl radicals in the presence of transition metal ions such as Fe^{2+} in cells although some chemical studies demonstrated that the ginsenoside PPT possesses strong iron-chelating activity (Kang *et al.* 2007). PPT may affect genomic expression, which in turn regulates the expression of certain cytoprotective enzymes (i.e., GPx, GR, or catalase). A certain period of time may thus be required for protein expression. So, direct interaction of PPT with H_2O_2 in our cell model should be excluded. In this circumstance, the redox status-modulating activity of PPT is suspected. Recent findings suggest that NAD^+ can modulate many different cellular functions, including cell death, by regulating the activity of NAD^+-dependent enzymes such as the mammalian silent information regulator 2 (SIRT1). As a result, the restoration of intracellular NAD^+ levels by the ginsenoside PPT may also restore the SIRT1 activity and enhance cell survival. Ginsenoside PPT can partially inhibit PARP-1 overactivation during oxidative stress; this may also help explain the therapeutic effects of ginseng on cardiovascular diseases (Kwok *et al.* 2010).

Ginsenosides are steroid-like molecules which have a four trans-ring structure with sugar residues and many reports suggest that ginseng saponins are capable of accessing

intracellular locations thanks to their steroid-like structures, justifying their ability to attenuate the oxidative stress caused by diverse stimuli (Tang and Eisenbrand 1992; Liu *et al.* 2003). In previous studies, ginsenosides were able to modulate angiogenesis mediated by genomic and nongenomic pathways upon binding to nuclear hormone receptors. For example, Rg_1 can induce vascular endothelial growth factor expression and promote angiogenesis *in vitro* (Leung *et al.* 2006).

In some experiments, the hormonal nature of these compounds did not result in concentration-dependent or time-dependent responses. This answer is not uncommon if we take into account the fact that ginsenosides exert their effect by acting as cellular signalling agents and not by directly binding onto the site of action. This mechanism implies the need of reaching an active concentration that could induce the opposite effect when levels are much higher, as was previously demonstrated (Yamaguchi *et al.* 1996; Kim *et al.* 2003; Xin *et al.* 2005; Chun *et al.* 2007; Shang *et al.* 2007). Moreover, the obtained effects were different depending on the experimental model: i.e. the *in vitro* vascular effects of ginsenosides (Chen *et al.* 1984), or in *Xenopus* oocytes ginseng saponins induced biphasic calcium entry (Jeong *et al.* 2004). This fact could explain the lack of relationship between several of the assayed ginsenosides concentrations and the obtained results.

Gastrointestinal, respiratory and urinary systems

Geng *et al.* (2010) have investigated the effect of ginsenosideRg_1 on experimental liver fibrosis in rats. Histological analysis revealed that ginsenosideRg_1 significantly improved the extent of liver fibrosis in rats induced by thioacetamide. Ginsenoside-Rg_1 markedly suppressed the serum levels of fibrotic markers and hepatic hydroxyproline content in rats treated with thioacetamide. GinsenosideRg_1 also reduced the serum levels of alanine transaminase, aspartate transaminase and alkaline phosphatase. Finally, ginsenosideRg_1 attenuated the levels of thiobarbituric acid reactive substances in livers of rats treated by thioacetamide. In cultured hepatic stellate cells, ginsenoside-Rg1 markedly inhibited cell proliferation, activation and formation of reactive oxygen species stimulated by platelet-derived growth factor-BB (PDGF-BB). Additionally, ginsenoside Rg_1 down-regulated the expression of PDGF receptor-β by reducing the nuclear factor-κB activity, which was required for the gene expression. These results suggest that ginsenosideRg_1, which exhibits its antioxidant and antifibrotic properties, may be of potential therapeutic value in protecting the liver fibrosis Gillis (1997) showed the protective effects of ginsenosides on an injured rabbit pulmonary endothelium induced by a variant of reactive oxygen species. He further reviewed other studies and confirmed that ginseng prevented manifestations of oxygen-derived free radical injury by promoting the release of NO. The endothelial dysfunction induced by homocysteine was blocked by Rb_1 (Zhou *et al.* 2005); this study proved that either high-concentration or low-concentration of Rb_1 fully blocked free radical production.

Evidence indicates that ginsenoside Rd exerts antioxidant effects in kidney injury models and in senescence-accelerated mice (Yokozawa *et al.* 1998, 1999, 2004).

All these studies strongly indicate that ginsenosides may function as protective substances for cells undergoing degeneration after injury.

CONCLUSION

The root of *P. ginseng* C.A. Meyer (*Araliaceae*) is the most widely used of several distinct species of plants known as "ginseng" and has a medical history of more than 5000 years. In addition, ginseng and its constituents have been thought to possess antineoplastic, antistress and antioxidant effects.

The antioxidant and protective effects of ginseng and ginsenosides have been studied on different cellular types, especially in neurons, to test their effects on calcium channels, neurotransmitters release and apoptotic-related enzymes.

As a result of our research, ginseng extract may attenuate pathophysiological changes caused by oxidative stress exposure *in vitro* and *in vivo*.

The major active components of ginseng – ginsenosides – may attenuate behavioral and pathophysiological changes caused by psychological stress exposure. Ginsenosides have proved to exert protective effects that are attributed to their antioxidant ability that prevents the decrease of antioxidant enzymes and act as a free-radicals scavenger. All the reviewed studies strongly indicate that ginsenosides may function as protective substances for cells undergoing degeneration after injury.

The exact mechanism of protection against oxidative stress remains unclear, so further experiments are needed to elucidate it.

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