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Original article:

PROTECTIVE EFFECT OF GINSENG AGAINST GAMMA-IRRADIATION-INDUCED OXIDATIVE STRESS AND ENDOTHELIAL DYSFUNCTION IN RATS

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ABSTRACT

This study investigated the potential protective effects of ginseng on gamma-irradiation-induced oxidative stress and endothelial dysfunction in rats. Twenty four male albino rats were divided into four groups. In the control group, rats were administered vehicle by tube for 7 consecutive days. The second group was administered ginseng extract (100 mg/kg, by gavage) for 7 consecutive days. Animals in the third group were administered vehicle by tube for 7 consecutive days, then exposed to single dose gamma-irradiation (6 Gy). The Fourth group received ginseng extract for 7 consecutive days, one hour later rats were exposed to gamma-irradiation. Oral administration of ginseng extract prior to irradiation produced a significant protection which was evidenced by a significant reduction in serum creatine kinase (CPK) and lactate dehydrogenase (LDH) activities and asymmetric dimethylarginine (ADMA), urea and creatinine levels with significant increase in serum total nitrate/nitrite (NO(x)) level. Moreover, ginseng significantly increased cardiac and renal superoxide dismutase (SOD) and glutathione peroxidase (GSHPx) activities, and reduced glutathione (GSH) content, associated with a significant depletion in malondialdehyde (MDA) and NO(x) levels compared to irradiated group. This study suggests that ginseng may serve as a potential protective agent against gamma-irradiation-induced cardio-nephrotoxicity via enhancing the antioxidant activity and inhibition of endothelial dysfunction.

Keywords: Ginseng, gamma-radiation, asymmetric dimethylarginine, nitric oxide, oxidative stress, rats

INTRODUCTION

Radiotherapy is frequently used as a part of cancer treatment to achieve tumor control. Although radiotherapy treatment has been widely used as an effective tool to kill tumor cells, it might produce harmful effects to surrounding healthy tissues (Ostrau et al., 2009; Sezen et al., 2008). It is well known that ionizing radiations induce oxidative stress on target tissues, mainly through the generation of reactive oxygen species (ROS) resulting in imbalance of the pro-oxidant and antioxidant in the cells, attack diverse cellular macromolecules such as DNA, lipids, and proteins, eventually inducing cell death (Boerma and Hauer-Jensen, 2011; Lee et al., 2010a; Sathyasaikumar et al., 2007).

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Several studies have shown that irradiation plays an important role in the development of endothelial dysfunction; in particular, reduced bioavailability of nitric oxide (NO) (Soucy et al., 2010). Asymmetric dimethylarginine (ADMA) is widely recognized as the major endogenous inhibitor of NO-synthase and is considered an emerging cardiovascular risk factor (Kiani et al., 2007). Plasma concentration of ADMA is markedly increased in patients with chronic renal failure, and in a variety of cardiovascular diseases and moderately increased in patients with many other diseases including hyperlipidemia, diabetes mellitus, arterial hypertension, hyperhomocysteinemia and heart failure, suggesting that ADMA is an early marker of atherosclerosis vascular disease (Beltowski and Kêdra, 2006).

Ginseng is one of the most highly valued natural dietary supplements (Yi et al., 2009; Wu et al., 2011). The term ginseng refers to the dried root of several species in the genus *Panax* of the Araliaceae family (Wang et al., 2007a) including two commonly used species, i.e., *Panax ginseng* C. A. Meyer (Asian ginseng) and *Panax quinquefolius* L (North American ginseng) (Lee and Lau, 2011). Ginseng contains many physiologically important constituents including saponin (ginsenosides), polysaccharides, peptides, polyacetylenes, alkaloids, nitrogen-containing compounds, fatty acids and phenolic compounds (Attele et al., 1999; Choi, 2008; Lee et al., 2010b). Ginseng has drawn attention worldwide for its invaluable medicinal potential including antidiabetic, anticarcinogenic (Xie et al., 2005; Wang et al., 2007b), analgesic, antipyretic, antistress, and antifatigue effects (Yi et al., 2009; Lee et al., 2005), as well as enhancement of immune-modulating capabilities (Hwanga et al., 2011). These multifold bioactive medicinal properties of ginseng have been closely linked to its antioxidative effects (Kang et al., 2007; Wang et al., 2007a). In addition, ginseng and its partially purified constituents have potential radioprotective properties (Lee et al., 2006; Syaifudin et al., 2008).

Therefore, the purpose of this study was to investigate the protective effect of ginseng against heart and kidney damage induced by gamma-radiation in rats.

MATERIALS AND METHODS

Chemicals

Panax ginseng was purchased from Eipico, Egypt. It was suspended in 1 % carboxy methyl cellulose (CMC) in water and administrated orally to rats at a dose of 100 mg/kg body weight for 7 consecutive days (Gadkariem et al., 2010).

Animals

Male Wistar rats (weighing 120–150 g) were obtained from the animal farm of the Egyptian Holding Company for Biological Products and Vaccines, Egypt. Upon arrival, the animals were allowed to acclimatize for 1 week before starting the experiment. Animals were kept under standard conditions and were allowed free access to a standard requirement diet and water ad libitum. Animals were kept under a controlled lighting condition (light: dark, 13 h-11 h). The animals' treatment protocol was approved by the Animal Care Committee of the National Center for Radiation Research and Technology (NCRRT), Cairo, Egypt.

Irradiation

Whole-body gamma-irradiation was performed at the National Centre for Radiation Research and Technology (NCRRT), Atomic Energy Authority, Cairo, Egypt, using (137 cesium) Gamma Cell-40 biological irradiator. Animals were irradiated at an acute single dose level of 6 Gy delivered at a dose rate of 0.012 Gy/s.

Experimental design

Male albino rats were divided into four groups, 6 rats in each. In the control group, rats were administered vehicle (0.5 ml of 1 % CMC suspension in water) by gavage for 7 consecutive days. The second group was administered ginseng (100 mg/kg, by gavage) for 7 consecutive days. Animals in the third group were administered vehicle

by gavage for 7 consecutive days, then exposed to single dose γ-irradiation (6 Gy). The Fourth group received ginseng (100 mg/kg, by gavage) for 7 consecutive days, one hour later rats were exposed to single dose γ-irradiation (6 Gy) (Mansour and Hafez, 2012).

Biochemical assays

Twenty-four hours after the last dose of the specific treatment, animals were anesthetized with ether, and blood samples were obtained by heart puncture and serum was separated by centrifugation (Sorvall TC centrifuge, Hamburg, Germany) at 750 g at room temperature for 10 min. Serum urea nitrogen, creatinine were determined according to the methods of Hallet and Cook (1971) and Bonsenes and Taussky (1945), respectively. Serum creatine phosphokinase (CPK) and Lactate Dehydrogenase were determined according to the methods of Swanson and Wilkinson (1972) and IFCC (1980) respectively. Serum total nitrate/nitrite (NO(x)) was measured as stable end product, nitrite, according to the method of Miranda et al. (2001). ADMA was estimated using a standard enzyme linked immunosorbent assay (ELISA) method according to the manufacturer's instructions (Immundiagnostik AG, Bensheim/Germany).

Hearts and kidneys were quickly excised, washed with saline, blotted with a piece of filter paper and homogenized in ice-cold 0.15 MTris-KCl buffer (pH 7.4) to yield a 20 % (w/v) homogenate using a Branson sonifier (250, VWR Scientific, Danbury, CT, USA). The homogenates were used for the determination of malondialdehyde (MDA) level, glutathione peroxidase (GSHPx) and superoxide dismutase (SOD) activities, total glutathione (GSH) content, and total nitrate/nitrite (NO(x)). The homogenates were centrifuged at 800 g for 5 min at 4 ºC to separate the nuclear debris. The supernatant so obtained was centrifuged (Eppendorf AG, centrifuge 5804R, Hamburg, Germany) at 15000 g for 30 min at 4 ºC to get the post mitochondrial supernatant which was used to assay superoxide dismutase (SOD) activity.

Reduced glutathione (GSH) and malondialdehyde (MDA) levels in heart and kidney homogenates were determined spectrophotometrically using the methods of Ellman (1959) and Buege and Aust (1978), respectively. Total nitrate/nitrite (NO(x)) was measured as the stable end product, nitrite, according to the method of Miranda et al. (2001). The activities of GSHPx and SOD were determined according to the methods of Lawrence and Burk (1976) and Minami and Yoshikawa (1979), respectively.

Statistical analysis

Results were expressed as mean ± SEM. The intergroup variation was measured by one way analysis of variance (ANOVA) followed by Tukey's Multiple comparison test. Statistical significance was considered at $p < 0.05$.

RESULTS

Table 1 shows the effects of ginseng, irradiation and their combination on serum creatine phosphokinase (CPK), lactate dehydrogenase (LDH), creatinine and urea. Gamma-irradiation (6 Gy) induced a significant increase in CPK and LDH activities and significant increase in the levels of serum urea nitrogen and serum creatinine compared to control ($P < 0.001$). Administration of ginseng for 7 consecutive days before irradiation significantly reduced the activities of CPK and LDH, and the levels of urea and creatinine in serum ($P < 0.001$) compared to the irradiated group (Table 1).

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Table 1: Effect of ginseng, irradiation (IR, 6 Gy) and their combination on serum creatine phosphokinase (CPK) and lactate dehydrogenase (LDH) activities, creatinine and urea levels

Parameters	Control	Ginseng	IR	Ginseng + IR
CPK (IU/L)	379.9 ± 30.4	398.3 ± 19.6#	841.8 ± 17.9*	439.8 ± 30.2#
LDH (IU/L)	1031 ± 44.4	1089 ± 22.8#	2180 ± 141.4*	1214 ± 65.8#
Creatinine (mg/dl)	0.85 ± 0.03	0.87 ± 0.02#	3.01 ± 0.09*	1.32 ± 0.07#
Urea (mg/dl)	52.6 ± 2.1	49.6 ± 0.95#	65.4 ± 3.0*	54.8 ± 2.1#

Data are presented as mean ± SEM, n = 6. * and # indicate significant changes from control and IR respectively at p ≤ 0.05 using ANOVA followed by Tukey-Kramer as a post ANOVA test.

Table 2 shows the effects of ginseng, gamma-irradiation and their combination on levels of GSH, MDA, and NO(x), the activity of SOD and GSHPx in kidney tissues. Gamma-irradiation exposure resulted in significant (54.9 %, 18 % and 40.3 %) decrease in SOD and GSHPx activities and GSH content and significant (67.7 % and 114.3 %) increase in MDA and NO(x), respectively, as compared to the control group. Treatment with ginseng for 7 consecutive days prior to irradiation resulted in a significant (100.4 %, 18.8 % and 60.7 %) increase in the activities of SOD and GSHPx and in GSH content, respectively, as compared with the irradiated group, and significant (29.6 % and 44.1 %) decrease in MDA and NO(x) levels ,respectively, compared to the irradiated group.

Table 3 shows the effects of ginseng, gamma-irradiation and their combination on levels of GSH, MDA, and NO(x), the activity of SOD and GSHPx in cardiac tissues. Gamma-irradiation exposure resulted in significant (33.8 %, 52.4 % and 50 %) decrease in SOD and GSHPx activities and GSH content and significant (65.9 % and 191.7 %) increase in MDA and NO(x), respectively, as compared to the control group. Treatment with ginseng for 7 consecutive days prior to irradiation resulted in a significant (31.9 %, 117.8 % and 87.5 %) increase in the activities of SOD and GSHPx and in GSH content, respectively as compared with the irradiated group, and significant (37.9 % and 43.2 %) decrease in MDA and NO (x) levels, respectively, compared to the irradiated group.

Table 2: Effect of ginseng, irradiation (IR, 6 Gy) and their combination on the levels of malondialdehyde (MDA), total nitrate/nitrite (NO(x)) and reduced glutathione (GSH), Superoxide dismutase (SOD) and Glutathione peroxidase (GSHPx) activities in rat kidney tissue

Groups	MDA (nmol/g tissue)	SOD (µg/g tissue)	GSHPx (mole/min/g tissue)	GSH (µmol/g tissue)	NO(x) (µmol/g tissue)
control	161.9 ± 2.6	90.85 ± 1.3	0.39 ± 0.003	0.149 ± 0.001	25.1 ± 0.6
Ginseng	145.3 ± 3.7#	87.10 ± 1.9#	0.38 ± 0.002#	0.151 ± 0.001#	26.1 ± 1.6#
IR	271.5 ± 7.1*	41.04 ± 0.7*	0.32 ± 0.01*	0.089 ± 0.001*	53.8 ± 1.5*
Ginseng+IR	191.1 ± 10.2#	82.23 ± 1.1*#	0.38 ± 0.003#	0.143 ± 0.001*#	30.1 ± 1.4#

Data are presented as mean ± SEM, n = 6. * and # indicate significant changes from control and IR respectively at p ≤ 0.05 using ANOVA followed by Tukey-Kramer as a post ANOVA test.

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Table 3: Effect of ginseng, irradiation (IR, 6 Gy) and their combination on the levels of malondialdehyde (MDA), total nitrate/nitrite (NO(x)) and reduced glutathione (GSH), Superoxide dismutase (SOD) and Glutathione peroxidase (GSHPx) activities in rat cardiac tissue

Groups	MDA (nmol/g tissue)	SOD (µg/g tissue)	GSHPx (mole/min/g tissue)	GSH (µmol/g tissue)	NO(x) (µmol/g tissue)
control	76.55 ± 0.8	82.55 ± 2.1	58.37 ± 1.2	0.192 ± 0.003	31.4 ± 1.1
Ginseng	74.70 ± 1.3$^{\#}$	80.00 ± 1.3$^{\#}$	55.06 ± 1.1$^{\#}$	0.176 ± 0.005$^{\#}$	33.1 ± 1.1$^{\#}$
IR	127.0 ± 2.8*	54.64 ± 1.4*	27.76 ± 1.8*	0.096 ± 0.004*	91.6 ± 4.3*
Ginseng + IR	78.84 ± 2.9$^{\#}$	72.06 ± 1.3$^{*\#}$	60.45 ± 0.5$^{\#}$	0.180 ± 0.006$^{\#}$	52.0 ± 2.6$^{*\#}$

Data are presented as mean ± SEM, n = 6. * and # indicate significant changes from control and IR respectively at p ≤ 0.05 using ANOVA followed by Tukey-Kramer as a post ANOVA test.

Figures 1 and 2 show the effects of ginseng, irradiation and their combination on serum NO(x) and ADMA. Gamma-irradiation exposure resulted in a significant increase in the level of serum ADMA and significant decrease in serum NO(x) level compared to control ($P < 0.001$). Administration of ginseng for 7 consecutive days before irradiation significantly reduced the level of serum ADMA and the increase in serum NO(x) level ($P < 0.001$) compared to the irradiated group.

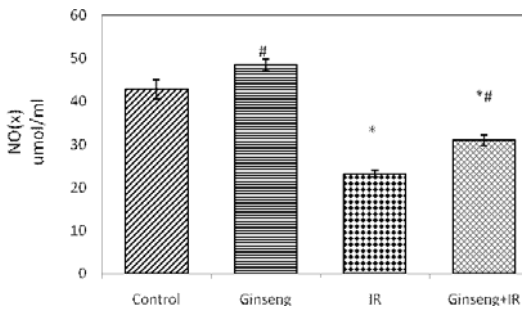

Figure 1: Effect of ginseng, irradiation (IR, 6 Gy) and their combination on serum total nitrate/nitrite (NO(x))

Data are presented as mean ± SEM, n = 6.
* and # indicate significant changes from control and IR respectively at p ≤ 0.05 using ANOVA followed by Tukey-Kramer as a post ANOVA test.

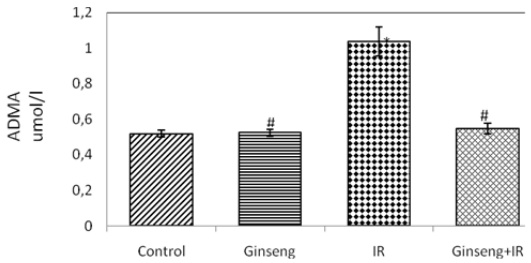

Figure 2: Effect of ginseng, irradiation (IR, 6 Gy) and their combination on serum asymmetric dimethylarginine (ADMA)

Data are presented as mean ± SEM, n = 6.
* and # indicate significant changes from control and IR respectively at p ≤ 0.05 using ANOVA followed by Tukey-Kramer as a post ANOVA test.

DISCUSSION

Ionizing radiation is known to induce oxidative stress through generation of ROS in an imbalance in pro-oxidant, antioxidant status in the cells (Bhosle et al., 2005). In the present study, Gamma-irradiation caused a marked increase in serum activities of LDH and CPK, levels of creatinine, urea and ADMA in parallel with a significant decrease in NO(x) level. These data agree with that reported in previous studies, which reported that IRR caused a significant increase in CPK and LDH activities (Sridharan and Shyamaladevi, 2002) and significant increase in urea and creatinine (Barakat et al., 2011). The excessive production of free radicals and lipid peroxides might have caused the leakage of cytosolic

enzymes such as lactate dehydrogenase, creatine kinase and phosphatases. Also, it could induce lipid peroxidation of cell membranes structure by oxygen derived free radicals leading to ionic leakage through cellular membranes and excessive calcium influx with ensuring cellular dysfunction and death from calcium overload (Ramadan et al., 1997). Increase in serum urea was due to increase in glutamate dehydrogenase enzyme as a result of irradiation and this may increase carbamoyl phosphate synthetase activity leading to increase in urea concentration (Ramadan et al., 2001).

Treatment with ginseng for 7 consecutive days prior to irradiation ameliorated the activites of serum CPK and LDH and the levels of serum creatinine and urea. This effect might be related to the antioxidative properties of ginseng, which protect the outer membrane of mammalian cells (Block and Mead, 2003). The antioxidative ability of ginseng is closely related to its ginsenoside content. Ginsenosides have the ability to intercalate into the plasma membrane, change its fluidity, and inhibit lipid peroxidation by chelating transition metals and scavenging ROS (Kang et al., 2007), ginsenosides thus affect membrane function, eliciting cellular responses to cytotoxic stresses (Attele, 1999).

In the present study, the γ-irradiated rats showed a significant increase in serum ADMA concomitantly with a significant decrease in NO(x) level. The effect is probably mediated by oxidative stress (Maas et al., 2007). In agreement with our results, previous studies of Schnabel et al. (2005), Busch et al. (2006), Ueda et al. (2007) and Wilcox (2012) have reported elevated ADMA levels and decreased NO(x) levels in states of cardiovascular diseases and chronic kidney disease in human and rat and also in response of endothelial cells to ionizing radiation (Lanza et al., 2007). Elevated levels of ADMA inhibit NO synthesis and therefore impair endothelial function (Sibal et al., 2010). Reduction of NO(x) levels might be due to both decreased production and increased consumption, with possible endothelial dysfunction and vascular impairment (Soloviev et al., 2003).

Consistent with previous studies (Kim and Lee, 2010; Pan et al., 2012), the present study showed that the endothelial NO(x) and ADMA levels were ameliorated by the administration of ginseng. Pan et al. (2012) reported that, ginseng reduced ROS production and increased NO(x) levels, thus ameliorating endothelial dysfunction. Panax ginseng is known to enhance the release of nitric oxide (NO) from endothelial cells of the rat aorta and kidney, and to protect the heart from injury via up-regulation of endothelial NO synthase (eNOS) expression (Wu et al., 2011; Razavi et al., 2005; Hare and Stamler, 2005; Han and Kim, 1996), resulting in Ca(2+) channel inhibition, activation of cardiac potassium channels and protection against ischemia-reperfusion injury (Wang et al., 2008; Szelid et al., 2010) and this protection is caused by reducing oxidative stress (Kim and Lee, 2010). These findings suggest that some of the observed effects of ginseng are possibly mediated through its antioxidant property.

Consistent with previous studies (Mansour and Hafez, 2012; Pradeep et al., 2012), the present study showed a significant depletion in the antioxidant system accompanied by enhancement of lipid peroxides and NO(x) levels in cardiac and renal tissues after whole body gamma-irradiation. Ionizing radiation is known to induce oxidative stress through generation of ROS in an imbalance in pro-oxidant, antioxidant status in the cells (Bhosle et al., 2005). The increase in lipid peroxidation levels in γ-irradiated rats might be due to the interaction of free radicals with polyunsaturated fatty acids in the phospholipids portion of cellular membranes (Prasad et al., 2005; Spitz et al., 2004). The decrease in the activities of SOD and GSHPx and the decreased level of GSH might be due to their utilization by the enhanced production of ROS, which interacts with the enzyme molecules causing their denaturation and partial inactivation (Kregel and Zhang, 2007;

Maurya et al., 2006). Under normal conditions the inherent defense system, including glutathione and the antioxidant enzymes, protects against oxidative damage. GSH, a well-known antioxidant, provides major protection in oxidative injury by participating in the cellular system of defense against oxidative damage (Sener et al., 2006). It was reported that tissue GSH levels and the activities of glutathione reductase and glutathione peroxidase, which are critical constituents of GSH redox cycle, were significantly reduced due to oxidative stress, permitting enhanced free radical-induced tissue damage (Reiter et al., 2001).

Our results show that whole body gamma-irradiation of rats at 6 Gy enhanced the formation of cardiac and renal NO(x). Similar results have been reported by Gorbunov et al. (2000). Gamma-irradiation may enhance endogenous NO biosynthesis in liver, intestine, lung, kidney, brain, spleen or heart of the animals, presumably by facilitating the entry of Ca^{2+} ions into the membrane as well as the cytosol of NO-producing cells through irradiation-induced membrane lesions. The enhancement of NO production following exposure to a high dose (6 Gy) of gamma rays was attributed to high levels of expression of the inducible nitric oxide synthase (Ibuki and Goto, 1997).

Consistent with previous studies (Abdel-Wahhab and Ahmed, 2004; Kim et al., 2011; Park et al., 2011; Ramesh et al., 2012), administration of ginseng markedly elevated the levels of antioxidant enzymes, indicating the antioxidant potential of the ginseng. This might be due to metabolized ginsenosides, which protect the outer membrane of mammalian cells (Block and Mead, 2003; Lee et al., 2009), scavenge free radicals, restore the GSH level and inhibit NO production (Zhu et al., 2009; Zhang et al., 2010). Panax ginseng extract has been shown to inhibit lipid peroxidation through transition metal chelation and scavenging of hydroxyl and superoxide radicals (Kitts et al., 2000). It has been also reported that Panax ginseng administration increased the activity of the antioxidant enzymes SOD and GSHPx in rats (Han et al., 2005; Sena et al., 2012; Sun, 2011).

Kumar et al. (2003) found that administration of panax ginseng root extract before irradiation significantly decreased lipid peroxidation levels and reduced the radiation damage in mice testes.

The results from the present investigation indicate that ginseng pretreatment protects against radiation damage by inhibiting radiation-induced oxidative stress and endothelial dysfunction by decreasing serum ADMA, increasing serum NO(x) and ameliorating the antioxidant system in cardiac and renal tissues.

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