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Inhibition of *Lycium barbarum* polysaccharides and *Ganoderma lucidum* polysaccharides against oxidative injury induced by γ-irradiation in rat liver mitochondria

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Abstract

Lycium barbarum and *Ganoderma lucidum*, the two precious traditional Chinese medicines, possesses the antitumor activity, antioxidant activity and the capability of modulating the immune system. In the present study, the possible antioxidant effects of *L. barbarum* polysaccharides (LBP) isolated from dried *L. barbarum* fruits and *Ganoderma lucidum* polysaccharides (GLP) isolated from dried *G. lucidum* against membrane damage induced by the free radicals generated during γ-irradiation were examined in rat liver mitochondria. Using an effective dose of 450 Gy, antioxidant effects of GLP and LBP were studied against oxidative damage in terms of protection against lipid peroxidation, protein oxidation, depletion of protein thiols and the levels of the antioxidant enzyme, such as superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px). The results showed that both GLP and LBP significantly protects against irradiation-induced loss of protein thiols and inactivation of SOD, CAT and GSH-Px in a dose-dependent manner. The inhibitory effects of GLP, at the entire concentration range, are stronger than LBP. Moreover, the two polysaccharides are more effective than α-tocopherol (VE) in inhibiting irradiation-induced oxidative injury. Hence our results indicate that GLP and LBP have potent antioxidant properties in vitro in mitochondrial membranes of rat liver.
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Keywords: Irradiation; Rat; SOD; LBP; GLP; Polysaccharides

1. Introduction

In radiotherapy, ionizing irradiation is used to damage target cells or tissues. However, the irradiation also damages nontarget cells and/or tissues. Oxidative stress occurs when there is excessive free radical production and/or low antioxidant defense, and results in chemical alterations of bio-molecules causing structural and functional modifications (Cuzzocrea & Reiter, 2001). The generation of the reactive oxygen metabolites (ROMs) plays an important role in the pathogenesis of irradiation-induced tissue injury (Agrawal, Chandra, & Kale, 2001). An extensive literature implicates cellular DNA as the primary target for the biological and lethal effects of ionizing irradiation (Daly, Bertagnoli, De Cosse, & Morton, 1999). Besides DNA, lipids and proteins are also attacked by free radicals induced by ionizing irradiation (Edwards, Chapman, Cramp, & Yatvin, 1984; Reiter, Calvo, Karbownik, Qi, & Tan, 2000). Because irradiation of the body results in oxidative stress due to the formation of oxygen radicals, damage due to irradiation could be controlled by antioxidants and antioxidative enzymes, resulting in successful radiotherapy. Thus, it is important to clarify the relationship between the treatment of antioxidants and oxidative damage of biomolecules in both target and nontarget tissues due to irradiation at the practical irradiation doses that are applied in radiotherapy.

Many naturally occurring compounds with antioxidant activity are known to protect cellular components from

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oxidative damage and prevent diseases (Lu, Nie, Belton, Tang, & Zhao, 2006). Fruit from *Lycium barbarum* in the family Solanaceae is well-known in traditional Chinese herbal medicine and nowadays has been widely used as a popular functional food, with a large variety of beneficial effects, such as reducing blood glucose and serum lipids, anti-aging, immuno-modulating, anticancer, anti-fatigue, hepatitis, thrombosis and male fertility-facilitating (Gao, Xu, & Li, 2000; Gan & Zhang, 2003; Peng, Huang, Qi, Zhang, & Tian, 2001a, Peng, Qi, Tian, & Zhang, 2001b; Wang, Wang, Zhang, & Zhang, 2002; Wang et al., 2002). The earliest Chinese medicinal monograph documented medicinal use of *L. barbarum* around 2300 years ago. *L. barbarum* fruits can be used to produce various types of healthy products and foods, e.g., medicinal beverages and drinks, and healthy dietary soups (Li, 2001). Some constituents of *L. barbarum* fruits have been chemically investigated, especially *Lycium barbarum* polysaccharide (LBP) components which are the most important functional factor (Gan & Zhang, 2003; Gan, Zhang, Yang, & Xu, 2004; Qi et al., 2001; Zhang et al., 2005). Five polysaccharides (glycoconjugates) (LbGp1-LbGp5) were isolated and structurally elucidated (Gan & Zhang, 2003; Wang et al., 2002).

Ganoderma lucidum, which belongs to the Polyporaeae family, has been used for thousands of years in traditional oriental medicine and this medicinal mushroom has been considered as a panacea in Chinese medicine (Noguchi et al., 2005). Earlier investigation have demonstrated that the fruiting bodies of *G. lucidum* occurring in South India possessed significant antioxidant, anti-inflammatory, antinociceptive and antitumor activities (Jones & Janardhanan, 2000; Sheena, Ajith, & Janardhanan, 2003). Their antioxidative, anti-inflammatory and immune enhancing properties, along with no toxicity, raise the possibilities that they could be effective in preventing cancer or related diseases.

The purpose of this study was to establish the impact of oxidative stress in irradiation-induced tissue damage in rat liver mitochondria. In addition, we also aimed to investigate the possible radioprotective effect of GLP and LBP administration on irradiation-induced damage of these tissues.

2. Materials and methods

2.1. Materials

2-Thiobarbituric acid, ethylenediaminetetraacetic acid (EDTA), guanidine hydrochloride, metaphosphoric acid, butylated hydroxytoluene, superoxide dismutase, catalase, glutathione peroxidase, epinephrine, tetraethoxypropane and dinitrophenyl hydrazine were purchased from the NanJing JianChen Biotechnology Co., Ltd, (Sigma Chemical, USA). Tetraethoxypropane was used as the standard for estimating malonaldehyde equivalents. Other chemicals used were of analytical grade. *G. lucidum* was purchased in the local medicinal markets. *L. barbarum* fruits, growing in JingHe county, XinJiang province in China, was kindly provided by Institute of ShengNong Biotechnology.

2.2. Isolation of mitochondrial fraction

Three-month-old Wistar rats (weighing about 250–300 g) comprising of 50% female and 50% male animals obtained from the Central Animal House of the Department of Biochemistry, were used for the preparation of mitochondria. Isolation of mitochondrial fraction was done by consulting the method of Kamat, Boloor, Devasagayam, and Venkatachalam (2000). In brief, rat liver was isolated, homogenised in 0.3 M sucrose containing 0.5 mM EDTA and 2 mM Mops, pH 7.4 at 4 °C. The homogenate was centrifuged at 4000*g* for 15 min to remove cell debris and the nuclear fraction. The resultant supernatant was centrifuged thrice at 10,000*g* for 15 min to sediment mitochondria. The mitochondria were washed three times (in 120, 60 and 30 ml pyrophosphate buffer, respectively) to remove sucrose. Protein contents were assayed (Lowry, Rosebrough, Farr, & Randall, 1951) and the mitochondria were suspended in the above buffer at the concentration of 12 mg protein/ml.

2.3. Exposure of mitochondria to irradiation and assays of oxidative damage

Consulting the method of Kamat et al. (2000), oxidative damage was induced by exposure to γ rays from a 60Co source (dose rate 13 Gy/min). The mitochondria (final concentration 4 mg protein/ml) were suspended in the above buffer and exposed to irradiation, with or without samples tested. After irradiation exposure lipid peroxidation (hepatic microsomal fraction) as an index of oxidation damage was determined fluorometrically (exitation wavelength at 513 nm; emission at 553 nm) as thiobar bituric-acid-reactive substances (TBARs) and lipid hydroperoxides (LOOH), using a modified method of Ohkawa, Ohishi, and Yagi (1979) and Vajragupta et al. (2000) and Jiang, Hunt, and Wolf (1992). The level of the protein oxidation products as protein carbonyls were measured spectrophotometrically by using the method of Kamat et al. (2000). The level of protein thiols, another protein oxidation products were assayed by the method of Gregor, Staniek, Nohl, and Gille (2006). The activities of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GSH-Px) were also measured by the methods of Marklund and Marklund (1974), Sinha (1972) and Rotruck, Pope, and Ganther (1973), respectively.

2.4. Preparation of GLP and LBP

Dried *G. lucidum* (400 g) were powdered and sequentially extracted in a sohxlet with boiling water for 4 h. This extract was filtered and an excess of ethanol was added. The precipitate obtained was filtered, washed. The combined extracts

were concentrated and deproteinated by the Sevag method (1934) and the resulting aqueous fractions was extensively dialyzed against running distilled water for 2 days, dried to obtain desired product (GLP). The product was hazel powder with a molecular weight of 584,847 and contained 16 different amino acid residues. The ratio of polysaccharides to peptides is 94.2:6.1%. The polysaccharides consisted of D-rhamnose, D-xylose, D-fructose, D-galactose, D-mannose, and D-glucose with molar ratio of 0.778:0.953:2.893:0.155:0.393:7.83 and linked together by β-glycosidic linkages.

The dried fruit of *L. barbarum* (400 g) was powdered with a blender and the ground samples were put in boiling water and decocted by a traditional method for Chinese medicinal herbs. The combined extracts were concentrated and deproteinated by the Sevag method (1934) and the resulting aqueous fractions was extensively dialyzed against running distilled water for 2 days. The retentate was concentrated to 400 ml under reduced pressure and precipitated by addition of three volumes of 95% ethanol. After filtering and centrifuging, the resulting precipitate was collected and vacuum-dried at 40 °C, giving a brown powder (LBP, 1.9% from the material, i.e. 7.4 g) with a molecular weight of 24,132. The polysaccharides consisted of D-rhamnose, D-xylose, D-arabinose, D-fucose, D-glucose, and D-galactosc with molar ratio of 1:1.07:2.14:2.29:3.59:10.06 and linked together by β-glycosidic linkages.

2.5. Statistics

The statistical analyses were performed by two-way ANOVA, followed by Dunnett's *t*-test. The results were expressed as the means ± SD to show variations in a group. Differences are considered significant when $p < 0.05$.

3. Results and discussion

The steadily increasing use of irradiation technologies in medicine, industry, agriculture and scientific research has been paralleled by increasing potential risk for irradiation over exposure. There is now considerable evidence indicating that the cytotoxic effects of ionizing irradiation are mediated almost entirely by oxygen free radicals (Epperly et al., 1998). Under basal conditions, oxidant-generating systems are compensated for by the presence of complex sets of protective mechanisms that prevent or limit oxidative damage, mechanisms that include antioxidant enzymes (superoxide dismutase, catalase, glutathione peroxidase) and nonenzymatic scavengers (Rybak, Husain, Whitworth, & Somani, 1999; Somani et al., 2000). However, in conditions of severe oxidative stress such as irradiation treatment, reactive oxidant species such as superoxide (O_2^-) and hydroxyl radical (^-OH) are excessively accumulated, resulting in increased lipid peroxidation in irradiation-treated rats. The supplement of nonenzymatic free radical scavengers, such as naturally occurring polysaccharides isolated from plants, microorganism and animals, have long been identified to be effective in enhancing the cellular defense mechanism against endogenous and exogenous oxidants (Nardini et al., 1997).

The anti-irradiation activity of LBP and GLP has already been studied, including reduction of the frequency of radiation-induced DNA breaks in murine lymphocytes (Kim & Kim, 1999; Lakshmi, Tilak, Adhikari, Devasagayam, & Janardhanan, 2005), protection from 137Cs-induced micronuclei in human lymphocytes (Ramarathnam, Osawa, Ochi, & Kawakishi, 1995; Urnysheva, Guliaeva, & Kushnireva, 2002), and enhancement of proofreading activity of eukaryotic DNA polymerase (Pillail, Salvi, Maurya, Krishnan Nair, & Janardhanan, 2006, 2003). However, the anti-irradiation compariation effect of the two polysaccharide has not previously been investigated. Anti-lipid peroxidation studies were performed in rat liver mitochondria using the TBARS and LOOH method (Jiang et al., 1992; Ohkawa et al., 1979; Vajragupta et al., 2000). Effect of irradiation-induced (450 Gy) lipid peroxidation was found to be significant ($p < 0.01$) as compared to the nonirradiation control (Tables 1 and 2). We observed decreased concentrations of TBARS and lipid hydroperoxides on antioxidants (GLP, LBP and VE) supplementation. Vitamin E is an efficient antioxidant, which prevents lipid peroxidation. Przbyszewski, Widel, and Koterbicka (1994) reported that the TBA-reactive substances (TBARS) concentration in serum and heart increased about 4 days after fractionated γ-irradiation, and this increase was diminished by Vitamin E supplementation. This is in agreement with the results of our work. When the formation of TBARS was used as a measure of peroxidation in our study, a lower concentration of VE (10 mg/ml) gave an inhibition of 25.1% whereas a higher concentration of VE (60 mg/ml) resulted in a 43.9% protection (Table 1). As was shown in Tables 1 and 2, an increase of antioxidant dose (LBP and GLP) resulted in a decrease of the concentration of TBARS and LOOH, which is in close agreement with the earlier observations that administration of antioxidant decreased the level of

Table 1
Effect of GLP and LBP on irradiation-induced TBARS formation in rat liver mitochondria

Group	TBARS (nmol/mg protein)
Nonirradiation control	0.11 ± 0.02
Irradiation control	1.87 ± 0.10[b]
Irradiation + LBP (10 mg/ml)	1.61 ± 0.26[c]
Irradiation + LBP (30 mg/ml)	1.01 ± 0.15[d]
Irradiation + LBP (60 mg/ml)	0.71 ± 0.05[d]
Irradiation + GLP (10 mg/ml)	1.24 ± 0.13[d]
Irradiation + GLP (30 mg/ml)	0.95 ± 0.12[d]
Irradiation + GLP (60 mg/ml)	0.55 ± 0.03[d]
Irradiation + VE (10 mg/ml)	1.40 ± 0.16[d]
Irradiation + VE (30 mg/ml)	1.27 ± 0.12[d]
Irradiation + VE (60 mg/ml)	1.05 ± 0.06[d]

Each value represents mean ± SD; $n = 8$. Irradiation dose used was 450 Gy. (b) $p < 0.01$, compared with nonirradiation control; (c) $p < 0.05$; (d) $p < 0.01$; compared with irradiation control.

Table 2
Effect of GLP and LBP on irradiation-induced LOOH formation in rat liver mitochondria

Group	LOOH (nmol/mg protein)
Nonirradiation control	0.10 ± 0.02
Irradiation control	6.39 ± 0.51^{b}
Irradiation + LBP (10 mg/ml)	3.25 ± 0.40^{d}
Irradiation + LBP (30 mg/ml)	1.02 ± 0.11^{d}
Irradiation + LBP (60 mg/ml)	0.07 ± 0.03^{d}
Irradiation + GLP (10 mg/ml)	1.09 ± 0.08^{d}
Irradiation + GLP (30 mg/ml)	0.22 ± 0.01^{d}
Irradiation + GLP (60 mg/ml)	0.04 ± 0.01^{d}
Irradiation + VE (10 mg/ml)	5.72 ± 0.74
Irradiation + VE (30 mg/ml)	4.95 ± 0.33^{d}
Irradiation + VE (60 mg/ml)	3.38 ± 0.12^{d}

Each value represents mean ± SD; $n = 8$. Irradiation dose used was 450 Gy. (b) $p < 0.01$, compared with nonirradiation control; (d) $p < 0.01$; compared with irradiation control.

lipid peroxidation after be exposed (Kamat et al., 2000). Our results demonstrated that the two polysaccharides were effective in inhibiting lipid peroxidation at the concentrations of 10, 30 and 60 mg/ml in the reaction mixture (Table 1 and 2). The LBP, at the concentration of 10 mg/ml, decreased peroxidation by 13.9%, while at 30 and 60 mg/ml it showed a decrease of 46% and 62%, respectively. The GLP, at the concentration of 10 mg/ml, decreased peroxidation by 33.7%, while at 30 and 60 mg/ml it showed a decrease of 49.2% and 70.6%, respectively. It was noteworthy that protective effects of both polysaccharides were significantly stronger than VE at same concentration tested. LOOH is often present in biological membrane systems. The data on antioxidant effects of the LBP and GLP on the irradiation-induced formation of LOOH are given in Table 2. The standard antioxidant VE, at the concentration of 10, 30 and 60 mg/ml, gave 10.5%, 22.5% and 47.1% protection, respectively. The LBP showed 49.1%, 84% and 98.9% protection against peroxidation induced by 450 Gy irradiation dose at the concentrations tested. The GLP, at the concentration of 10 mg/ml, gave 82.9% protection and that at 30 and 60 mg/ml showed 96.5% and complete protection (100%), respectively. At the concentrations tested, the two polysaccharides showed complete inhibition of lipid peroxidation at a higher concentration of 60 mg/ml. The exact radioprotective mechanism of polysaccharides is a matter of hot debate at present. It has been suggested that polysaccharides, including LBP and GLP, protect animals against radiation through the elevation of endogenous production of antioxidants, which may reduce the oxidative damage via induction of mitochondrial enzymes and other scavenging proteins (Chen & Dong, 2004; Trommer & Neubert, 2005). In the present studies, we observed that the activities of superoxide dismutase, catalase and glutathione peroxidase were stimulated in the irradiated rats that had received polysaccharides (Table 5). Therefore, we suggest that polysaccharides (LBP and GLP) can suppress radiation-induced lipid peroxidation via a radical scavenging mechanism.

On the other hand, the decrease in protein thiols content, SOD, CAT and GSH-Px activities, and the increase in protein carbonyls levels in liver mitochondria postirradiation as recorded in the present study are in agreement with those recorded by (Lee et al., 2003), (Pleshakova, Kutsyi, Sukharev, Sadovnikov, & Gaziev, 1998) and (Klein, Walt, & Richter, 1997). They recorded a significant depletion in the antioxidant system accompanied by enhancement of lipid peroxides after mitochondria γ-irradiation. Under normal conditions the inherent defense system, including glutathione and the antioxidant enzymes, protects against oxidative damage. Table 3. describes the protective effect of LBP and GLP on protein oxidation. The formation of protein carbonyls was significantly enhanced at a dose of 450 Gy ($p < 0.01$). The presence of LBP and GLP during irradiation could effectively and concentration-dependently prevent the oxidation of protein. The two polysaccharides treatment showed 60% and 86.3% protection against the formation of protein carbonyls at the concentrations of 60 mg/ml bringing the levels to nonirradiation control values ($p < 0.05$). The VE, on the other hand, was relatively less effective, at 10, 30 and 60 mg/ml concentrations. Table 4. demonstrates the depleted levels of protein thiols following irradiation exposure and their restoration by LBP and GLP. A decrease in the concentration of protein thiols with irradiation at the dose of 450 Gy was quite significant ($p < 0.05$). The LBP and GLP, at the lower concentration of 10 mg/ml, gave 11.9% and 47.5% protection respectively and that observed at a higher concentration of 60 mg/ml was 60% and 86.3% respectively. The VE was relatively less effective. The protection afforded by VE treatment, at 10 and 60 mg/ml, was 1.3% and 23.8%, respectively. Our results showed that administration of polysaccharides produced a profound effect in protecting mitochondria against radiation-induced protein oxidation (Table 4). These results have corroborated earlier reports (Yuan et al., 2003; Yan, Xu, & Lu, 2002).

Table 3
Effect of GLP and LBP on irradiation-induced formation of protein carbonyls in rat liver mitochondria

Group	Protein carbonyls (nmol/mg protein)
Nonirradiation control	1.17 ± 0.18
Irradiation control	1.60 ± 0.19^{b}
Irradiation + LBP (10 mg/ml)	1.41 ± 0.17
Irradiation + LBP (30 mg/ml)	1.32 ± 0.16^{d}
Irradiation + LBP (60 mg/ml)	0.64 ± 0.08^{d}
Irradiation + GLP (10 mg/ml)	0.84 ± 0.11^{d}
Irradiation + GLP (30 mg/ml)	0.39 ± 0.02^{d}
Irradiation + GLP (60 mg/ml)	0.22 ± 0.02^{d}
Irradiation + VE (10 mg/ml)	1.58 ± 0.22
Irradiation + VE (30 mg/ml)	1.42 ± 0.14^{c}
Irradiation + VE (60 mg/ml)	1.22 ± 0.12^{d}

Each value represents mean ± SD; $n = 8$. Irradiation dose used was 450 Gy. (b) $p < 0.01$, compared with nonirradiation control; (c) $p < 0.05$, (d) $p < 0.01$; compared with irradiation control.

Table 4
Effect of GLP and LBP on irradiation-induced depletion of protein thiols in rat liver mitochondria

Group	Protein thiols (nmol/mg protein)
Nonirradiation control	14.03 ± 1.81
Irradiation control	8.21 ± 1.12[b]
Irradiation + LBP (10 mg/ml)	8.76 ± 1.02
Irradiation + LBP (30 mg/ml)	9.33 ± 1.22
Irradiation + LBP (60 mg/ml)	11.16 ± 1.09[d]
Irradiation + GLP (10 mg/ml)	9.28 ± 0.65[c]
Irradiation + GLP (30 mg/ml)	11.49 ± 0.72[d]
Irradiation + GLP (60 mg/ml)	13.42 ± 0.91[d]
Irradiation + VE (10 mg/ml)	8.18 ± 0.57
Irradiation + VE (30 mg/ml)	8.96 ± 1.08
Irradiation + VE (60 mg/ml)	9.33 ± 0.52[d]

Each value represents mean ± SD; $n = 8$. Irradiation dose used was 450 Gy. (b) $p < 0.01$, compared with nonirradiation control; (c) $p < 0.05$; (d) $p < 0.01$; compared with irradiation control.

Several authors have suggested a possible correlation between lipid and protein oxidation (Kamat et al., 2000; Viljanen, Kivikari, & Heinonen, 2004). Both primary (hydroperoxides) and secondary (aldehydes and ketones) lipid oxidation products can react with proteins and in that way cause protein oxidation (Kikugawa, Kato, & Hayasaka, 1991). The carbonyl complexes due to protein–lipid interaction are rapidly formed and are relatively stable, these protein carbonyl compounds being convenient markers of protein oxidation (Viljanen et al., 2004). In our study, a significant correlation coefficients was found between protein oxidation and lipid oxidation induced by free radical caused under irradiation (Table 1–4). These correlations reinforce the link between lipid and protein oxidation. Furthermore, all these parameters (TBARS, LOOH, protein carbonyls and protein thiols in irradiation-induced lipid and protein oxidation; protective effect of GLP, LBP and α-tocopherol) seem to be suitable methods to predict not only the oxidative susceptibility of lipids and proteins in rat liver mitochondria to irradiation treatment, but also protection of antioxidants from irradiation-induced oxidative injury. From these correlations, it seems clear that supplement with antioxidants (GLP, LBP and α-tocopherol) are more effective in preventing lipid and protein oxidation in irradiation-treated rat liver mitochondria.

ROS is generated from O_2 by multiple pathways (Evans & Halliwell, 2001; Freidovich, 1999; Gilbert, 2000; Wu & Morris, 1998). Under physiological conditions, there is a well managed balance between the formation and neutralization of ROS by these systems. Oxidative stress can occur when ROS production is accelerated or when the mechanisms involved in scavenging ROS is impaired. Increased production of ROS is thought to occur more frequently than diminished antioxidant defence, and is postulated to play a role in the pathogenesis of several irradiation-induced diseases (Shao, Dai, Xu, Lin, & Gao, 2004). SOD, GSH-Px and CAT are important antioxidant enzymes to inhibit oxyradical formation and usually used as biomarkers to indicate ROS production (Oost, Beyer, & Vermeulen, 2003; Regoli et al., 2003; Zhang, Shen, Wang, Wu, & Xue, 2004). Superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GSH-Px) constitute the enzymic antioxidant system, which scavenges ROS and lipid peroxidation. SOD is the only enzymes that disrupts superoxide radicals and are present in all cells with high amounts in erythrocytes (Bradford, 1976). The primary role of CAT is to abolish H_2O_2 that has been generated by free radicals or by SOD in removal of superoxide anions and to convert it to water. (Urso & Clarkson, 2003). GSH-Px is an equally important antioxidant, which reacts with hydrogen peroxide thus preventing intracellular damage caused by the same.

Free radicals generated by irradiation would attack the membrane and cause its peroxidation. Peroxidation of membrane lipids could disturb the anatomical integrity of the membrane and diminishes its fluidity leading to inhibition of several membrane bound enzymes (Burk, 1983). For example, irradiation-induced ROS markedly alters the physical, chemical and immunologic properties of endogenetic antioxidant enzymes (SOD, CAT, and GSH-Px), which further increase oxidative damage in cells. The cytotoxic effect of free radicals is deleterious to mammalian cells. Some laboratory studies have reported an increase or decrease in their activities in rat liver mitochondria exposed

Table 5
Effect of GLP and LBP on irradiation-induced depletion of antioxidant enzymes in rat liver mitochondria

Group	SOD (U/mg protein)	GSH-Px (U/mg protein)	CAT (U/mg protein)
Nonirradiation control	5.85 ± 0.60	6.93 ± 0.32	3.33 ± 0.36
Irradiation control	1.91 ± 0.22[b]	2.35 ± 0.20[b]	0.95 ± 0.26[b]
Irradiation + LBP (10 mg/ml)	2.49 ± 0.27[d]	2.64 ± 0.15[d]	1.20 ± 0.21
Irradiation + LBP (30 mg/ml)	3.34 ± 0.31[d]	2.74 ± 0.22[d]	3.88 ± 0.26[d]
Irradiation + LBP (60 mg/ml)	5.57 ± 0.41[d]	3.94 ± 0.21[d]	4.51 ± 0.27[d]
Irradiation + GLP (10 mg/ml)	3.07 ± 0.29[d]	2.66 ± 0.11[d]	3.25 ± 0.11[d]
Irradiation + GLP (30 mg/ml)	3.89 ± 0.22[d]	2.83 ± 0.22[d]	5.33 ± 0.22[d]
Irradiation + GLP (60 mg/ml)	6.11 ± 0.21[d]	4.77 ± 0.29[d]	7.08 ± 0.55[d]
Irradiation + VE (10 mg/ml)	1.92 ± 0.22	2.75 ± 0.40[c]	0.99 ± 0.16
Irradiation + VE (30 mg/ml)	2.25 ± 0.27[c]	2.99 ± 0.12[d]	1.58 ± 0.25[d]
Irradiation + VE (60 mg/ml)	2.56 ± 0.22[d]	3.85 ± 0.17[d]	2.94 ± 0.18[d]

Each value represents mean ± SD; $n = 8$. Irradiation dose used was 450 Gy. (b) $p < 0.01$, compared with nonirradiation control; (c) $P < 0.05$; (d) $p < 0.01$, compared with irradiation control.

to irradiation (Hill & Burk, 1984). Table 5 presents the antioxidant activities of LBP, GLP and VE in rat liver mitochondria. Compared with the nonirradiation control group, SOD, GSH-Px and CAT activities decreased by 67.4%, 66.1% and 71.5%, respectively, in irradiation group. Therefore, it is perhaps not surprising that these antioxidant enzymes showed much or significant response to irradiation-induced oxidative injury in rat liver mitochondria. The abnormal alterations associated with irradiation were effectively prevented with two polysaccharides treatment. Our results demonstrated that the protective effect of LBP on SOD, GSH-Px and CAT activities inactivation, at the lower concentration of 10 mg/ml, was in turn the recovery in activity of about 14.7%, 6.3%, and 7.5% while that obtained at the higher concentration of 60 mg/ml was 92.9%, 34.7% and 149.5% (Table 5), respectively. In addition, the protective effect of GLP on SOD, GSH-Px and CAT activities inactivation, at the lower concentration of 10 mg/ml, was in turn the recovery in activity of about 29.4%, 6.8%, and 96.6% while that obtained at the higher concentration of 60 mg/ml was 106.6%, 52.8% and 257.6% (Table 5), respectively. The VE, at entire concentration range, was relatively less effective. It, at 60 mg/ml, shows 16.5%, 32.8%, and 83.6% restoration in the activities of SOD, GSH-Px and CAT, respectively. The increased activity of antioxidant enzymes in polysaccharides-treated irradiated rats may be attributed to two effects, either by facilitating the replacement of lost antioxidase activity in irradiated tissue or by enhancing synthesis of essential repair enzymes (Ohkawa et al., 1979). It can be concluded that these polysaccharides treatment might facilitate the synthesis of essential antioxidant enzymes which have a role in preventing the accumulation of pathological concentrations of oxygen radicals or ameliorating biochemical changes caused by irradiation-induced bond homolysis leading to free radicals in radiosensitive tissues.

4. Conclusion

In this context, we have already established in our laboratory that polysaccharides inhibits lipid peroxidation and scavenges reactive oxygen species. The present results showed that treatment with polysaccharides improved most parameters investigated in the irradiated rat liver mitochondria. Moreover, among three tested antioxidants, GLP exhibits the highest antioxidant activity, followed by LBP and VE. In conclusion, both GLP and LBP are effective antioxidants which can protect rat liver mitochondria from irradiation-induced lipid peroxidation and protein oxidation by enhancing endogenetic antioxidant enzymes.

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