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Influence of *Lycium barbarum* Extract Intake on Oxidative Stress in *Wistar* Rats

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HIGHLIGHTS

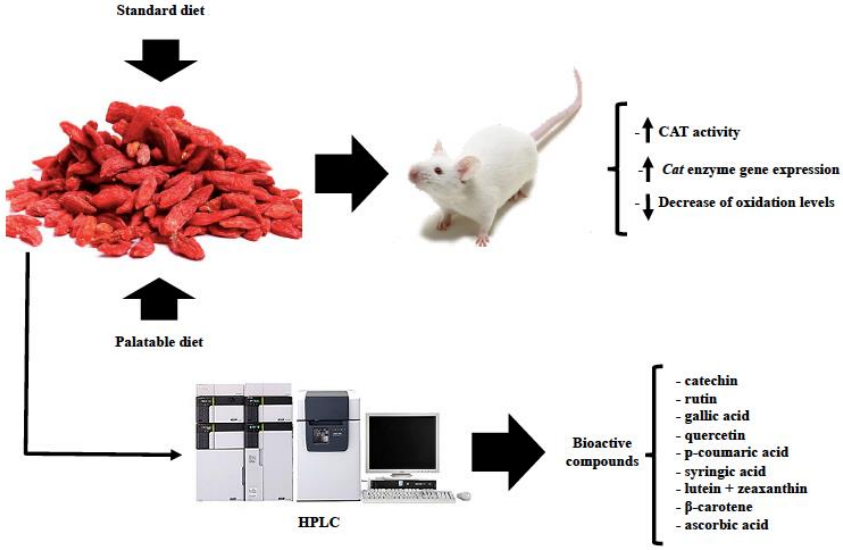
- *L. barbarum* promote the increase in catalase enzyme activity in the liver of rats.
- *L. barbarum* increase mRNA expression of catalase enzyme in the liver of rats.
- *L. barbarum* extract produced a significant oxidation decrease in the liver of rats.
- *L. barbarum* extract has a significant amount of bioactive compounds.

Abstract: This study aimed to analyze the effects of Goji Berry extract (GB, *Lycium barbarum*) gavage administration on liver tissue oxidative stress in *Wistar* rats as well as to identify and quantify the content of the major bioactive compounds of the fruit. Four diets were applied: SW - standard diet + water; SG - standard

diet + Goji Berry extract (125 mg/kg of animal); PW - palatable diet + water; PG - palatable diet + Goji Berry extract (125 mg/kg of animal). Results showed a significant increase in catalase enzyme activity in the liver of rats treated with GB and also in those intaking the palatable diet without GB when compared to the SW group. An increased mRNA expression of this enzyme in the same tissue and groups was also verified. Regarding lipid peroxidation, the GB extract produced a significant decrease in the oxidation state in the SG and PG groups. The results also showed a significant amount of bioactive compounds in GB extract.

Keywords: Goji Berry; Oxidative damage; Lipid peroxidation; Antioxidant enzymes.

GRAPHICAL ABSTRACT



INTRODUCTION

The generation of free radicals is a continuous and physiological process, fulfilling relevant biological functions. Free radicals are by-products generated in the respiratory chain when there is an imbalance between the entry of electrons and its transfer through the chain (electrons leak) [1]. During metabolic processes, these radicals act as mediators for electron transfer in various biochemical reactions. For example, the production of free radicals, in appropriate proportions, enables the generation of adenosine triphosphate, through the electron transport chain. However, the excessive production of these compounds can lead to oxidative damage [2]. The continuous production of free radicals during metabolic processes culminates in the development of antioxidant defense mechanisms [3, 4], which aid in limiting intracellular reactive species levels and controlling tissue damage [2]. These compounds can basically be divided into enzymatic (endogenous) and non-enzymatic (exogenous - obtained through the diet) antioxidants.

Alternative antioxidant capacity markers include endogenous changes in glutathione related enzymes (i.e. glutathione peroxidase (Gpx)), superoxide dismutase (SOD), catalase (CAT) and changes in DNA oxidation, prostaglandin levels and lipid peroxidation (as indicated by malonaldehyde (MDA) and other markers) [5]. Although the physiological significance of these *in vivo* antioxidant markers has not yet been fully understood, they are widely used as a first step in antioxidant evaluation in conditions associated with diseases *in vivo*. The identification of these markers may be important for disease risk reduction and prevention [6].

The overconsumption of calories and nutrients, mainly from ultra-processed foods, high in sugar and fat, with high palatability, leads to an imbalance of energy intake and energy expenditure, which results in the overproduction of oxidative stress and fat storage in the adipose tissue, possibly leading to insulin resistance and chronic disease [7, 8]. *In vivo* studies demonstrated that the ingestion of high energy dense diets can lead to increased lipoperoxide and hydroperoxide levels, a decrease in antioxidant markers, such as Gpx and CAT, and an increase in oxidative stress [9, 10].

The Goji Berry fruit (*Lycium barbarum*) has recently become popular due to public acceptance as a "super food" with high nutritional benefits and antioxidant properties, and has been widely used for medicinal proposals and as a functional food [11]. Its antioxidant polysaccharides, carotenoids and flavonoids protect

the body from free radical damage, which can cause disease and aging [12, 13]. Several studies are being conducted to evaluate the bioactive compounds content and to demonstrate the benefits of *Lycium barbarum* to human and animal health [11, 13, 14].

However, *in vivo* studies investigating the relationship of its beneficial health effects and antioxidant activity are insufficient. Thus, there is a clear need for studies that increase the understanding of the beneficial attributes of the consumption of the *Lycium barbarum*-based products, increasing the range of information and ensuring safe consumption without health risks. In this context, the present study aimed to assess the *in vivo* effects of *Lycium barbarum* extracts on oxidative stress in the liver of *Wistar* rats as well as to identify and quantify the content of the major bioactive compounds of the fruit.

MATERIAL AND METHODS

Sample characterization and preparation

Lycium barbarum fruits were bought at the Pelotas Public Market, RS, Brazil, obtained from China as dried fruits. The fruits were sampled in bulk and placed in hermetically closed jars and taken to the Laboratory of Experimental Nutrition, at the Nutrition School of the Federal University of Pelotas (UFPEL). It was used the extract of *Lycium barbarum* prepared with 20 g of the fruit diluted in 80 mL of water at room temperature (concentration 20% (v/v)) which was prepared daily for administration to the animals (2 mL of extract per day per animal/ 125 mg/kg of animal).

Conditions and experimental model

This study was approved by the Ethics Committee on Animal Experimentation (UFPEL), under the registration number 9662-2015. Twenty-eight female *Wistar* rats (*Rattus novergicus*), 60 days old, obtained from the UFPEL Central Animal Laboratory were used. The animals were maintained in polypropylene boxes in ventilated cabinets, with controlled temperature and relative humidity conditions (23 ºC±1 ºC and 65-75 %), under a *12-hour light*/*dark cycle*. After five days of adaptation, the animals were randomly divided into four groups, comprising seven animals each, namely SW: standard diet + water; SG: standard diet + Goji Berry; PW: palatable diet + water; PG: palatable diet + Goji Berry. The palatable diet was composed of 25% standard diet, 34% condensed milk, 23% corn starch, 8% sucrose and 10% lipid content (soybean oil) [15]. *Lycium barbarum* extracts were prepared daily at a 20% concentration (v/v) using water at room temperature, and applied by gavage (2 mL of extract per day per animal/ 125 mg/kg of animal). “All animals received water *ad libitum*. At the end of the experimental period (60 days), all animals were euthanized according to the Resolution Protocol nº 714 of June 2002 of the Federal Council of Veterinary Medicine, following the Ethical Principles in Animal Experimentation adopted by the Brazilian College of Animal Experimentation [16].

Catalase (CAT) determination

CAT activity was carried out using homogenized liver samples which were incubated in 14 mM phosphate buffer in the presence of hydrogen peroxide according Aebi [17]. The determination was performed by monitoring H_2O_2 decomposition spectrophotometrically at 240 nm, expressed as units (U) per mg^{-1} of protein.

Superoxide Dismutase (SOD) determination

The SOD activity in the homogenized liver samples was determined by the method described by Klamt and coauthors [18]. The absorbance of the inhibition rate of adrenochrome self-catalytic reaction was monitored at 480 nm for 60 s in a buffer solution containing 1 mM of Madrenaline/50 glycine-NaOH (pH 10.2)/ 1 mM catalase, and expressed as U mg^{-1} of protein.

Glutathione (GSH) determination

The glutathione content in the liver samples were determined according to the method described by Browne and Armstrong [19]. The samples were incubated in sodium phosphate buffer (0.1M, pH 8.0) containing 5 mM EDTA and homogenized. Then, the fluorescence was measured using 350 and 420 nm excitation and emission wavelengths, respectively; and these concentrations were expressed as nmol mg^{-1} of protein.

Lipid peroxidation determination by the thiobarbituric acid reactive substances (TBARS) method

The formation of malondialdehyde (MDA), a lipid peroxidation index, was used to determinate TBARS in the liver fragments as described by Esterbauer and Cheeseman [20]. The liver samples were homogenized and incubated with 10% trichloroacetic acid (TCA); then MDA levels were measured at 532 nm and results expressed in nmol of MDA mg^{-1} protein.

Gene expression determination of oxidative stress- related enzymes

To determine gene expression, liver samples were obtained and immediately stored at -80ºC. The samples were subsequently homogenized with Qiazol (Qiagen, Valencia, USA), and total RNA was isolated and purified following the Qiazol protocol. RNA was quantified and adjusted to 200 ng μL^{-1} on a spectrophotometer (Nanodrop Lite, Thermo Fischer Scientific Inc., USA) applying the 260/280 nm absorbance ratio. RNA quality was assessed by electrophoresis on agarose gels. Reverse transcription reactions were performed using 1 µg of RNA with a reverse transcription kit containing RNase inhibitor (Applied Biosystems, Foster City, USA) in 10 µL. Real-time PCR was performed to assess the expression of the target genes Copper/Zinc Superoxide Dismutase (*Sod1*); Catalase (*Cat*); Glutathione peroxidase 1 (*Gpx1*) and internal control beta-actin (*Actb*) (Table 1).

Table 1. Oligonucleotide primers used in the gene expression analysis by real-time PCR.

Primers	Sequence (5'-3')	Product (pb)	GenBank accession number
Actb (F)	TCACCACCACAGCCGAGAGA	72	NM_031144.3
Actb (R)	CGAAATCCAGTGCGACGTAGC		
Cat (F)	GAATGGCTATGGCTCACACA	100	NM_012520.2
Cat (R)	CAAGTTTTTGATGCCCTGGT		
Sod1 (F)	GGTGGTCCACGAGAAACAAG	98	NM_017050.1
Sod1 (R)	CAATCACACCACAAGCCAAG		
Gpx1 (F)	CTCTCCGCGGTGGCACAGT	290	NM_030826
Gpx1 (R)	CCACCACCGGGTCGGACATAC		

PCR reactions were performed in triplicate in 12 µL using a SYBR Green Mastermix (Applied Biosystems) and fluorescence was quantified on an Eco Real Time (Illumina, San Diego, California, USA). For each test, 40 cycles (95°C for 10 seconds and 60°C for 30 seconds) were carried out and a dissociation curve was included at the end of the reaction, in order to verify the amplification of a single PCR product. The coefficient of variation was less than 3% for all the pairs of primers used. Relative expression was calculated as $2^{A-B}/\ 2^{C-D}$, according to Masternak and coauthors [21]. Each assay plate included a negative control comprising water.

Identification and quantification of bioactive compounds by high-performance liquid chromatography (HPLC)

Individual Phenolic Compounds

Extraction of phenolic compounds from *Lycium barbarum* fruit was carried out according to the method described by Häkkinen and coauthors [22], with some modifications. Five grams of sample were dissolved in 30 mL of methanol, to which 4.9 mL of hydrochloric acid p.a. was added. The extract was homogenized in a water bath at 35˚C and left in the dark for 24 hours. After this period, the mixture was filtered, and the supernatant was concentrated in a rotary evaporator at 40˚C for 30 minutes. The concentrated residue was redissolved in methanol to a final volume of 5 mL and centrifuged at 7000 rpm for 10 minutes. An aliquot of the supernatant (30 µL) was injected into the chromatograph. The chromatograph consisted of a Shimadzu-HPLC system with an automatic injector, a UV-visible detector set at 280 nm, and an RP-18 CLC-ODS column (5 mm, 4.6 mm × 150 mm) with the stationary phase and an octadecyl guard column CLC-GODS (4)

with the octadecyl stationary phase surface, both of which were pre-conditioned at 25˚C. The mobile phase consisted of an elution gradient of aqueous acetic acid (99:1, v/v) (A) and methanol (B) with a flow of 0.8 mL/min and a total running time of 45 minutes, according to the methodology described by Zambiazi [23]. The mobile phase consisted of 100% A for 25 minutes, 60% B and 40% A from 25 to 37 minutes, 95% A and 5% B from 37 to 42 minutes, and then the initial mobile phase (100% A) after 45 minutes. Individual phenolic compounds were identified and quantified based on the calibration curves of standards, including syringic acid, p-coumaric acid, quercetin, rutin, gallic acid, catechin. The results are expressed in mg of phenolic compound per 100 g of fruit.

Individual Carotenoids

Determination of individual carotenoids was performed by the method described by Rodriguez-Amaya [24], with modifications. Five grams of each sample and 3 g of celite were added to 20 mL of cold acetone, and the mixture was shaken for 10 minutes. The material was filtered with a Buchner funnel with filter paper, washing the sample with acetone until the extract was colorless. The filtrate was transferred to a separatory funnel, to which 30 mL of petroleum ether and 30 mL of distilled water were added. The lower phase was discarded, then distilled water was added; this procedure was repeated four times to achieve total removal of the acetone. The upper phase was transferred to a 50 mL volumetric flask, and the volume was completed with petroleum ether.

Afterwards, 25 mL of this extract was mixed with 25 mL of 1.5 N KOH in ethanol, and the samples were then subjected to cold saponification for 18 hours in the dark. After saponification, petroleum ether and water were added for phase separation; the upper phase was concentrated in a rotary evaporator at 35˚C and dissolved in the initial mobile phase (methanol: acetonitrile, 30:70 v/v). The diluted extract was transferred to an Eppendorf tube and centrifuged at 9000 rpm for 6 minutes. Aliquots of the supernatant (25 µL) were injected in a Shimadzu HPLC system with a UV-visible detector set at 450 nm. The separation was performed using a elution gradient of methanol, acetonitrile and ethyl acetate with a 1 mL/min flow rate. The mobile phase consisted of 30% methanol and 70% acetonitrile for 10 minutes, 10% methanol, 80% acetonitrile and 10% ethyl acetate from 10 to 35 minutes, 5% methanol, 80% acetonitrile and 15% ethyl acetate from 35 to 40 minutes, and then the initial mobile phase (30% methanol and 70% acetonitrile) from 40 to 45 minutes. For the identification and quantification of the compounds, a standard curve prepared with the chromatographic standards corresponding to β-carotene, lutein and zeaxanthin was used. The results are expressed in µg of compound per g of fruit.

Ascorbic acid analysis

The ascorbic acid was analyzed according to the methodology described by Vinci and coauthors [25], with few modifications, as described by Jacques and coauthors [26]. Five grams of ground sample was weighed, and 30 mL of metaphosphoric acid (4.5% in ultrapure water) solution was added, before the solution was left to rest for an hour in an amber flask. The solution was then transferred to a 50 mL volumetric flask, and the volume was completed with ultrapure water. The sample was filtered through filter paper, and the supernatant was centrifuged at 7000 rpm for 10 min (microcentrifugue NT800 Nova Técnica -São Paulo, Brazil). After that, the sample was transferred to 1.5 mL vial. Ten milliliters of the sample, which consisted of the modules described previously, was injected into the system of high-performance liquid chromatography. A spectrophotometric detector UV/V SPD-10AVVP with wavelength at 254 nm was employed. Ultrapure water with 0.1% of acetic acid as a mobile phase was employed with a flow of 0.8 $mL.min^{-1}$. The data were acquired and processed using the Class-VP software. The peaks were identified by comparing the retention time of the standards of L-ascorbic and dehydroascorbic acid. The vitamin C quantification was evaluated according to the external standard calibration curve of L-(+)-ascorbic acid. The results were expressed in $mg.g^{-1}$ of fruit.

Statistical analyses

Data were tested statistically by an analysis of variance (ANOVA) and Tukey test, at a 5% of significance level to compare means, using the Graph Pad Prism software, version 6.0. Likewise, gene expression was evaluated after normalization of the target gene expressions by beta-actin applying the one-way ANOVA using the Graph Pad Prism 6.0 software.

RESULTS

Antioxidant capacity was investigated by measuring CAT (Figure 1A) and SOD (Figure 1B) enzymatic activities in the liver. Significant increases in CAT activity in liver (P<0.01) was observed for the SG and PW groups when compared to the SW group (Figure 1A). *Lycium barbarum* had a significant effect only when administered in the standard diet (SG). Regarding SOD, no *Lycium barbarum* effect (P>0.05) were observed for both standard and palatable diets (Figure 1B), with only a small decrease observed when comparing PG with SW (P< 0.05).

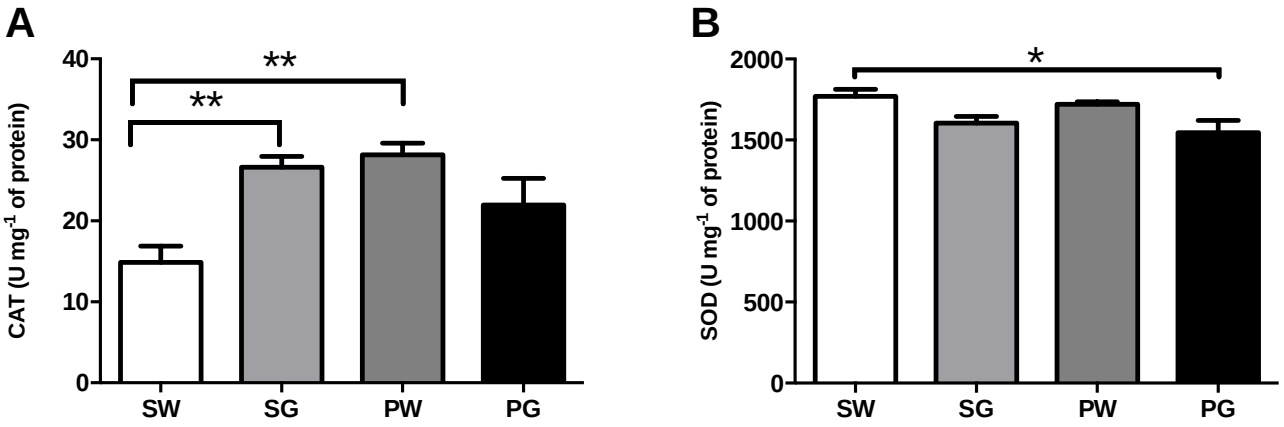


Figure 1. Effects of the ingestion of Goji Berry (*Lycium barbarum*) extract on Catalase (CAT) (A) and superoxide dismutase (SOD) (B) in the liver of *Wistar* rats. Results are expressed as means ± standard error. SW: standard diet + water; SG: standard diet + Goji Berry; PW: palatable diet + water; PG: palatable diet + Goji Berry. *, P<0.05, **, P<0.01, n = 7.

Regarding GSH, an important tripeptide antioxidant, no effect of *Lycium barbarum* extract for the standard diet (SW and SG) in liver was observed. However, when evaluating in the palatable diet, a significant decrease in GSH levels was observed in the PG group (P<0.05) compared to the PW group (Figure 2A).

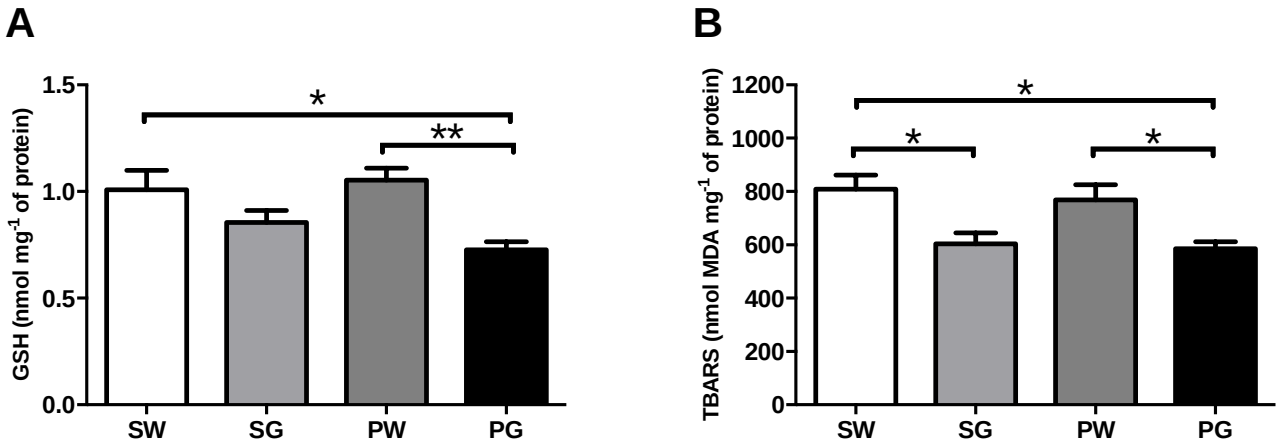


Figure 2. Effects of the ingestion of Goji Berry (*Lycium barbarum*) extract on the non-enzymatic redox state evaluated as glutathione (GSH) (A) and TBARS levels (B) in the liver of *Wistar* rats. Results are expressed as means ± standard error. SW: standard diet + water; SG: standard diet + Goji Berry; PW: palatable diet + water; PG: palatable diet + Goji Berry. *, P<0.05, **, P<0.01, n = 7.

Concerning lipid peroxidation (TBARS) (Figure 2B), *Lycium barbarum* extracts produced a significant decrease in oxidation levels (P<0.01) in liver in the SG group when compared to the SW group. The same was observed for the palatable diet, with the PG group displaying a significant decrease in lipid peroxidation compared to the PW group.

Gene expression involved in the production of antioxidant enzymes *Cat*, *Sod1* and *Gpx1* in liver were also evaluated concerning dietary *Lycium barbarum* extract administration (Figure 3). A significant increase (P<0.01) in the *Cat* enzyme gene expression in the liver was observed in the SG and PG groups when

compared to the SW group. However, when analyzing the *Cat* enzyme gene expression among diets, *Lycium barbarum* extract was effective only when administered with the standard diet (Figure 3A). On the other hand, data from *Cat* gene expression agreed with liver CAT enzyme quantification measurements (Figure 1A). The slight increase in the *Sod1* gene expression was not significant (P=0.06) (Figure 3B), while *Gpx1* gene expression was not significantly different (P>0.01) among the four groups (Figure 3C).

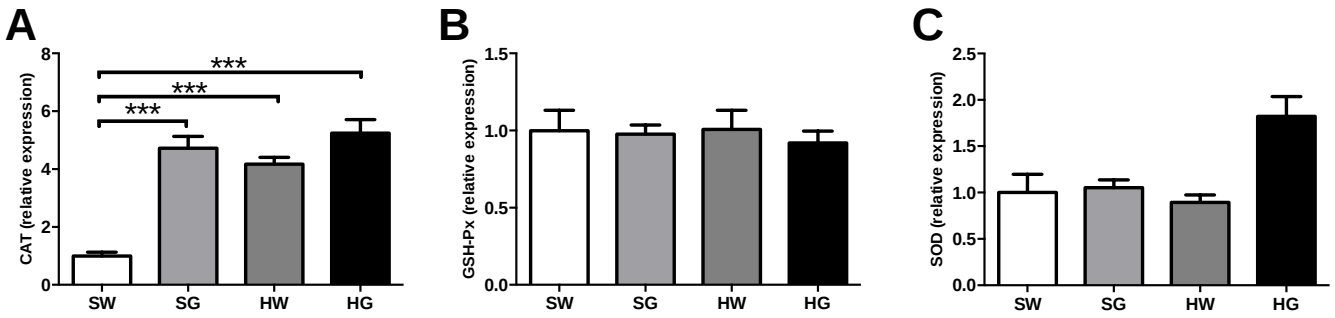


Figure 3. Effects of the ingestion of Goji Berry (*Lycium barbarum*) extract on the gene expression of Cat (A), Gpx1 (B) and SOD1 (C) in the liver of *Wistar* rats. Results are expressed as means ± standard error. SW: standard diet + water; SG: standard diet + Goji Berry; HW: palatable diet + water; HG: palatable diet + Goji Berry. ***, P<0.001, n = 7.

In order to identify the main bioactive compounds into the *Lycium barbarum* fruit which it can explain the antioxidant effect presented *in vivo* by increase CAT activity together with increase *Cat* enzyme levels and inhibition of the lipid peroxidation in the liver. The content of individual phenolic compounds, carotenoids and ascorbic acid were analyzed by HPLC (Figure 4). The results showed a significant amount of flavonols catechin (434.63 ± 81.03mg/100g), rutin (401.73 ± 43.86mg/100g) and gallic acid (328.03 ± 34.12mg/100g); also, the results showed a lower content of the quercetin, p-coumaric acid and syringic acid (Table 2). Likewise, it was identified the lutein + zeaxanthin and β-carotene as carotenoids, and a significant amount of ascorbic acid (05.25 ± 01.88mg/g) (Table 2).

Table 2. Determination and content of the bioactive compounds in *Lycium barbarum* extract by HPLC.

Bioactive compounds	**Chromatogram identification (n)**	**Content of individual phenolic compounds**
		----- mg 100g $^{-1}$ -----
Catechin	1	434.63 ± 81.03
Rutin	2	401.73 ± 43.86
Gallic acid	3	328.03 ± 34.12
Quercetin	4	11.15 ± 02.58
p-Coumaric acid	5	08.06 ± 01.22
Syringic acid	6	02.17 ± 00.06
		Content of individual carotenoids
		----- µg g^{-1} -----
Luteina + Zeaxantina	1	02.04 ± 00.19
β-caroteno	2	00.33 ± 00.03
		Ascorbic acid content
		----- mg g^{-1} -----
L-(+)-ascorbic acid	1	05.25 ± 01.88

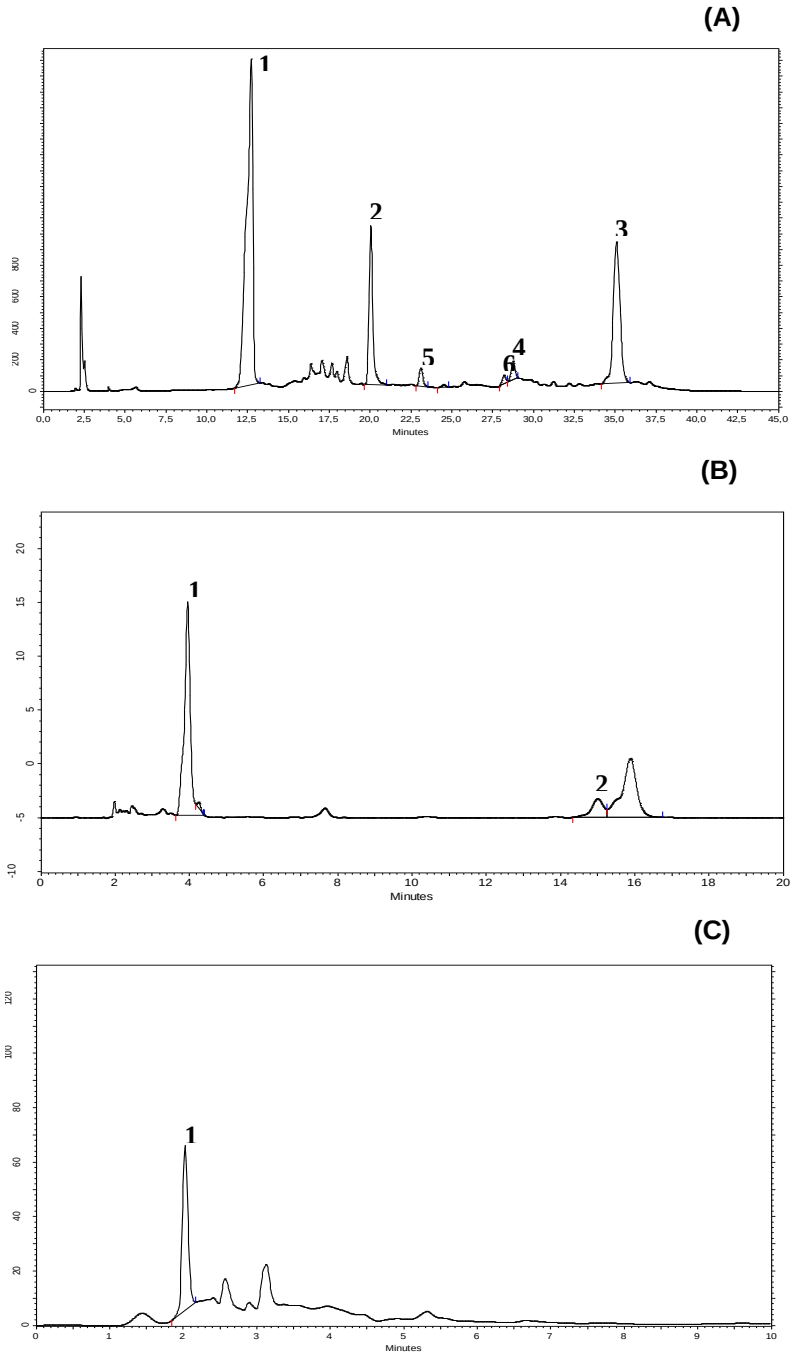


Figure 4. HPLC chromatogram of individual phenolic compounds (A), individual carotenoids (B) and ascorbic acid (C) of *Lycium barbarum* sample. The respective numbers corresponding to the identified compounds are described in Table 2 (Chromatogram identification).

DISCUSSION

The antioxidant enzymes SOD, CAT and Gpx are considered the first line of antioxidant defense system against reactive oxygen species generated *in vivo* during oxidative stress [27]. Among these antioxidant enzymes, SOD dismutase superoxides radicals to form hydrogen peroxide, which in turn is decomposed into water and oxygen by Gpx and CAT, thereby preventing the formation of hydroxyl radicals [28]. In the current study, rats fed a *Lycium barbarum* exhibited increased *Cat* gene expression and CAT enzyme activity in liver and decreased lipid peroxidation.

Increased liver CAT activity was observed in the standard diet and *Lycium barbarum* fed (SG group). According to the study carried out by Ming and coauthors [29], a diet rich in fat causes decreases in Gpx, CAT and SOD activity, and *Lycium barbarum* supplementation increases the activity of these antioxidant enzymes. In another study, the effects of *Lycium barbarum* supplementation on oxidative stress related to old age in mice, a condition evaluated by increasing endogenous lipid peroxidation and decreased activity of the antioxidant enzymes, were evaluated, and increased SOD, CAT and Gpx activities in different tissues,

such as liver, brain and lungs, were detected [30]. According to Amagase and coauthors [6], in a study with healthy human adults, SOD activity was significantly higher in groups treated with *Lycium barbarum* (about 8%) compared to levels observed in the control group ($p<0.01$), while Gpx activity was increased 9.04% in the group treated with *Lycium barbarum* compared to the control group ($p<0.01$).

According to Matsuzawa-Nagata and coauthors [31], the increase in oxidative stress caused by a high fat diet is the primarily mechanism triggering the development of insulin resistance and fatty acid metabolism dysregulation. Mice fed a high fat diet had a significant increase of the expression of genes responsible for reactive oxygen species production in both liver and adipose tissue, which occurred before the onset of insulin resistance and obesity. When compared with mice in the control diet, the high fat diet led to an up-regulation of genes encoding key regulators of mitochondrial fatty acid β-oxidation in the liver, such as PPARα, carnitine palmitoyltransferase 1a (CPT-1a), Acox1, and CYP2E1. In the adipose tissue of the mice in the high fat diet group, gene expression levels of Acox1, CYP2E1, Nox4, and p22 were significantly up-regulated, when compared with mice in the control diet group. Thus, a high fat diet may increase reactive oxygen species production and oxidative stress formation through different mechanisms, and it may vary according to specific organs or tissues.

In the present study, lipid peroxidation (TBARS) in the liver was significantly decreased in the standard (SG) and palatable (PG) groups treated with *Lycium barbarum*. These results are significant, since *Lycium barbarum* may possibly contains protective components against cellular oxidation. According to Pike and coauthors [32], lipid peroxidation may lead to the loss of the fluidity and increased permeability of cell membranes, leading to nutrient release into the extracellular space, and even membrane rupture, with consequent cell death. Furthermore, Draper and Hadleyos [33], reported that the main biological markers of this type of injury in cell membranes are substances reactive to thiobarbituric acid (TBARS). Niu and coauthors [34] described *Lycium barbarum* intake effects under an exhaustive exercise regime, a condition that causes greater oxidative stress in skeletal muscle, confirmed by decreases in muscle glycogen and increased malondialdehyde (MDA) concentrations. After treatment with *Lycium barbarum* extract, decreased MDA levels and increased CAT activity and GSH levels were observed. This suggests that *Lycium barbarum* bioactive compounds may inhibit the formation of reactive oxygen species (ROS) and consequently, antioxidant enzyme activity. Antioxidants may also chelate metal ions and prevent lipid peroxidation, leading to an increase in H_2O_2 and O_2- concentrations as a consequence of reduced SOD and GPx activities [35].

In a study reported by Yang and coauthors [36], ischemia-reperfusion injury (IRI) significantly decreased antioxidant enzyme activity and increased MDA levels in rat tissues. However, treatment with *Lycium barbarum* significantly reduced MDA levels in the analyzed tissues and increased the activity of antioxidant enzymes, which was confirmed by the expression of the protein levels of the evaluated antioxidant enzymes.

In the present study, the analysis of the expression of genes related to oxidative stress, *Sod1*, *Cat* and *Gpx1*, in rat livers was carried out. The results indicate that only *Cat* expression increased in the group supplemented with a standard diet and *Lycium barbarum* extract (SG), and both groups treated with the palatable diet (PW and PG). Few studies in the literature have evaluated the consumption of *Lycium barbarum* extracts and their effects on endogenous antioxidant enzyme gene modulation. In a study carried out by Ross and Kasum [37], increases in SOD and Gpx activity were detected in human erythrocytes after apigenin intake, a flavonoid found in fruits and herbs. In the present study, *Lycium barbarum* extract and its antioxidant components increased *Cat* enzyme gene expression, confirming the increases in CAT activity in the liver.

The results showed in the present study by the HPLC analysis revealed that the extract of *Lycium barbarum* has a significant amount of catechin, rutin and gallic acid. This extract promoted an increase in the activity of the CAT enzyme, and also it increased the expression of the *Cat* enzyme and showed a reduction of the potential of oxidation by TBARS analysis. This increase was also found in a review conducted by Tian and coauthors [12].

According to Tsao and Li [38], dietary phenolics are pontential antioxidants for *in vitro* analysis, being able to neutralize free radicals by donating an electron or hydrogen atom to a wide range of reactive oxygen, nitrogen and chlorine species, including $O_2^{\bullet-}$, $OH^{\bullet}$, peroxyl radicals $RO_2^{\bullet}$, hypochlorous acid (HOCl) and peroxynitrous acid (ONOOH). Phenolics compounds interrupt the propagation stage of the lipid autoxidation chain reaction as effective radical scavengers, also actting as metal chelators to convert hydroperoxides or metal prooxidants into stable compounds. Phenolic compounds, as metal chelators, can directly inhibit Fe^{3+} reduction, decreasing the production of reactive $OH^{\bullet}$ of Fenton reaction [39]. Both phenolic acids and flavonoids possess effective radical scavenging activity; however, the metal chelating potential and reduction power can vary depending on their structural features [40].

Other studies reported that dietary polyphenols are one of the most important xenobiotics with physiological relevance to human health [40, 41]. Consumption of dietary polyphenols has been shown to be able to restore the redox homeostasis and prevent systemic or localized inflammation by enhancing activities of the antioxidant enzymes SOD, CAT and GPx. Expressions of these detoxifying and antioxidant enzymes are modulated by a key transcription factor, nuclear factor and erythroid-related factor (Nrf2) which can be activated by ROS at cellular level. Nrf2 translocates into nucleus and regulates antioxidant-responsive elements (ARE)-mediated transcriptions of various genes encoding the above mentioned antioxidant enzymes. Furtheremore, it was described that polyphenols may induce Nrf2 activation to up-regulate cellular antioxidant enzymes [40, 41]. Dietary polyphenols and especially flavonoids are also capable of triggering Nrf2 translocation to induce subsequent activation of the endogenous antioxidant actions through ligand interaction with cytosolic aryl hydrocarbon receptor (AhR) [40, 42]. Flavonols and isoflavones and also their derivatives have shown agonistic potential to regulate AhR mediated signalling in cells [43]. Several flavonols and flavones, including quercetin, luteolin, apigenin, chrysin [40, 42] and catechin [44] have been confirmed as AhR agonistic regulators.

Some studies attributed many of the benefits of *Lycium barbarum* to their high amount of polyssacarydes [12, 45]. Polyssacarydes are carbohydrates formed by chains of monosaccharides linked together by glycosidic bonds, and they have many biological activities, such as high antioxidant capacity, antiaging and antiglaucoma effects, imune regulation, anticancer effects, neuroprotective and cardioprotective properties, antidiabetic and hypolipidaemic effects, among others [12, 39, 46, 47, 48, 49, 50].

CONCLUSIONS

The increase in CAT activity together with increased *Cat* enzyme gene expression, decrease in GSH levels and decrease in lipid peroxidation in liver observed in the present study suggest that *Lycium barbarum* displays significant antioxidant potential. Its effects might be due to the presence of the bioactive compounds found into it, even in its dehydrated form, which can be beneficial in various oxidative stress conditions, thus decreasing oxidative stress-related damage. Among the bioactive compounds found, those in greater quantity were catechin, rutin and gallic acid, in addition to high doses of the antioxidant micronutrient ascorbic acid. These results showed a possible applicability of *Lycium barbarum* extract in diseases of oxidative character, however, further studies are necessary to confirm this hypothesis.

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REFERENCES

1. Drose S, Brandt U. Molecular mechanisms of superoxide production by the mitochondrial respiratory chain. Adv Exp Med Biol. 2012 May;748:145-69.
2. Pham-Huy L, He AH, Pham-Huy C. Free Radicals, Antioxidants in Disease and Health. Int J Biomed Sci. 2008 Jun;4:89-96.
3. Phillips M, Cataneo RN, Cheema T, Greenberg J. Increased breath biomarkers of oxidative stress in diabetes mellitus. Clin Chim Acta. 2004 Jun;344:189-94.
4. Asfandiyarova N, Kolcheva N, Ryazantsev I, Ryazantsev V. Risk factors for stroke in type 2 diabetes mellitus. Diab Vasc Dis Res. 2006 May;3:57-60.
5. Selman C, McLarena JS, Collinsb AR, Duthieb GG, Speakmana JR. Antioxidant enzyme activities, lipid peroxidation, and DNA oxidative damage: the effects of short-term voluntary wheel running. Arch Biochem Biophys. 2002 May;401:255-226.
6. Amagase H, Sunb B, Borekc C. *Lycium barbarum* (goji) juice improves in vivo antioxidant biomarkers in serum of healthy adults. Nutr Res. 2009 Jan;29:19-25.
7. Azeez O, Adeshina A, Oyagbemi A, Ajibade O, Omobowale O, Nabofa E. Oxidative stress in concurrent hypercaloric diet and caffeine consumption in Wistar rats: Possible modulatory effects of aqueous extract of curcuma longa. FASEB J. 2019 Apr;3:694.15.

8. Taherkhani S, Suzuki K, Ruhee RT. A Brief Overview of Oxidative Stress in Adipose Tissue with a Therapeutic Approach to Taking Antioxidant Supplements. Antioxidants. 2021 Apr;10:594-615.
9. Burneiko RC, Diniz YS, Galhardi CM, Rodrigues HG, Ebaid GM, Faine LA, et al. Interaction of hypercaloric diet and physical exercise on lipid profile, oxidative stress and antioxidant defenses. Food Chem Toxicol. 2006 Jul;44:1167-72.
10. Novelli E, Fernandes A, Campos K, Diniz, Y, Almeida J, Faine L, et al. The Adverse Effect of a High Energy Dense Diet on Cardiac Tissue. J Nutr Environ Med. 2009 Jul;12:287-94.
11. Larramendi C, García-Abujeta JL, Vicario S, García-Endrino A, López-Matas MA, García-Sedeño MD, et al. Goji Berries: Risk of allergic reactions in individuals with food allergy. J Investig Allergol Clin Immunol. 2012 Oct;22:345-50.
12. Tian X, Liang T, Liu Y, Ding G, Zhang F, Ma Z. Extraction, Structural Characterization, and Biological Functions of *Lycium barbarum* Polysaccharides: A Review. Biomolecules. 2019 Aug;9:389.
13. Peng Y, Ma C, Li YW, Leung KSY, Jiang ZH, Zhao ZZ. Quantification of zeaxanthin dipalmitate and total carotenoids in *Lycium* fruits (Fructus Lycii). Plant Foods Hum Nutr. 2005 Aug;60:161-4.
14. Skenderidis P, Lampakis D, Giavasis I, Leontopoulos S, Petrotos K, Hadjichristodoulou C, et al. Chemical Properties, Fatty-Acid Composition, and Antioxidant Activity of Goji Berry (*Lycium barbarum* L. and *Lycium chinense* Mill.) Fruits. Antioxidants. 2019 Mar;8:1-13.
15. Souza CG, Moreira JD, Siqueira IR, Pereira AG, Rieger DK, Souza DO, et al. Highly palatable diet consumption increases protein oxidation in rat frontal cortex and anxiety-like behavior. Life Sci. 2007 Jun;81:198-203.
16. Cobea. Ethical principles for animal experimentation; 2008. Avaliable from: http://www.cobea.org.br.
17. Aebi H. Catalase in vitro. Meth Enzymol. 1984;105:121-6.
18. Klamt F, Dal-pizzol F, Conte Da Frota ML, Walz R, Andrades ME, Da Silva EG, Brentani RR, Izquierdo I, Fonseca Moreira JC. Imbalance of antioxidant defense in mice lacking cellular prion protein. Free Radic Biol Med. 2001 May;30:1137-44.
19. Browne RW, Armstrong D. Reduced glutathione and glutathione disulfide. Methods Mol Biol. 1998;108:347-52.
20. Esterbauer H, Cheeseman KH. Determination of aldehydic lipid peroxidation products: malonaldehyde and 4-hydroxynoneal. Meth Enzymol. 1990;186:407-21.
21. Masternak MM, Al-Regaiey KA, Del Rosario Lim MM, Bonkowski MS, Panici JA, Przybylski GK, et al. Caloric restriction results in decreased expression of peroxisome proliferator-activated receptor superfamily in muscle of normal and long-lived growth hormone receptor/binding protein knockout mice. J Gerontol A Biol Sci Med Sci. 2005 Oct;60:1238-45.
22. Häkkinen SH, Kärenlampi SO, Heinonen M. HPLC Method for Screening of Flavonoids and Phenolic Acids in Berries. J Sci Food Agric. 1998 Mar;77:543-51.
23. Zambiazi RC. The Role of Endogenous Lipid Components on Vegetable Oil Stability [Ph.D. Thesis]. University of Manitoba, Winnipeg; 1997.
24. Rodriguez-Amaya DB. A Guide to Carotenoid Analysis in Foods. International Life Sciences Institute (ILSI) Press, Washington DC; 1999.
25. Vinci G, Botre F, Mele RG. Ascorbic acid in fruits: a liquid chromatographic investigation. Food Chem. 1995 Aug;53:211-4.
26. Jacques AC, Pertuzatti PB, Barcia MT, Zambiazi RC, Chim JF. Stability of bioactive compounds in frozen pulp of blackberry (*Rubus fruticosus*) cv. Tupy. Quím Nova. 2010 Aug;33:1720-5.
27. Yao DC, Shi WB, Gou YL, Zhou XR, Tak YA, Zhou YK. Fatty acid-mediated intracellular iron translocation: a synergistic mechanism of oxidative injury. Free Radic Biol Med. 2005 Nov;39:1385-98.
28. Zhang XY, Tan YL, Cao LY, Wu GY, Xu Q, Shen Y, et al. Antioxidant enzymes and lipid peroxidation in different forms of schizophrenia treated with typical and atypical antipsychotics. Schizophr Res. 2006 Jan;81:291-300.
29. Ming M, Guanhua L, Zhanhai Y, Guang C, Xuan Z. Effect of the *Lycium barbarum* polysaccharides administration on blood lipid metabolism and oxidative stress of mice fed high-fat diet in vivo. Food Chem. 2009 Apr;113:872-7.
30. Li XM, Ma YL, Liu XJ. Effect of the *Lycium barbarum* polysaccharides on age-related oxidative stress in aged mice. J Ethnopharmacol. 2007 May;111:504-11.
31. Matsuzawa-Nagata N, Takamura T, Ando H, Nakamura S, Kurita S, Misu H, et al. Increased oxidative stress precedes the onset of high-fat diet-induced insulin resistance and obesity. Metabolism. 2008 Aug;57:1071-7.
32. Pike LS, Smift AL, Croteau NJ, Ferrick DA, Wu M. Inhibition of fatty acid oxidation by etomoxir impairs NADPH production and increases reactive oxygen species resulting in ATP depletion and cell death in human glioblastoma cells. Biochim Biophys Acta. 2011 Jun;1807:726-734.
33. Draper, HH, Hadley M. Malondialdehyde determination as index of lipid peroxidation. Meth Enzymol. 1990;186:421-31.

34. Niu A, Wub J, Yuc D, Wang R. Protective effect of *Lycium barbarum* polysaccharides on oxidative damage in skeletal muscle of exhaustive exercise rats. Int J Biol Macromol. 2008 Jun;42:447-9.
35. Skrzydlewska E, Ostrowska J, Farbiszewski R, Michalak K. Protective effect of green tea against lipid peroxidation in the rat liver, blood serum and the brain. Phytomedicine. 2002 May;9:232-8.
36. Yang X, Bai H, Cai W, Li J, Zhou Q, Wang Y, et al. *Lycium barbarum* polysaccharides reduce intestinal ischemia/reperfusion injuries in rats. Chem Biol Interact. 2013 Aug;204:166-72.
37. Ross JA, Kasum CM. Dietary flavonoids: bioavailability, metabolic effects, and safety. Annu Rev Nutr. 2002 Jan;22:19-34.
38. Tsao R, Li H. Antioxidant properties in vitro and in vivo: realistic assessments of efficacy of plant extracts. In: Plant Sciences Reviews Edited by Hemming D. Oxfordshire UK: CABI. 2012 Mar;7:11-7.
39. Perron NR, Brumaghim JL. A review of the antioxidant mechanisms of polyphenol compounds related to iron binding. Cell Biochem Biophys. 2009 Jan;53:75-100.
40. Zhang H, Tsao R. Dietary polyphenols, oxidative stress and antioxidant and anti-inflammatory effects. Curr Opin Food Sci. 2016 Apr;8:33-42.
41. Kansanen E, Kuosmanen SM, Leinonen H, Levonen, AL. The Keap1-Nrf2 pathway: mechanisms of activation and dysregulation in cancer. Redox Biol. 2013 Jan;1:45-49.
42. Köhle C, Bock KW. Activation of coupled Ah receptor and Nrf2 gene batteries by dietary phytochemicals in relation to chemoprevention. Biochem Pharmacol. 2006 Sep;72:795-805.
43. Zhang S, Qin C, Safe SH. Flavonoids as aryl hydrocarbon receptor agonists/antagonists: effects of structure and cell context. Environ Health Perspect. 2003 Dec;111:1877-82.
44. Palermo CM, Hernando JI, Dertinger SD, Kende AS, Gasiewicz TA. Identification of potential aryl hydrocarbon receptor antagonists in green tea. Chem Res Toxicol. 2003; Jul16:865- 72.
45. Masci A, Carradorim S, Casadei MA, Paolicelli P, Petralito S, Ragno R, et al. *Lycium barbarum* polysaccharides: Extraction, purification, structural characterization and evidence about hypoglycaemic and hypolipidaemic effects. A review. Food Chem. 2018 Jul;15:377-89.
46. Tian XM, Wang R, Zhang BK, Wang CL, Guo H, Zhang SJ. Impact of *Lycium Barbarum* Polysaccharide and Danshensu on vascular endothelial growth factor in the process of retinal neovascularization of rabbit. Int J Ophthalmol. 2013 Feb;6:59-61.
47. Lu SP, Zhao PT. Chemical characterization of *Lycium barbarum* polysaccharides and their reducing myocardial injury in ischemia/reperfusion of rat heart. Int J Biol Macromol. 2010 Dec;47:681-4.
48. Xin YF, Wan LL, Peng JL, Guo C. Alleviation of the acute doxorubicin-induced cardiotoxicity by *Lycium barbarum* polysaccharides through the suppression of oxidative stress. Food Cheml Toxicol. 2011 Jan;49:259-64.
49. Gong H, Shen P, Jin L, Xing C, Tang F. Therapeutic effects of *Lycium barbarum* polysaccharide (LBP) on irradiation or chemotherapy-induced myelosuppressive mice. Cancer Biother Radiopharm. 2005 Apr;20:155-62.
50. Chang RC, So KF. Use of anti-aging herbal medicine, *Lycium barbarum*, against aging-associated diseases. What do we know so far? Cell Mol Neurobiol. 2008 Aug;28:643-52.