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ORIGINAL CONTRIBUTION

Effects of *Lentinula edodes* consumption on biochemical, hematologic and oxidative stress parameters in rats receiving high-fat diet

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Abstract
Purpose Functional foods can prevent/reduce the risks related to obesity. *Lentinula edodes* is a highly nutritious mushroom rich in protein, vitamins and minerals. Some studies have demonstrated the hypocholesterolemic effects from *L. edodes* in high doses, which does not represent the consumption in humans. We evaluated ingestion of a realistic dose of *L. edodes* associated with a high-fat diet (HFD) on hematologic, biochemical and oxidative stress parameters.
Methods Eighteen male Wistar rats were divided into three groups: control (normal diet); HFD; and HFD + *L. edodes* (100 mg/kg/day). After 30 days, blood was collected. Biochemical and hematologic parameters were analyzed, as well as oxidative stress biomarkers.
Results The HFD increased levels of total cholesterol and triglycerides. *Lentinula edodes* reduced these parameters significantly to concentrations found in the control group. The HFD increased levels of alanine transaminase and aspartate transaminase (markers of liver damage). *Lentinula edodes* returned the levels of these enzymes to normal levels and normalized serum levels of urea (which were also increased owing to consumption of the HFD). *Lentinula edodes* reduced levels of urea and glucose. Lipid peroxidation was increased in rats receiving the HFD, and *L. edodes* reduced malondialdehyde levels, thereby preventing oxidation of fatty acids.
Conclusions *Lentinula edodes* was shown to have hypolipidemic, hypoglycemic, hepatoprotective and renoprotective features in doses that are suitable for humans.

Keywords Nutraceuticals · *Lentinula edodes* · Metabolic disorder · Oxidative stress · Glucans

Introduction

Obesity is a major public health problem worldwide. Environmental and genetic factors have important roles in obesity, but inappropriate eating habits and sedentary lifestyles also contribute [1]. Cavalheira et al. [2] stated that excessive ingestion of food and, consequently, storage of carbohydrate and fat result in metabolic dysfunction, which activates innate and adaptive inflammation in adipose tissue. The inflammatory reaction promotes the resistance to insulin and metabolic diseases caused by obesity [3].

Hutcheson and Rocic [4] have hypothesized that the metabolic syndrome is associated with oxidative stress, which is a risk factor for the development of cardiovascular diseases. They have suggested that antioxidants can be useful for the treatment and prevention of cardiovascular diseases in patients with the metabolic syndrome.

Several resources have been used for the prevention and treatment of obesity and related diseases, including "nutraceuticals" [2]. For example, mushrooms have been used as food for thousands of years and have nutritional and medical properties [5, 6]. *Lentinula edodes* is a mushroom low in calories and lipids yet rich in proteins, vitamins, minerals and fibers [7–9].

β-Glucans are polysaccharides found in the cellular walls of *L. edodes.* They are bioactive compounds that can activate leukocytes, stimulate the immune system to

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produce anti-inflammatory mediators and have anticarcinogenic activity [10]. β-Glucans can also participate in lipid metabolism to reduce levels of total cholesterol and reduce body weight [11].

Lentinula edodes also contains glycoproteins as well as compounds with antioxidant [12], antitumor [13] and antibiotic activities [14, 15]. *Lentinula edodes* has antiviral [16], antihypertensive [17], antihypercholesterolemic [18, 19], hypoglycemic [20, 21] and antithrombotic activities [17, 18]. However, many of the studies mentioned were carried out in vitro or with high concentrations of *L. edodes*, which does not correspond with human consumption.

We wished to evaluate the effects of ingestion of *L. edodes* under biochemical, hematologic and oxidative stress parameters in rats receiving a high-fat diet (HFD).

Materials and methods

Reagents and solutions

Ethyl ether, sulfuric acid, copper sulfate, potassium sulfate, boric acid, hydrochloric acid (HCl), acetic acid, nitric acid, trichloroacetic acid (TCA), potassium hydroxide (KOH), sodium acetate buffer (SAB), Triton X-100, potassium phosphate buffer (TFK), 5-5-dithiobis(2-nitrobenzoic acid) (DTNB), ethylenediamine tetra-acetic acid (EDTA), reduced glutathione (GSH), glutathione reductase (GR), nicotinamide adenine dinucleotide phosphate (NADPH), sodium azide, hydrogen peroxide, exo-β-1,3-glucanase, β-glycosidase, glucose oxidase/peroxidase, amiloglycosides and invertase, sodium hydroxide (NaOH), malondialdehyde (MDA), thiobarbituric acid (TBA), phosphoric acid (H_3PO_4), sodium dodecyl sulfate (SDS) were purchased from Sigma-Aldrich (St. Louis, MO, USA) or from Yeast and Mushroom, Megazyme, Bray, Eire.

Ethical approval of the study protocol

The study protocol was approved by the Commission of Ethics in the Usage of Animals of the University of Sorocaba (approval number 008/2012; São Paulo, Brazil).

Preparation of *Lentinula edodes*

Lentinula edodes (Berk.) Pegler-cultivated strain H600 (Hokken, Shimotsuga-gun, Japan) was provided by the commercial company Yuri Cogumelos (São Paulo, Brazil). Fresh samples of *L. edodes* were sliced and dehydrated in a greenhouse at 38 ± 2 °C. Material was ground in a mill to obtain a fine, homogeneous powder and then diluted in water.

To verify humidity, samples (4 g) were dried at 105 °C until they reached constant weight. To obtain ashes, 3 g of the sample was carbonized and incinerated at 550 °C. For extraction of lipids, ethyl ether was employed for 6 h [22].

Nutritional composition of *Lentinula edodes*

Proteins were quantified using a nitrogen distiller following the method of Zhou et al. [23] with some alterations. This method comprised three phases: digestion, distillation and titration. Digestion was based on oxidation of 70 mg of sample at 390 °C with sulfuric acid, cooper sulfate and potassium sulfate. In the distiller, the nitrogen in proteins was reduced to ammonium sulfate. Upon contact with boric acid, the ammonium sulfate forms ammonium borate, with release of HCl. Centesimal composition of *L. edodes* was determined by summating the humidity, ashes, lipids, proteins and carbohydrates [24].

Fibers were quantified following the method of Mccleary et al. [25]. Dry defatted samples were digested with glacial acetic acid, nitric acid and trichloroacetic acid with heating for 40 min. Samples were filtered, desiccated, weighed and washed with boiling water until pH neutralization. Then, samples were washed (alcohol and ether), dried and incinerated at 550 °C until ashes were obtained. The difference between the initial weight of the sample and the weight of the ashes was the quantity of total fibers.

Total glucans were determined using a β-Glucan Assay kit (Yeast and Mushroom; Megazyme, Bray, Eire). Briefly, *L. edodes* (100 mg) and 1.5 mL of 37 % HCl were incubated for 45 min at 30 °C. Volume was adjusted to 10 mL with deionized water followed by incubation for 2 h. Then, 10 mL of KOH (2 M) was added and sample volume adjusted to 100 mL with SAB (200 mM, pH 5.0). The solution was centrifuged at 1500×*g* for 10 min at room temperature. The supernatant (0.1 mL) was mixed with 0.1 mL of exo-β-1,3-glucanase and β-glycosidase and incubated for 60 min at 40 °C. Then, 1.5 mL of glucose oxidase/peroxidase was added followed by incubation for 20 min at 40 °C. Absorbance was measured at 510 nm against a blank reagent.

For α-glucans, 0.2 mL of KOH (2 M) was added to 100 mg of sample. After 20 min of agitation, 0.2 mL of aminoglycosides and invertase were added and incubated for 30 min at 40 °C. Tubes were centrifuged at 1500×*g* 10 min at room temperature. To 0.1 mL of supernatant were added 0.1 mL of SAB and 0.3 mL of enzymatic reagent and incubated for 20 min at 40 °C. Absorbance was measured at 510 nm. α-Glucans were subtracted from total glucans for quantification of β-glucans.

Animal experiments

The dose of *L. edodes* (100 mg/kg) was chosen based on a study from our research team [26]. A HFD (60 % of lipids) [27] and control diet (10 % lipids) were used (Table 1).

Table 1 Composition and energy content of the standard diet and high-fat diet according to Fraulob et al. [27]

Components (g/kg)	Standard diet	High-fat diet
Casein	140	190
Cornstarch	620.7	250.7
Sucrose	100	100
Soybean oil	40	40
Lard	–	320
Fiber	50	50
Total vitamins	10	10
Mineral	35	35
L-cystine	1.8	1.8
Choline	2.5	2.5
Carbohydrates (%)	76	26
Proteins (%)	14	14
Lipids (%)	10	60

Male Wistar rats (45 ± 3 days) were obtained from Anilab (São Paulo, Brazil). They were maintained individually in cages at the Laboratory of Toxicological Research at a controlled temperature (21 ± 2 °C), humidity (50 ± 5 %) and lighting (12-h light–dark cycle). Standard rat chow and filtered tap water were provided ad libitum. Eighteen rats were allocated randomly (random number table generated by computer) into three groups of six: control (standard diet); HFD (60 % lipids); and HFD + *L. edodes* (100 mg/kg). Administration of *L. edodes* was by oral gavage at <3 mL/kg body weight. Mushroom powder was reconstituted in water every day. Rats were treated for 30 days. Rats were weighed every week. At the study end, rats were killed with an overdose of ketamine (100 mg/kg) and xylazine (6 mg/kg). Blood was collected and stored at −80 °C.

Hematologic parameters

Hematologic parameters were measured using a Sysmex XS 1000i™ Hematology Analyzer (Roche, Basel, Switzerland). Parameters were: white blood cells (WBCs) or leukocytes; red blood cells (RBCs) or erythrocytes; hemoglobin (Hb); hematocrit; mean corpuscular volume; mean corpuscular hemoglobin; mean corpuscular hemoglobin concentration (MCHC); platelets.

Biochemical profile

Levels of glucose, triglycerides, cholesterol (total and fractions), fructosamine and gamma-glutamyl transferase (γ-GT) were evaluated using commercial kits according to manufacturers Bioclin (Pretoria, South Africa), in vitro (Placentia, CA, USA), and Wiener Laboratorios (Buenos Aires, Argentina) instructions. Analyses were undertaken on a spectrophotometer (Lambda 35; PerkinElmer, Waltham, MA, USA).

Hepatic enzymes aspartate (aminotransferase (AST), alanine aminotransferase (ALT)) and renal biomarkers (urea, creatinine) were analyzed on automated equipment (Cobas C111; Roche).

Oxidative stress

Determination of reduced glutathione (GSH) levels was based on quantification of total reduced thiols following the method of Ellman [28]. Briefly, 150 μL of blood maintained in an ice bath was vortex-mixed with 100 μL of 10 % Triton X100 (10 %) and 100 μL of 30 % trichloroacetic acid. The solution was centrifuged at 4000×*g* for 10 min at 4 °C. In the cuvette, 900 μL of trifluoromethyl ketone (TFK; 1 M), 50 μL of the supernatant and 50 μL of 5,5′-dithiobis-(2-nitrobenzoic acid) (10 mM) were pipetted and formed a yellow complex. Absorbance was measured at 412 nm using a spectrophotometer. To calculate the GSH concentration, a calibration curve with predefined concentrations of GSH (0.005, 0.01, 0.025, 0.05 and 0.1 mM) was employed.

Levels of the antioxidant enzyme glutathione peroxidase (GSH-Px) were determined based on the oxidation of the reduced form of nicotinamide adenine dinucleotide phosphate (NADPH) following the method of Paglia and Valentine [29]. Blood was diluted (1:40) in TFK (pH 7.0). Then, 20 μL of the diluted sample was added to 880 μL of a solution containing GSH, glutathione reductase, NADPH, sodic azide and 100 μL of hydrogen peroxide (H_2O_2). GSH-Px level was monitored in a spectrophotometer at 340 nm for 2 min.

Measurement of catalase levels followed the method of Aebi [30], which is based on H_2O_2 decomposition by catalase at 240 nm. Briefly, blood was diluted (1:60) in TFK 50 mM. An aliquot of 20 μL was mixed to 1910 μL of TFK, and 70 μL of H_2O_2 was added, thereby initiating a reaction that was monitored for 5 min. A constant of variation (k), which is related to Hb, was used to obtain a value for blood activity (k/g Hb).

Thiobarbituric acid reactive substances (TBARS) are an important method to evaluate the lipid peroxidation, including malondialdehyde [31]. Plasma aliquots (150 μL) were mixed with 50 μL of NaOH and 50 μL of Milli-Q Water™ (Millipore, Billerica, MA, USA). The mixture was incubated for 20 min at 60 °C with agitation. Then, 6 % H_3PO_4 (250 μL), 0.8 % thiobarbituric acid (TBA; 250 μL) and 100 μL of 10 % sodium dodecyl sulfate were added to samples, which were bathed for 1 h at 80 °C. Lipid peroxidation products reacted with TBA in acidic conditions to form a pink substance, the absorbance of which was read at 532 nm in a spectrophotometer. A calibration curve was used (predefined concentrations of 0.28, 0.56, 1.7, 3.4 and 6.6 μM) to calculate the concentration of TBARS in plasma.

Statistical analyses

Data are the mean ± standard deviation. Results were analyzed using one-way analysis of variance followed by Duncan's test to identify differences among groups. Differences with a p value of <0.05 were considered to be significant. Results were analyzed using Statistica version 8.0 (Dell, Round Rock, TX, USA) and GraphPad Prism version 6.0 (San Diego, CA, USA).

Results

Nutritional composition of *Lentinula edodes*

Nutritional composition of *L. edodes* was based on dry weight (with the exception of humidity analyses) and is shown in Table 2 (which also shows the nutritional composition of other varieties of the same mushroom).

Body weight

Rat weights are presented in Fig. 1. Besides lipid content, the HFD administered to the two treatment groups caused a significant increase in body weight only in weeks 3 and 4 compared with control ($p = 0.03$ and $p = 0.04$ in HFD; $p = 0.02$ and $p = 0.05$ in HFD + *L. edodes*, respectively). On the other hand, oral administration *L. edodes* (100 mg/kg) for 30 days was not sufficient to reduce body weight compared with the HFD group ($p = 0.68$; 0.98; 0.67; 0.81; 0.97 in week 0, 1, 2, 3 and 4, respectively).

Hematologic parameters

Data for hematologic parameters are shown in Table 3. The group receiving a HFD + *L. edodes* displayed a significant increase in the number of WBCs compared with the control group ($p = 0.03$). The MCHC showed a significant ($p = 0.04$) increase in the HFD group compared with the control group. Values for RBC and platelet counts, as well as those for hemoglobin, hematocrit, mean corpuscular volume, and mean corpuscular hemoglobin, did not show significant differences among the three groups. Hence, apart from WBC count, a HFD and co-exposure of *L. edodes* did not modify hematologic parameters.

Liver function

Rats receiving the HFD had a significant increase in levels of ALT ($p = 0.0001$; $p = 0.0002$) and AST ($p = 0.003$; $p = 0.002$) compared with the control group and HFD + *L. edodes* group, respectively (Fig. 2a). In animals who received *L. edodes*, hepatic enzymes were preserved, without significant differences compared with the control group ($p = 0.61$ to ALT; $p = 0.97$ to AST). There were no significant differences in γ-GT levels among groups when compared with the control group ($p = 0.53$; $p = 0.09$).

Kidney function

Urea levels were increased in the group receiving a HFD in comparison with the control group ($p = 0.05$) (Fig. 2b). There was no significant difference in creatinine levels among the study groups ($p = 0.68$; $p = 0.72$, compared with control). Urinary glucose levels in the group receiving a HFD did not show a significant difference in comparison with the control group ($p = 0.73$). Animals that consumed a HFD + *L. edodes* had reduced urinary glucose levels compared with the control group ($p = 0.04$), suggesting that *L. edodes* has a hypoglycemic action.

Lipid profile

A significant increase in levels of total cholesterol ($p = 0.027$) and triglycerides ($p = 0.042$) was noted in the group receiving the HFD compared with that of the control

Table 2 Nutritional composition of *L. edodes* (based on 100 g of the dry sample (%)) and comparison with other studies using other varieties of the same mushroom

	This study	Regula; Siwulski [8]	Mattila et al. [32][a]	Reis et al. [33][a]	Gaitán-Hernández et al. [34]
Humidity	90.6 ± 0.1	92.9	91.6	91.6	91.7
Ashes	5.3 ± 0.04	6.7	0.5	1.4	5.4
Lipids	2.9 ± 1.0	2.9	0.3	0.35	2.15
Proteins	18.4 ± 1.2	17.2	1.8	1.1	17.2
Carbohydrates	38.6 ± 2.0	19.85	5.8	17.6	75.3
Fibers	33. ± 0.1	46.15	3.3	–	–
α-Glucan	1.01 ± 0.02	–	–	–	–
β-Glucan	34.5 ± 0.01	–	–	–	–

[a] Results of Matilla et al. [32] and Reis et al. [33] were presented on fresh base and the other ones on dry base

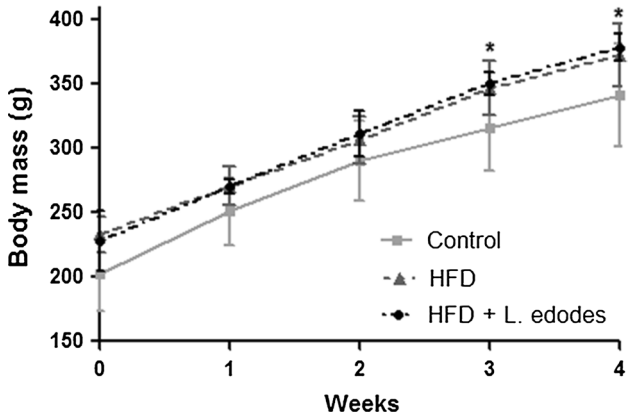

Fig. 1 Body mass (g) in the control group, high-fat diet (HFD) and HFD + *L. edodes* (100 mg/kg) group through the weeks of the treatment. Data are the mean ± standard deviation. *Statistically different from control group: week 3 $p = 0.03$ in HFD; $p = 0.023$ in HFD+ *L. edodes*; week 4 $p = 0.043$ in HFD; $p = 0.049$ in HFD+ *L. edodes*. No differences were observed comparing HFD+ *L. edodes* to HFD

Table 3 Hematologic parameters of rats treated 30 days with a standard diet (control), high-fat diet (HFD) and HFD + *L. edodes* (100 mg/kg)

	Control	HFD	HFD + *L. edodes*	*p* value[b]
WBC ($10^3/\mu L$)	5.66 ± 0.9	6.56 ± 0.5	7.60 ± 2.0*	0.25; 0.026; 0.186
RBC ($10^6/\mu L$)	7.49 ± 0.7	7.35 ± 0.3	7.48 ± 0.5	0.66; 0.98; 0.667
Hb (g/dL)	14.5 ± 1.2	14.10 ± 0.9	14.3 ± 0.7	0.52; 0.72; 0.747
HCT (%)	44.9 ± 3.6	43.0 ± 2.8	44.0 ± 1.9	0.27; 0.55; 0.554
MCV (fL)	60.6 ± 2.3	58.4 ± 2.2	58.9 ± 2.9	0.16; 0.23; 0.758
MCH (pg)	19.3 ± 0.7	19.2 ± 0.7	19.1 ± 0.9	0.74; 0.67; 0.908
MCHC (g/dL)	32.2 ± 0.6	32.8 ± 0.2[a]	32.5 ± 0.5	0.041; 0.26; 0.262
PLT ($10^3/\mu L$)	617 ± 196	656 ± 217	741 ± 107	0.73; 0.3; 0.447

Data are the mean ± standard deviation

WBC white blood cells, *RBC* red blood cells, *Hb* hemoglobin, *HCT* hematocrit, *MCV* mean corpuscular volume, *MCH* mean corpuscular hemoglobin, *MCHC* mean corpuscular hemoglobin concentration, *PLT* platelet

[a] Statistically different from the control group

[b] *p* value of HFD and HFD + *L. edodes* compared to the control, and HFD + *L. edodes* compared to HFD, respectively

group. In animals receiving a HFD + *L. edodes*, levels of cholesterol and triglycerides did not show a significant difference compared with those of the control group ($p = 0.59$; $p = 0.08$, respectively), thereby suggesting a hypocholesterolemic action from *L. edodes* (Fig. 3a). Furthermore, *L. edodes* administration contributed to a reduction in the level of very-low-density lipoprotein ($p = 0.002$ when compared with the HFD group) and an increase in those of high-density lipoprotein (HDL) compared with the control group ($p = 0.01$) and HFD group ($p = 0.03$). These effects could be attributed to the high concentration of fibers and β-glucans in *L. edodes*.

Glycemic profile

The fructosamine concentration (Fig. 3b) was not significantly different among the three groups ($p = 0.27$; 0.73, compared with the control group). The serum concentration of glucose (Fig. 3c) was reduced significantly in the HFD + *L. edodes* group compared with the HFD group (0.043). These findings suggested a hypoglycemic action arising from β-glucans.

Oxidative stress

The HFD increased TBARS levels significantly compared with those of the control group ($p = 0.02$) and *L. edodes* group ($p = 0.006$) (Fig. 4a). Nevertheless, the *L. edodes* group had decreased levels of TBARS similar to those of the control group ($p = 0.45$). No significant differences were observed among the groups with respect to catalase (Fig. 4b) ($p > 0.38$), reduced GSH (Fig. 4c) ($p > 0.43$) or GSH-Px (Fig. 4d) ($p > 0.11$).

Discussion

Our findings for the nutritional composition of *L. edodes* exhibited similarities and differences compared with those from other studies. Differences in the concentrations of macronutrients are dependent upon the location, substrate, weather, crop conditions, phase of growing and harvest of mushrooms [35].

We found high concentrations of β-glucans that were comparable with those observed by Bak et al. [36] (29.7–56.5 %) in ten crops of *L. edodes*. Brauer and colleagues observed, in extracts and lyophilized samples of *L. edodes*, 5.0 and 5.6 % of β-glucans, respectively [37]. Manzi et al. [38] found 13.9–66.6 % of β-glucans in samples of *Pleurotus ostreatus*. Those wide variations of concentrations of bioactive compounds are due to differences in cultivation and species.

Handayani et al. [39] evaluated the ingestion of powdered *L. edodes* (7, 20 and 60 g/kg) in rats receiving a HFD (50 %) during 6 weeks, along with mushrooms. The group receiving the higher dose of *L. edodes* gained less corporeal weight in comparison with all other groups. Despite those

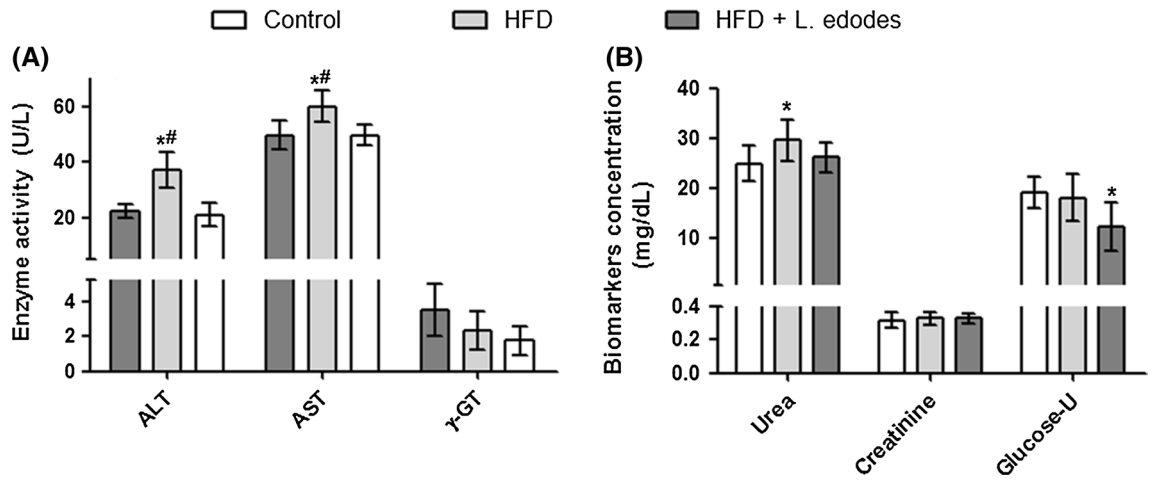

Fig. 2 **a** Enzyme activities [alanine transaminase (ALT); aspartate transaminase (AST); gamma-glutamyl transferase (γ-GT)]. **b** Kidney biomarkers concentrations of rats treated for 30 days with a standard diet (control), high-fat diet (HFD) and HFD + *L. edodes* (100 mg/kg). Data are the mean ± standard deviation. *Statistically different from control group ($p = 0.0001$ for ALT; $p = 0.003$ for AST; $p = 0.047$ for urea; $p = 0.044$ for Glucose-U). #Statistically different from HFD+ *L. edodes* ($p = 0.0002$ for ALT; $p = 0.002$ for AST)

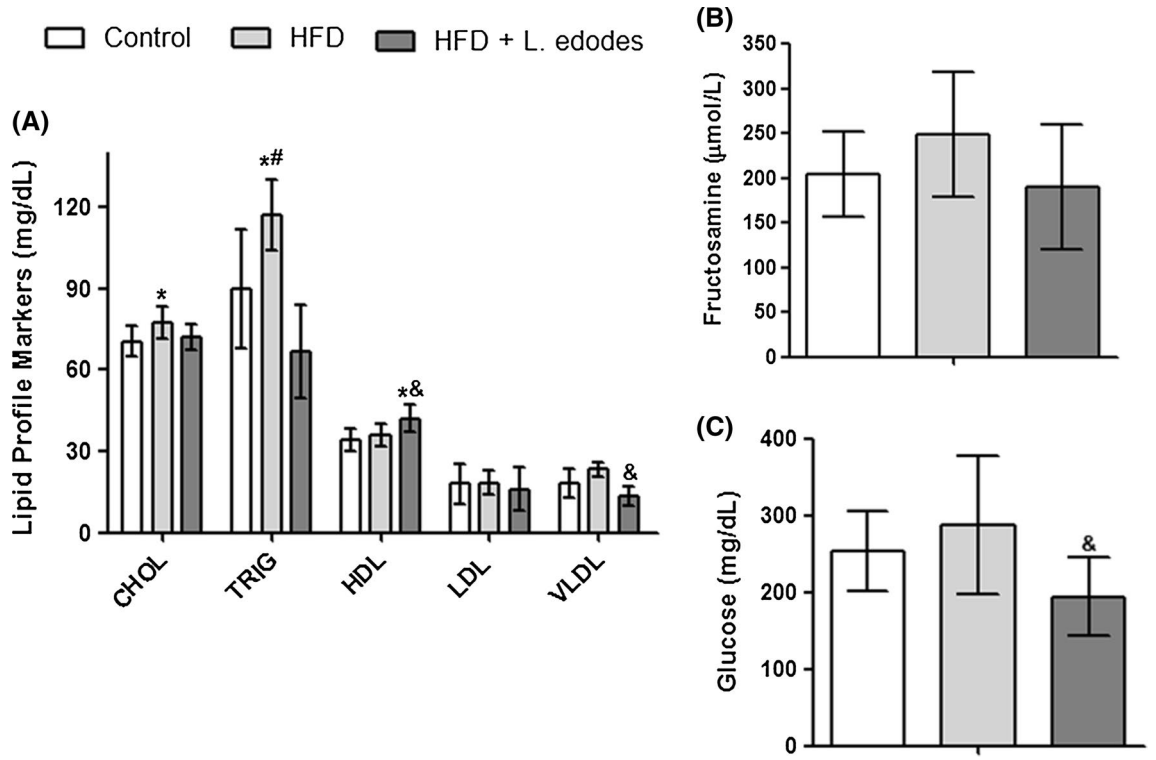

Fig. 3 **a** Lipid profile [cholesterol (COL); triglycerides (TRIGL); high-density lipoprotein (HDL); very-low-density lipoprotein (VLDL); low-density lipoprotein (LDL)]. **b**, **c** Glycemic profile of rats after 30 days receiving a standard diet (control group), high-fat diet (HFD) and HFD + *L. edodes* (100 mg/kg). Data are the mean ± standard deviation. *Statistically different from control group ($p = 0.027$ for CHOL; $p = 0.049$ for TRIG; $p = 0.001$ for HDL). #Statistically different from HFD+ *L. edodes* ($p = 0.0018$ for TRIG). &Statistically different from HRD ($p = 0.03$ for HDL; $p = 0.002$ for VLDL; $p = 0.043$ for Glucose)

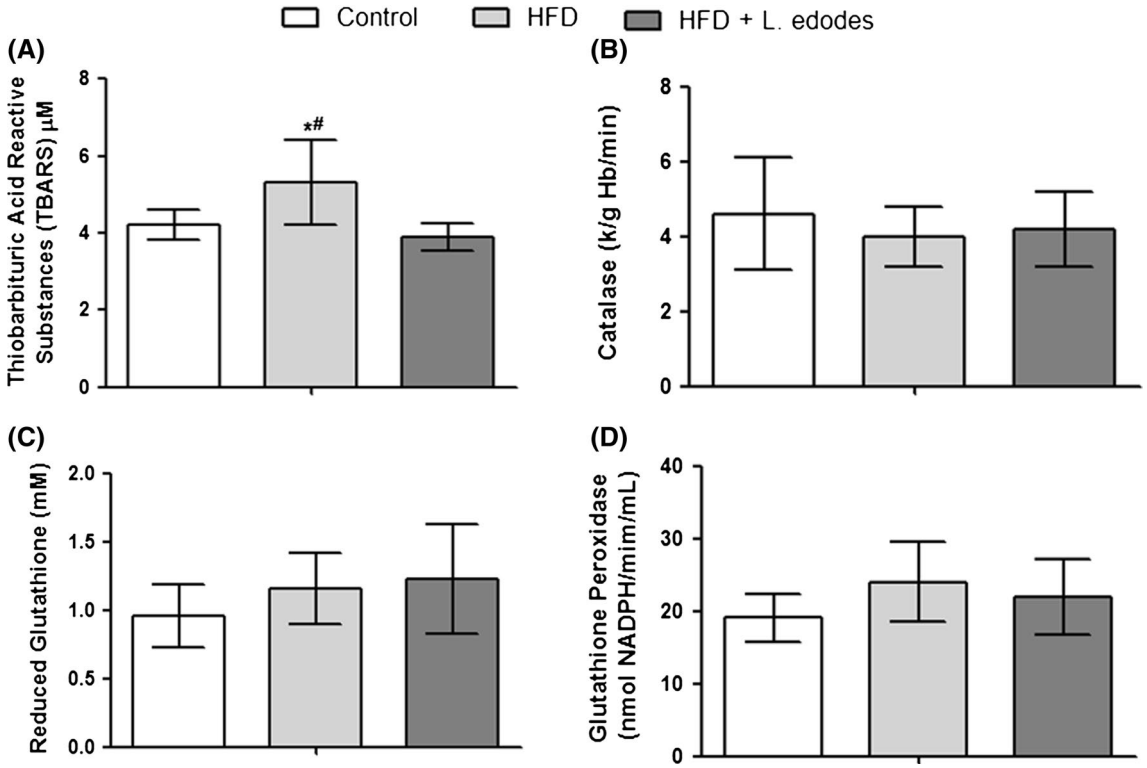

Fig. 4 Biomarkers of oxidative stress in rats treated for 30 days with a standard diet (control), high-fat diet (HFD) and HFD + *L. edodes* (100 mg/kg). Data are the mean ± standard deviation. *Statistically different from control group ($p = 0.02$ for TBARS). #Statistically different from HFD + *L. edodes* ($p = 0.006$ for TBARS)

results, the consumption reported does not match realistic consumption of mushrooms in humans.

Increases in WBC count can be related to: (i) activation of inflammation from excessive consumption of lipids and (ii) stimulation of the immune system by *L. edodes* [40, 41]. Harada et al. [42] made rats eat extracts of β-glucans from mushrooms to induce leukopenia. They noted improvement in the hematopoietic system, suggesting that this extract could contribute to the increase in production and maturation of thymocytes [42, 43], results that were corroborated by our study. β-Glucans could activate the hematopoietic system.

The MCHC was increased significantly in the HFD group compared with the control. The high concentration of fat could have caused an increase in the level of hemoglobin, causing blood thickening [44]. Tai et al. [45] showed that the lipid source can influence hematologic parameters, since they observed increase in the viscosity of blood, serum and plasma in rats receiving soy oil, palm oil and vegetable fat.

Levels of ALT and AST in blood can be increased if the metabolism of the liver is compromised or if the liver is injured [46, 47]. Increased levels of saturated fatty acids induce liver damage, which results in high levels of AST and ALT in blood [48]. The HFD increased ALT and AST levels by 74 and 20 %, respectively, compared with the control group. *L. edodes* reduced levels of this enzyme by 63 and 20 %. Chung et al. [49] presented that 250 mg/kg of *L. edodes* reduced the levels of ALT and AST along with a reduction in inflammation, necrosis and fatty deposition in liver tissue in rats chemically injured.

In the present study, the main reason for increases in urea levels is the kidney damage triggered by excessive amounts of lipids, which hinders blood filtration (Fig. 2b). Urea and creatinine are sensitive markers of early damage to the kidneys [50]. *L. edodes* acted to reduce urea levels. To verify the renoprotective properties of *Inonotus obliquus*, researchers induced diabetes in rats using streptozotocin. Results showed reductions in urea levels in groups eating *I. obliquus* compared with the control group [51], following the same line our findings. In another study, *Ganoderma lucidum* decreased concentrations of creatinine and urea in the nephrotoxicity induced by cisplatin [52], thereby denoting kidney protection by mushroom ingestion. Moreover, Vaidya et al. [50] showed significant protection of *L. edodes* against the kidney injuries induced by carbon tetrachloride (CCl_4) by normalizing the levels of urea and creatinine.

Fukushima et al. [53] detailed hypocholesterolemic effects in the same line to our findings. Upon feeding of isolated fibers of *Grifola frondosa* and *Flammulina velutipes* mushrooms to rats, the concentration of total cholesterol was reduced 11 and 25 %, respectively. Similarly, Yoon et al. [54] administered 5 % *L. edodes* in powder form to hypercholesterolemic rats and found reductions in levels of triglycerides and cholesterol, findings that are in accordance with our data. However, the concentration of *L. edodes* used was very high and not consistent with ingestion in humans.

Hyperlipidemia and high blood pressure are the two main risk factors for atherosclerosis. In excess, low-density lipoprotein (LDL) is deposited in the walls of blood vessels and becomes an important component to platelet injuries. HDL facilitates translocation of cholesterol from peripheral tissue to be catabolized in the liver [54]. Yang et al. [55] used 5, 10 and 20 % *L. edodes* (from total ingestion of food) to feed rats with hypercholesterolemia. The hypocholesterolemic effect was proportional to the increase in percentage of *L. edodes* administered. Authors have proposed that such action is due to eritadenine and β-glucans in mushrooms. Viscosity and gel-formation properties of β-glucans facilitate fat excretion to reduce the time cholesterol is present in the organism and thus diminish its absorption [56]. Mircea et al. [20] evaluated the effects of three species of fungi—extracts and powders—on levels of glucose and cholesterol. The hypoglycemic effect was more pronounced in groups treated with extracts, whereas groups treated with powder had better results for hypocholesterolemia. This hypoglycemic action could be attributed to the insulin secretion and action after mushroom extracts ingestion [21].

TBARS is a technique that evaluates products of lipid peroxidation, which are involved in the oxidative degradation of polyunsaturated fatty acids [57]. The diet given to rats was rich in lipids, so the increase in levels of total cholesterol and triglyceride was related to the increase in TBARS levels. Conversely, *L. edodes* could decrease lipid peroxidation levels to those seen in the control group, suggesting an antioxidant action of *L. edodes* that prevents oxidation of fatty acids. Jayakumar et al. [57] evaluated the CCl_4 effects in liver of rats exposed to *P. ostreatus.* They noticed as increase in liver MDA levels in rats exposed only to CCl_4. And the group co-exposed *P. ostreatus* had a significant reduction in MDA concentrations, suggesting a decreased oxidative damage to tissue liver, similar to our results in plasma.

Kanagasabapathy et al. [58] observed a significant reduction in hepatic and renal lipid peroxidation and an increase in catalase and GPx activities in rats treated with a HFD and three doses of *Pleurotus sajor-caju*. Acharya et al. [59] noted an increase in lipid peroxidation and decreases in levels of catalase and GSH in liver of rats treated with CCl_4 and the restored antioxidant status upon administration of *Macrocybe gigantean*, which was proposed to be a potent scavenger of free radicals.

Conclusions

This is the first study to report on the different parameters associated with HFD ingestion and *L. edodes*. The most important finding was the dose of *L. edodes* that is suitable for human consumption. HFD consumption induced alterations in the lipid profile, hepatic function, renal function and lipid peroxidation. *L. edodes* was shown to be a good nutraceutical against metabolic disorders through its hypocholesterolemic action as well as its ability to increase HDL levels, restore the concentration of urea to normal levels, decrease levels of transaminases and stimulate the immune system (by increasing the WBC count). *Lentinula edodes* protected against the oxidative damage induced by excessive ingestion of lipids, thereby suggesting an antioxidant effect.

Bioactive properties of *Lentinula edodes* can be suggested to the high concentrations of β-glucans and fibers, among other. Thus, even though *L. edodes* does not aid weight loss, it could be an excellent nutritional source in food supplements (especially in metabolic disorders associated with high consumption of fat).

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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