JOURNAL OF MEDICINAL FOOD
J Med Food 28 (11) 2025, 1079–1090

DOI: 10.1177/1096620X251370638

ORIGINAL ARTICLES

Antioxidant Properties of Mexican *Ganoderma lucidum* Extracts in Obese C57BL/6 Mice Fed with a High-Fat and Sugar Diet

Zoha Bautista-Montero,[1,*] María E. Meneses,[1,2] Daniel Martínez-Carrera,[1] Mónica Sánchez-Tapia,[3] Julieta Hernández-Acosta,[3] Nimbe Torres,[3] Myrna Bonilla,[1] Ivan Castillo,[1] Beatriz Petlacalco,[1] Nora Fernández,[1] Alfredo Morales,[1] Wilfrido Martínez,[1] Diana Coutiño-Hernández,[3] Mario Aliphat,[1] Miguel Sánchez,[1] Armando R. Tovar,[3] and Aleyda Pérez-Herrera[4]

[1]*Centro de Biotecnología de Hongos Comestibles, Funcionales y Medicinales (CB-HCFM), Campus Puebla, Colegio de Postgraduados (CP), Puebla, Mexico.*
[2]*SECIHTI-Colegio de Postgraduados (CP), Campus Puebla, Puebla, Mexico.*
[3]*Departamento de Fisiología de la Nutrición, Instituto Nacional de Ciencias Médicas y Nutrición Salvador Zubirán (INCMNSZ), Mexico City, Mexico.*
[4]*SECIHTI-Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR), Unidad Oaxaca, Instituto Politécnico Nacional (IPN), Oaxaca, Mexico.*

ABSTRACT Oxidative stress is a key factor of comorbidities in obesity. A Mexican genotype of the medicinal mushroom *Ganoderma lucidum* (*Gl*) contains bioactive compounds showing antioxidant, hypocholesterolemic, anti-inflammatory, anticancer, and prebiotic properties. We assessed the effect of standardized *Gl*-1 and *Gl*-2 extracts (1%) consumed by obese C57BL/6 mice fed with a high-fat and sugar diet (HFSD), on weight increase, serum parameters, liver lipid accumulation, and the expression of antioxidant genes (glutathione peroxidase 1, catalase [CAT], superoxide dismutase [SOD] 1, SOD2) and proteins (CAT, SOD2, nuclear factor erythroid 2-related factor 2) in the liver. Fifty-six male C57BL/6 mice were randomized into seven groups involving the control and treatments during 17 weeks, as well as using metformin (Met, 250 mg/kg/day) as a reference drug. Serum lipids and glucose levels decreased in mice groups consuming *Gl* extracts and metformin, in comparison with the HFSD group, as follows: total cholesterol (−11.8% to −35.7%), triglycerides (−8.3% to −24.8%), low-density lipoprotein cholesterol (−19.6% to −51.6%), and glucose (−3.1% to −25.7%). *Gl*-1 and *Gl*-2 extracts showed antioxidant properties and prevented lipid accumulation in the liver. The expression of antioxidant genes and proteins was significantly higher ($P < .001$) in the mice groups consuming *Gl* extracts, as compared to the HFSD group. This evidence showed that *Gl*-1 and *Gl*-2 extracts prevented oxidative stress in the *in vivo* model of obesity induced by an HFSD.

KEYWORDS: • ***antioxidant*** • ***Ganoderma lucidum*** • ***gene expression*** • ***high-fat and sugar diet*** • ***obesity*** • ***protein expression***

INTRODUCTION

Obesity is considered one of the main public health problems, since it affects ∼671 million people in the world[1] and is associated with the development of pathologies, such as nonalcoholic fatty liver disease (NAFLD), diabetes, and cardiovascular disease.[2] The relationship between the presence of oxidative stress in obesity and the development of comorbidities is close; if the release of reactive oxygen species (ROS) exceeds endogenous antioxidant capacity, it leads to cell death.[3] Oxidative stress is strongly implicated in myocardial infarction, ischemia/reperfusion, and heart failure. Studies for the treatment of obesity have been carried out, focused on finding safer and more effective medical treatments, such as the consumption of bioactive compounds from natural sources showing antioxidant activity.[4]

The medicinal mushroom *Ganoderma lucidum* (*Gl*) *sensu lato* has been used in traditional medicine to prevent or treat various diseases. Bioactive compounds from *Gl* have been effective in the prevention and treatment of obesity and associated disorders, without presenting side effects or toxicity.[5,6] Chang et al.[7] showed that the aqueous extract of *Gl* inhibits obesity through modulation of the gut microbiota, which is mainly due to the antioxidant activity of the

*Master of Sciences scholarship (no. 788247) from the National Council of Science and Technology (CONACYT) at CIIDIR, IPN, Oaxaca, Mexico.

Article received August 23, 2023. Revision accepted July 13, 2025.

Address correspondence to: María E. Meneses, PhD, Centro de Biotecnología de Hongos Comestibles, Funcionales y Medicinales (CB-HCFM), Campus Puebla, Colegio de Postgraduados (CP), Boulevard Forjadores de Puebla no. 205, Puebla 72760, Mexico, E-mail: meneses.eugenia@colpos.mx *or Aleyda Pérez-Herrera, PhD, SECIHTI-Centro Interdisciplinario de Investigación para el Desarrollo Integral Regional (CIIDIR), Unidad Oaxaca, Instituto Politécnico Nacional (IPN), Oaxaca, Mexico, E-mail:* alperezh@ipn.mx

polysaccharides. It has also been reported that a *Gl* ethanolic extract increases antioxidant enzymes while decreasing the production of lipid peroxides, which effectively counteracts oxidative stress and optimizes the antioxidant capacity.[8] At present, pharmacological studies have demonstrated the antioxidant activity of *Gl*, mainly attributed to polysaccharides, polyphenols, terpenoids, and sterols.[8–10] Our research work has shown that standardized extracts (*Gl*-1, *Gl*-2) of Mexican *Gl* exert hypocholesterolemic and prebiotic effects in animal models fed with a high-cholesterol diet, reducing hepatic steatosis and observing better responses using the *Gl*-2 extract obtained from basidiocarps cultivated on a substrate treated with acetylsalicylic acid (ASA, 10 mM).[11–13] Furthermore, these *Gl*-1 and *Gl*-2 extracts have been shown to be safe without adverse, toxic, or harmful effects at effective doses.[14]

In an oxidative stress state, ROS generators contribute to NAFLD, inflammatory and fibrotic progression linked to the lipotoxic liver injury. The antioxidant defense system is usually capable of protecting hepatic cells from damaging effects.[15] We assessed the effects of two standardized extracts, obtained from a Mexican genotype of *Gl*, on weight increase, lipid accumulation in the liver, serum lipid parameters, and determined their antioxidant properties through the expression of specific genes (GPX1 [glutathione peroxidase 1], CAT [catalase], SOD1 [superoxide dismutase 1], SOD2 [superoxide dismutase 2]) and proteins (CAT, SOD2, NRF2 [nuclear factor erythroid 2-related factor 2]) in the liver of obese C57BL/6 mice induced by a high-fat and sugar diet (HFSD).

MATERIALS AND METHODS

Standardized Gl *extracts*

The Mexican CP-145 strain of *Gl* (Curtis) P. Karst was provided by the Genetic Resources Platform from the Centre of Biotechnology of Medicinal, Functional and Edible Mushrooms (CB-HCFM) at the Puebla Campus, CP, Mexico. Its ITS1-5.8S-ITS2 rDNA gene sequence is deposited at the GenBank (www.ncbi.nlm.nih.gov/genbank/), with the accession number LN998989. Mushroom cultivation and harvesting have been previously described.[11,14]

Dried mushroom slices were chopped in a stainless steel blender for the preparation of extracts, and the product (10 g) was placed in a filter paper (8 μm) bag for maceration (24 h). Hydroalcoholic extracts, 32% v/v (alcohol: water), were obtained according to a previous patent (MX322035-B).[16] Mushroom extracts were then concentrated to 10 mL in a rotary evaporator (R-220 Pro; Buchi, Switzerland), 90 rpm, at 40°C water bath, 23–25°C sample vapor, and 10°C chiller temperature. They were then filter-sterilized (0.45 μm; Merck Millipore, Mexico) and stored at 4°C until use. In this study, standardized extracts of *Gl* cultivated on the control substrate were labeled as *Gl*-1, whereas those cultivated on the treated substrate (ASA, 10 mM) were labeled as *Gl*-2. The standardized extracts of *Gl* were prepared at a concentration of 250 mg/mL.

Nutrient composition of Gl *extracts*

Standardized extracts of *G. lucidum* (*Gl*-1 and *Gl*-2) were previously analyzed by the authors according to certified protocols,[11,14] as shown in Supplementary Table S1. *Gl*-1 and *Gl*-2 showed different nutrient profiles, containing carbohydrates, glucans, total protein, total dietary fiber, fat, vitamins (B complex, D), minerals, and several organic acids. The total content of polyphenols in both extracts, as well as their antioxidant capacity, was also different. The addition of ASA (10 mM) to the cultivation substrate generated these differences.

Animals and treatments

The testing of *Gl* extracts was performed according to the national regulatory policy, and standard studies were performed at the institution (CP). Experimental protocols were approved by the Internal Bioethics Committee, CB-HCFM (Permit number: CIB-CB-HCFM-002). Fifty-six male C57BL/6 mice (*Mus musculus*), 6 weeks old, were provided by the INCMNSZ, Mexico City. They were kept at 22 ± 2°C (relative humidity: 45–55%), on a 12-h light/12-h dark cycles. Standard AIN-93 diet was given to all experimental groups.[17] Basic ingredients per 1000 g of diet were L-cysteine, choline, vitamins, cellulose, minerals, soybean oil, starch, dextrin, saccharose, and casein. Extracts (*Gl*-1 or *Gl*-2) were homogeneously mixed with AIN-93 and administered orally. There were seven random and independent experimental mice groups of eight animals each ($n = 8$), which were treated with one type of mushroom extract, as follows: (1) Control diet (Ctrl): AIN-93; (2) HFSD: 30% lard, 6% soybean oil, 6% coconut oil, and water containing 50 g/L of fructose and saccharose at a ratio of 55:45, respectively; (3) HFSD + *Gl*-1: HFSD plus the *Gl*-1 extract (1% of the total diet); (4) HFSD + *Gl*-2: HFSD plus the *Gl*-2 extract (1%); (5) HFSD + *Gl*-1 Tx: HFSD for 13 weeks followed by HFSD + *Gl*-1 for 4 weeks; (6) HFSD + *Gl*-2 Tx: HFSD for 13 weeks followed by HFSD + *Gl*-2 for 4 weeks; and (7) HFSD + Met Tx: HFSD for 13 weeks followed by HFSD plus metformin (250 mg/kg) for 4 weeks. The composition of different experimental diets is described in Table 1. Mice groups were fed *ad libitum* for 17 weeks, providing water and making sure that the full dose of *Gl*-1 or *Gl*-2 extracts had been eaten per day according to experimental diets. Food consumption was recorded every day, while body weight was registered twice weekly. All 56 mice finished experiments, and at the end of the study, they were deprived of food and water for 12 h before blood sampling and then sacrificed on day 119 (17 weeks). Sodium pentobarbital was administered intraperitoneally at a dose of 100–150 mg/kg. Blood was collected via the eyes, using specific capillaries, centrifuged at 1000 *g* for 10 min, and the serum was stored at –70°C until analysis. The liver was excised, weighed, frozen (liquid N_2), and stored at –70°C. Efficient animal care and good laboratory practices were followed.

Table 1. Composition of Experimental Diets Consumed by C57BL/6 Mice Groups Studied

Ingredients (g/kg)	*Ctrl AIN-93G*	*HFSD*	*HFSD* + Gl-*1*	*HFSD* + Gl-*2*	*HFSD + Met Tx*
L-cysteine	3	3	3	3	3
Choline	2.5	2.5	2.5	2.5	2.5
Vitamins	10	10	10	10	10
Cellulose	50	50	50	50	50
Minerals	35	35	35	35	35
Soybean oil	70	35	35	35	35
Coconut oil	0	35	35	35	35
Starch	397.5	239.03	239.03	239.03	239.03
Dextrin	132	102.67	102.67	102.67	102.67
Saccharose	100	77.78	77.78	77.78	77.78
Casein	200	240	240	240	240
Lard	0	170	170	170	170
Gl-1 extract	—	—	10	—	—
Gl-2 extract	—	—	—	10	—
Metformin					0.250

Ctrl, Control diet: AIN-93; HFSD, high-fat and sugar diet; HFSD + *Gl*-1, HFSD plus the *Gl*-1 extract (1% of the total diet); HFSD + *Gl*-2, HFSD plus the *Gl*-2 extract (1%); HFSD + *Gl*-1 Tx, HFSD for 13 weeks followed by HFSD + *Gl*-1 for 4 weeks; HFSD + *Gl*-2 Tx, HFSD for 13 weeks followed by HFSD + *Gl*-2 for 4 weeks; HFSD + Met Tx, HFSD for 13 weeks followed by HFSD plus metformin (250 mg/kg) for 4 weeks.

Biochemical parameters

The concentrations of total cholesterol (TC), total triglycerides (TG), low-density lipoprotein cholesterol (LDL-c), glucose, alanine transaminase (ALT), and aspartate transaminase (AST) were measured in the serum following standard protocols in a COBAS C111 apparatus (Roche Diagnostics Ltd., Switzerland).[18]

Antioxidant activity

The serum antioxidant activity was assessed by the oxygen radical absorbance capacity assay, according to a protocol previously described.[19] Experimental data were processed using Trolox as a standard and expressed as micromoles of Trolox equivalents (TE) per milliliter.

Histological analysis

Livers were dissected, fixed with ice-cold paraformaldehyde (4% w/v), dissolved in phosphate buffer, and dehydrated and embedded in paraffin. Two sections (4 μm) were taken per block, stained with hematoxylin and eosin, and analyzed under a microscope (Leica DM750, Germany).[20] The severity of hepatic steatosis was quantified using the Brunt classification as follows: the amount of fat was graded from 1 to 3 according to the proportion of fatty droplets (1: 0–33%; 2: 34–66%; 3: 67–100%).[21]

Gene expression

RNA extraction. Total RNA was extracted from mouse livers using TRIzol reagent (Invitrogen, USA), following the manufacturer's instructions. The RNA was quantified in a Nanodrop 2000 spectrophotometer (Thermo Scientific, USA).

Gene expression by reverse transcription polymerase chain reaction. RNA was reverse-transcribed to complementary DNA (cDNA) using Moloney murine leukemia virus reverse transcriptase (Invitrogen, USA). Amplifications from real-time PCR analyses of cDNA were performed using TaqMan probes in a Light Cycler 4800 thermal cycler system (Roche Diagnostics Ltd., Switzerland). The TaqMan probes used are described in Supplementary Table S2, and the housekeeping gene was beta-2 microglobulin (β2M). Gene expression was calculated as the relative expression of the target gene minus the constitutively expressed β2M gene (relative expression = 2 − [Ct, Target gene − Ct, Reference gene]). Assays for each gene were performed in triplicate.

Protein expression

Western blot analysis. Protein concentrations were determined according to the Bradford assay (Bio-Rad, USA). Samples were stored at −80°C until use. The protein (30 μg) was loaded and separated on a sodium dodecyl sulfate–polyacrylamide gel electrophoresis gel (10%) and transferred to a polyvinylidene difluoride membrane (Millipore Sigma, USA). Membranes were blocked with 3% bovine serum albumin in Tris-buffered saline with Tween for 60 min at room temperature and incubated overnight at 4°C with the primary antibody and secondary antibodies in the blocking buffer: anti-NFR2 (Santa Cruz, SC-722, rabbit polyclonal immunoglobulin G [IgG]) at 1 : 3,500; anti-SOD2 (Santa Cruz, sc-30080 rabbit polyclonal IgG) at 1 : 2,500; and anti-CAT (Santa Cruz, sc-50508 rabbit polyclonal) at 1 : 50,000. Blots were incubated with anti-rabbit (Abcam, AB6885) secondary antibodies conjugated with horseradish peroxidase (1 : 20,000). Data were normalized using glyceraldehyde-3-phosphate dehydrogenase (1 : 40,000, ab181602 rabbit monoclonal IgG). Images were analyzed with ChemiDoc™ XRS +

System Image Lab™ software (Bio-Rad). All assays were performed in triplicate using independent blots.

Statistical analysis

A GraphPad Prism v. 8.0.2 was used (GraphPad Software, USA). Data were expressed as the mean ± standard error of the mean. Statistically significant data were determined by one-way analysis of variance followed by Tukey's multiple comparisons test. Significant differences were assessed at $P < .05$.

RESULTS

Effects of Gl *extracts on weight increase and food intake*

Weight gain was calculated by subtracting the initial weight from the final weight. At the end of the study, as expected, the Ctrl group had gained less weight than the rest of the groups fed with an HFSD ($P < .0001$). However, there was no significant difference between the Ctrl and the HFSD + *Gl*-1 Tx groups, showing a clear effect of the *Gl*-1 extract on the prevention of weight gain. There was no significant difference in weight gain between the HFSD group (20.13 g) and the HFSD + *Gl*-1 (22.33 g), HFSD + *Gl*-2 (16.02 g), HFSD + *Gl*-2 Tx (15.87 g), and HFSD + Met Tx groups (18.12 g). Interestingly, although mice from the HFSD + *Gl*-1 Tx group consumed an HFSD, the weight gain (12.15 g) was significantly different from that recorded in the HFSD group (20.13 g). There was a significantly greater weight gain in the HFSD + *Gl*-1 group compared with HFSD + *Gl*-1 Tx and HFSD + *Gl*-2 Tx groups (Fig. 1A).

Regarding food intake, the average consumption (g/day) was as follows: Ctrl group (3.90), HFSD (2.95), HFSD + *Gl*-1 (3.05), HFSD + *Gl*-2 (2.85), HFSD + *Gl*-1 Tx (2.74), HFSD + *Gl*-2 Tx (2.99), and the HFSD + Met Tx group

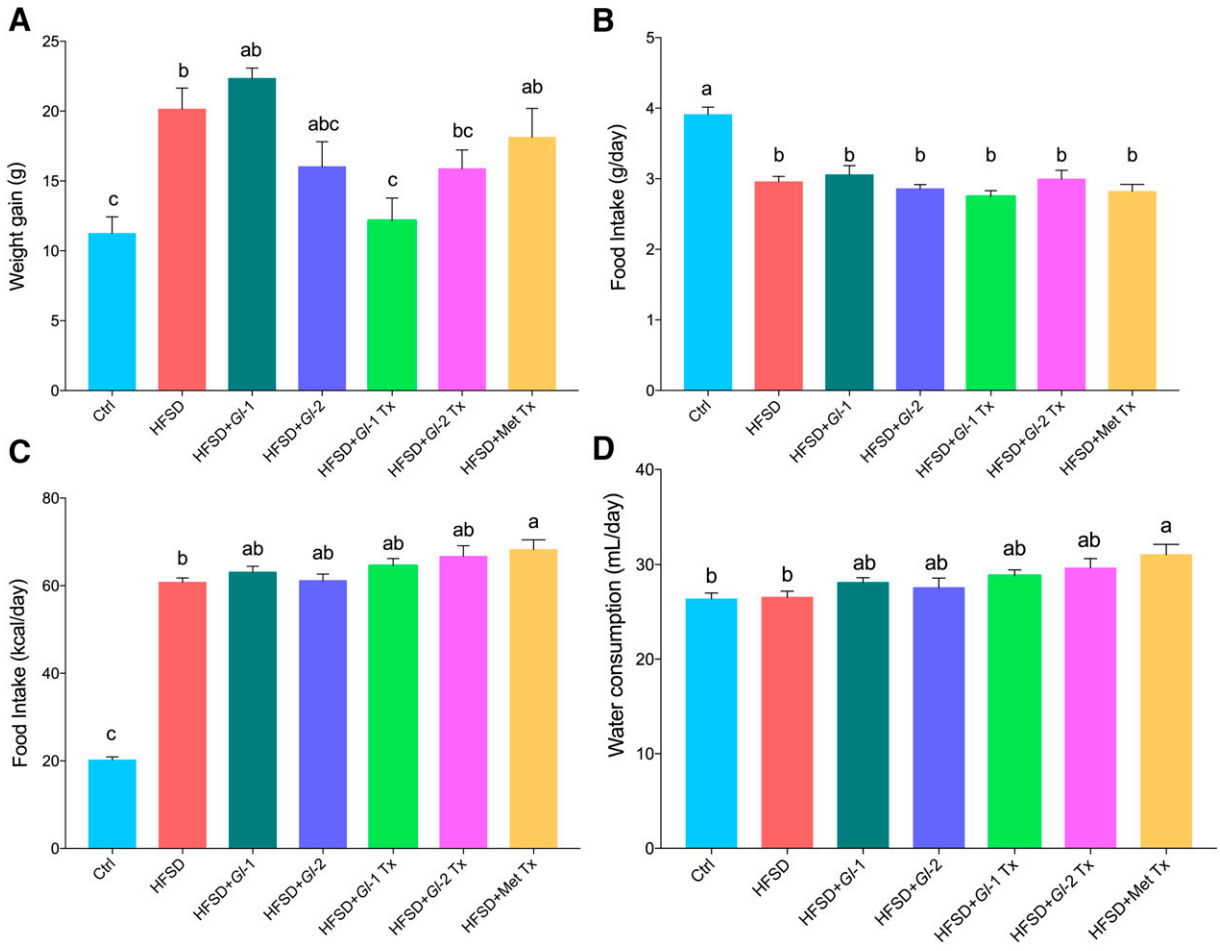

FIG. 1. Average data from experimental groups of obese C57BL/6 mice fed with an HFSD during 17 weeks. **(A)** Weight gain (g/day). **(B)** Food intake (kcal/day). **(C)** Food intake (g/day). **(D)** Water consumption (mL/day). Ctrl: Control diet: AIN-93; HFSD: high-fat and sugar diet; HFSD + *Gl*-1: HFSD plus the *Gl*-1 extract (1% of the total diet); HFSD + *Gl*-2: HFSD plus the *Gl*-2 extract (1%); HFSD + *Gl*-1 Tx: HFSD for 13 weeks followed by HFSD + *Gl*-1 for 4 weeks; HFSD + *Gl*-2 Tx: HFSD for 13 weeks followed by HFSD + *Gl*-2 for 4 weeks; HFSD + Met Tx: HFSD for 13 weeks followed by HFSD plus metformin (250 mg/kg) for 4 weeks. Data are presented as the mean ± SEM. Different letters on a column indicate significant differences, $P < .05$. Values (P) correspond to one-way ANOVA from Tukey's multiple comparison test. ANOVA, analysis of variance; HFSD, high-fat and sugar diet; *Gl*, *Ganoderma lucidum*; SEM, standard error of the mean.

(2.82). All treated mice groups fed an HFSD were significantly different ($P < .0001$) from the Ctrl group (Fig. 1B). As expected, the Ctrl diet had better palatability than HFSDs for rodents. The intake expressed as kilocalories per day, referring to the water containing fructose and saccharose given to all treated mice groups fed with a high-fat diet, showed that the Ctrl group was significantly lower ($P < .0001$) from the rest of experimental groups (Fig. 1C). The mice group fed with an HFSD + M Tx diet consumed significantly more kilocalories and water than the HFSD group (Fig. 1D).

Effects of Gl *extracts on serum parameters*

Antihyperlipidemic effects of *Gl* extracts were recorded in the obesity model of mice fed with an HFSD. Significantly lower concentration of cholesterol (mg/dL; $P < .0001$) was obtained in the HFSD + *Gl*-2 (134.0), HFSD + *Gl*-1 Tx (134.2), and HFSD + *Gl*-2 Tx (117.9) groups, compared with the HFSD group (183.40), showing no significant difference with the Ctrl group (92.2). The metformin also had a cholesterol-lowering effect, as there was a significant difference between the HFSD + Met Tx group (128.8) and the HFSD group (183.40). This trend was also observed in the HFSD + *Gl*-1 group (161.7) compared with the HFSD group (Fig. 2A).

The TG levels (mg/dL) recorded in HFSD + *Gl*-2 (55.0), HFSD + *Gl*-1 Tx (56.1), HFSD + *Gl*-2 Tx (57.8), and HFSD + Met Tx (54.0) experimental mice groups were significantly lower ($P = .0014$) than that from the HFSD group (71.9), but not significantly different compared with the Ctrl group (49.7). There was also a reduction of TG in the HFSD + *Gl*-1 group (65.9), as compared with the HFSD group (Fig. 2B).

Serum LDL-c levels (mg/dL) of the HFSD + *Gl*-2 Tx group (20.8) decreased significantly in comparison with the rest of the treated experimental groups, being equivalent to the Ctrl group (17.3; $P < .0001$). The HFSD + *Gl*-1 Tx (30.0) and HFSD + Met Tx (25.4) groups also showed significantly lower LDL-c levels compared with the HFSD group (43.0). The HFSD + *Gl*-1 (34.6) and HFSD + *Gl*-2 (32.0) groups had lower LDL-c values than the HFSD group (Fig. 2C).

Antihyperglycemic effects of *Gl* extracts were also observed, as significantly reduced glucose levels (mg/dL) were recorded in mice from the HFSD + *Gl*-2 (257.8) and HFSD + *Gl*-2 Tx (265.8) groups, in comparison with the HFSD group (334.5; $P =. 001$). This was also so for the mice group receiving the specific drug, the HFSD + Met Tx group (248.4), which was equivalent to the Ctrl group (239.4). The HFSD + *Gl*-1 (324.1) and HFSD + *Gl*-1 Tx (284.3) groups had lower glucose values than the HFSD group (Fig. 2D).

Serum levels of ALT (U/L) were significantly lower in the HFSD + *Gl*-2 Tx group (35.9; $P = .0119$) compared with the HFSD group (65.6), as well as the rest of experimental groups (Fig. 2E). There were no significant differences between the Ctrl group (41.4) and most experimental mice groups administered with the *Gl* extracts and metformin, namely, HFSD + *Gl*-1 (52.3), HFSD + *Gl*-2 (55.0), HFSD + *Gl*-1 Tx (50.9), and HFSD + Met Tx (41.8). Similarly, AST levels (U/L) of the HFSD + *Gl*-2 Tx group (73.3) were also significantly lower ($P = .0025$) than those from the HFSD (101.5), Ctrl (103.4), and HFSD + Met Tx group (100.7) groups. The HFSD + *Gl*-1 (93.4), HFSD + *Gl*-2 (93.7), and HFSD + *Gl*-1 Tx (77.7) groups had equivalent AST levels (U/L), which were lower than the HFSD and Ctrl groups (Fig. 2F).

Effects of Gl *extracts on hepatic lipids*

After euthanasia, a differential effect was clearly observed in the coloration of mice livers. The HFSD group showed a whitish liver. However, livers from mice groups consuming *Gl* extracts were as reddish as those from the Ctrl group, indicating less accumulation of lipids and a preventive effect against the development of hepatic steatosis (Fig. 3A). Histological analysis of liver sections from experimental mice groups revealed that the consumption of an HFSD for 17 weeks markedly increased the number of lipid vacuoles in the liver. Interestingly, the consumption of HFSD plus *Gl* extracts or the drug metformin prevented the accumulation of lipids in the liver (Fig. 3B). According to the histopathological experience, the Ctrl group showed grade 1 hepatic steatosis, HFSD group showed grade 3, while the rest of the experimental groups (HFSD + *Gl*-1, HFSD + *Gl*-2, HFSD + *Gl*-1 Tx, HFSD + *Gl*-2 Tx, HFSD + Met Tx) showed grade 1.

Antioxidant effects of Gl *extracts*

Effects of Gl *extracts on gene expression of antioxidant enzymes in the liver.* We observed a differential effect on the expression of antioxidant genes in the liver from mice groups consuming *Gl* extracts. The expression of GPX1 gene increased significantly in the HFSD + *Gl*-1 Tx, HFSD + *Gl*-2 Tx, and HFSD + Met Tx groups, in comparison with the HFSD group ($P = .0002$). This gene also increased its expression in the HFSD + *Gl*-1 and HFSD + *Gl*-2 groups, compared with the Ctrl group. Lower expression of the GPX1 gene was recorded in the HFSD and Ctrl groups (Fig. 4A). A similar expression trend was recorded in the CAT gene, which increased in mice groups consuming HFSD and *Gl* extracts. The *Gl*-2 extract significantly induced the highest expression of the CAT gene (HFSD + *Gl*-2, HFSD + *Gl*-2 Tx), followed by the HFSD + *Gl*-1 Tx, HFSD + Met Tx, and HFSD + *Gl*-1 groups. Lower expression of the CAT gene was recorded in the HFSD and Ctrl groups (Fig. 4B).

A significantly greater expression of the SOD1 gene was recorded in the HFSD + *Gl*-2 Tx group, in comparison with the rest of the experimental groups ($P < .0001$). The increased expression trend of the SOD1 gene was also observed in the other mice groups consuming *Gl* extracts (HFSD + *Gl*-1, HFSD + *Gl*-1 Tx, HFSD + *Gl*-2) or the specific drug (HFSD + Met Tx), compared with the HFSD and Ctrl groups (Fig. 4C). Similar was the case for the expression of the SOD2 gene, as

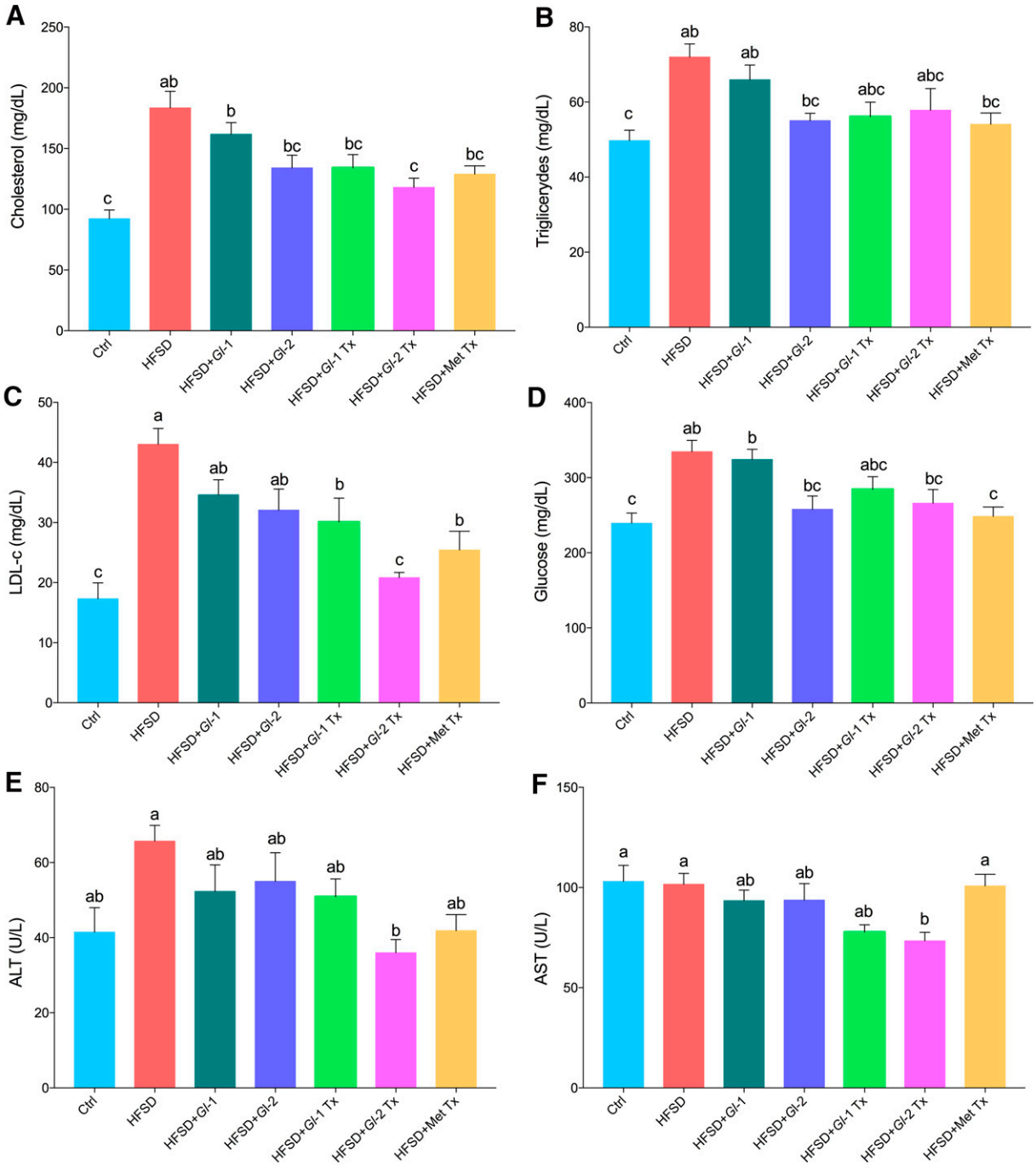

FIG. 2. Average data of serum biochemical parameters from experimental groups of obese C57BL/6 mice fed with a high-fat and sugar diet during 17 weeks. **(A)** Cholesterol (mg/dL). **(B)** Triglycerides (mg/dL). **(C)** LDL-c (mg/dL). **(D)** Glucose (mg/dL). **(E)** ALT (U/L). **(F)** AST (U/L). Blood samples were taken after 12 h of food deprivation, followed by mice euthanasia, and prepared for analyses. Ctrl: Control diet: AIN-93; HFSD: High-fat and sugar diet; HFSD + *Gl*-1: HFSD plus the *Gl*-1 extract (1% of the total diet); HFSD + *Gl*-2: HFSD plus the *Gl*-2 extract (1%); HFSD + *Gl*-1 Tx: HFSD for 13 weeks followed by HFSD + *Gl*-1 for 4 weeks; HFSD + *Gl*-2 Tx: HFSD for 13 weeks followed by HFSD + *Gl*-2 for 4 weeks; and HFSD + Met Tx: HFSD for 13 weeks followed by HFSD plus metformin (250 mg/kg) for 4 weeks. Data are presented as the mean ± SEM. Different letters on a column indicate significant differences, $P < .05$. Values (P) correspond to one-way ANOVA from Tukey's multiple comparison test.

the significant highest level was also recorded in the HFSD + *Gl*-2 Tx group ($P < .0001$). The increased expression trend of the SOD2 gene was also observed in the other mice groups consuming *Gl* extracts (HFSD + *Gl*-1, HFSD + *Gl*-1 Tx, HFSD + *Gl*-2) or the specific drug (HFSD + Met Tx), compared with the HFSD and Ctrl groups (Fig. 4D).

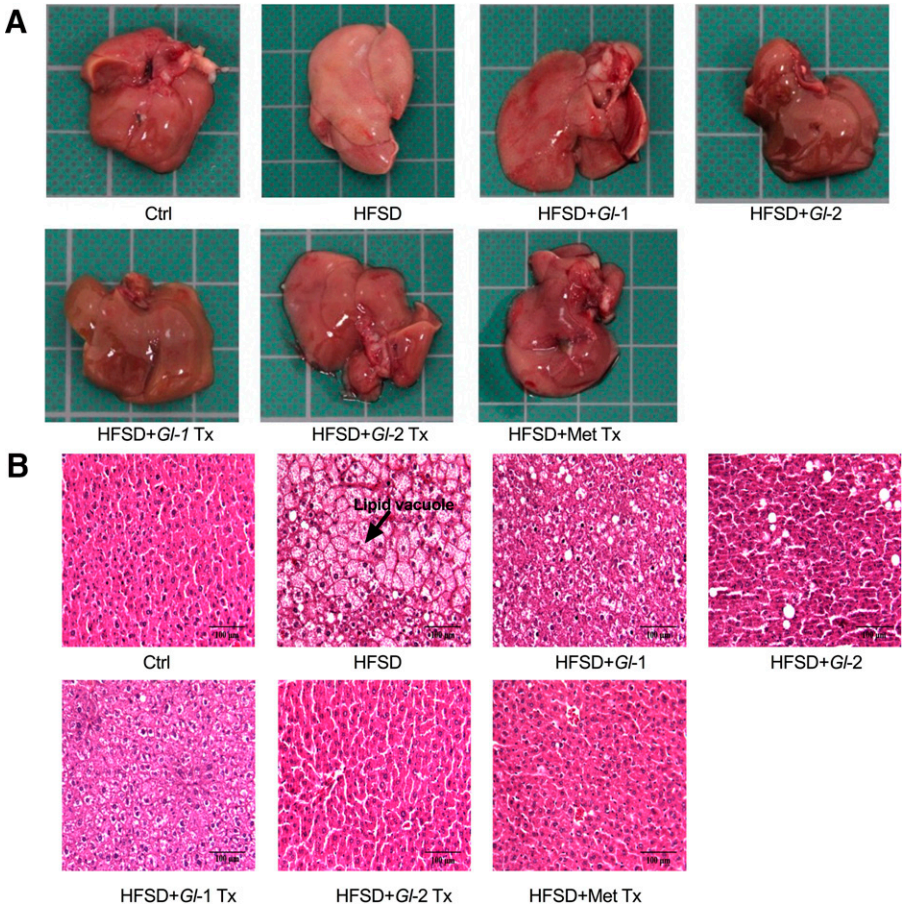

FIG. 3. Effects of experimental diets on lipid accumulation in the liver of obese C57BL/6 mice fed with a high-fat and sugar diet during 17 weeks. **(A)** Comparison of the color of livers after euthanasia among experimental groups. **(B)** Histological analysis of liver tissue stained with hematoxylin and eosin, showing differences in lipid accumulation among experimental groups. Ctrl: Control diet: AIN-93; HFSD: High-fat and sugar diet; HFSD + *Gl*-1: HFSD plus the *Gl*-1 extract (1% of the total diet); HFSD + *Gl*-2: HFSD plus the *Gl*-2 extract (1%); HFSD + *Gl*-1 Tx: HFSD for 13 weeks followed by HFSD + *Gl*-1 for 4 weeks; HFSD + *Gl*-2 Tx: HFSD for 13 weeks followed by HFSD + *Gl*-2 for 4 weeks; and HFSD + Met Tx: HFSD for 13 weeks followed by HFSD plus metformin (250 mg/kg) for 4 weeks.

Effect of Gl *extracts on antioxidant capacity of serum samples.* The antioxidant activity in serum samples showed significant differences between the Ctrl group and the rest of the treated groups ($P < .0001$). The HFSD + *Gl*-1 and HFSD + *Gl*-2 groups had higher antioxidant activity than the HFSD and HFSD + Met Tx groups. Similar trend of antioxidant activity was recorded in the HFSD + *Gl*-1 Tx and HFSD + *Gl*-2 Tx groups, which were greater than the HFSD group (Fig. 4E).

Effects of Gl *extracts on antioxidant protein expression in the liver.* The protein analysis showed that CAT protein expression in the liver was significantly higher in mice groups that consumed the HFSD plus the *Gl*-1 or *Gl*-2 extracts, compared with the HFSD group ($P = .0018$). This was so also for the HFSD + Met Tx group (Fig. 5A). The protein expression of SOD2 in the HFSD + *Gl*-1, HFSD + *Gl*-1 Tx, HFSD + *Gl*-2 Tx, and HFSD + Met Tx groups was significantly greater than that from the HFSD and Ctrl groups. This increasing trend was also recorded in the HFSD + *Gl*-2 group (Fig. 5B). Similar was the case for NRF2, as protein expression increased significantly in mice groups consuming the *Gl* extracts or metformin ($P < .0001$), compared with the HFSD group. The protein expression of NRF2 in the Ctrl group was lower than that of the HFSD + *Gl*-1, HFSD + *Gl*-2, HFSD + *Gl*-1 Tx, HFSD + *Gl*-2 Tx, and HFSD + Met Tx groups (Fig. 5C).

DISCUSSION

Obesity induces cellular oxidative stress, leading to the development of inflammation and cardiovascular diseases. Natural antioxidants consumed in the diet reduce oxidative stress processes and prevent associated comorbidities.[22] In the present study, we found antioxidant properties of standardized *Gl* extracts consumed by obese C57BL/6 mice, induced by an HFSD, as gene and protein expression increased significantly. We found that mice groups consuming an HFSD had statistically significantly higher caloric intake, in comparison with the Ctrl treatment. However, mice groups treated with *Gl* extracts consistently gained less weight. In fact, the HFSD + *Gl*-1 Tx group was not statistically different from the Ctrl

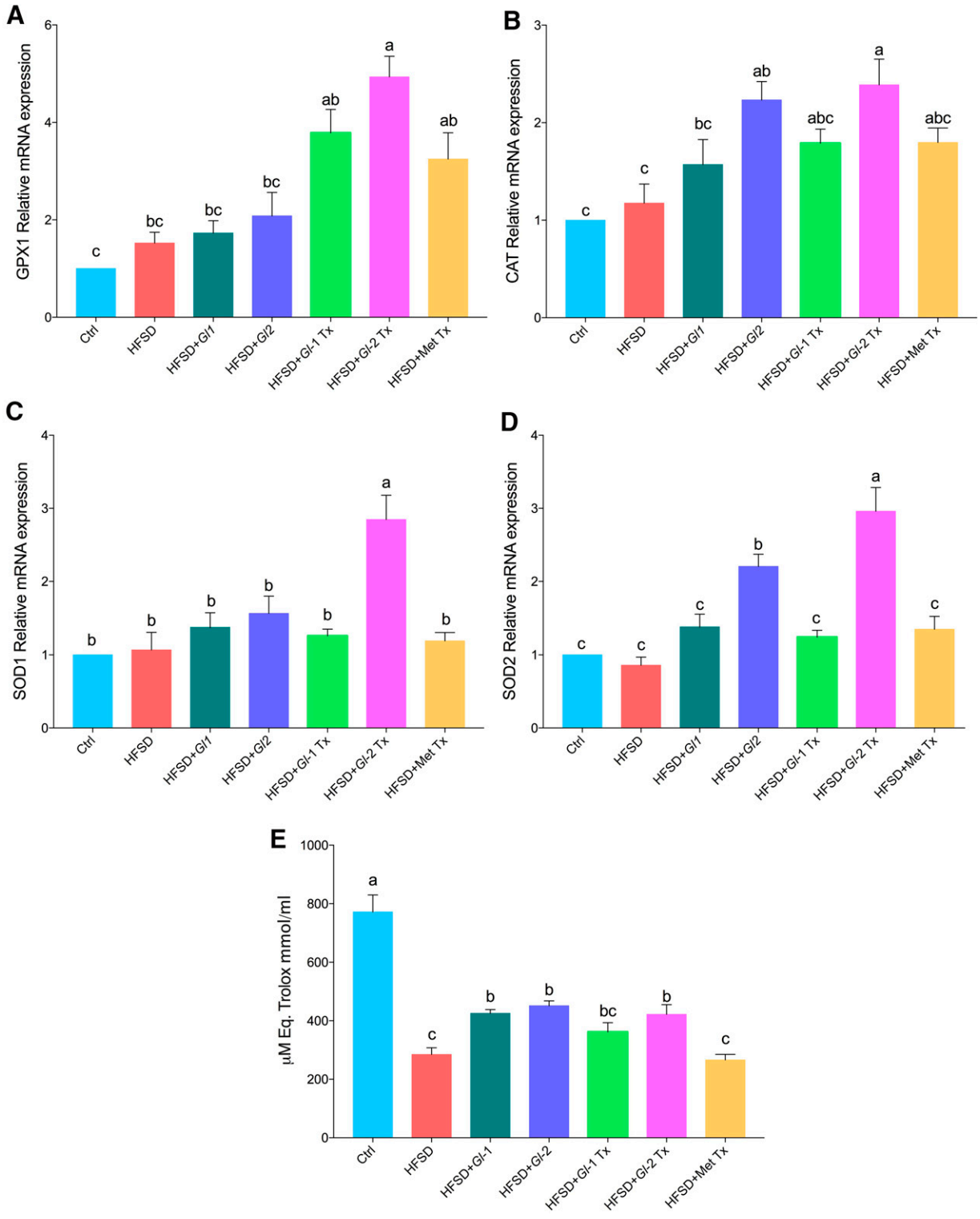

FIG. 4. Effects of experimental diets on the liver gene expression and the serum antioxidant capacity of obese C57BL/6 mice fed with a high-fat and sugar diet during 17 weeks. **(A)** GPX-1 gene. **(B)** CAT gene. **(C)** SOD1 gene. **(D)** SOD2 gene. **(E)** Antioxidant capacity in serum. Ctrl: Control diet: AIN-93; HFSD: high-fat and sugar diet; HFSD + *Gl*-1: HFSD plus the *Gl*-1 extract (1% of the total diet); HFSD + *Gl*-2: HFSD plus the *Gl*-2 extract (1%); HFSD + *Gl*-1 Tx: HFSD for 13 weeks followed by HFSD + *Gl*-1 for 4 weeks; HFSD + *Gl*-2 Tx: HFSD for 13 weeks followed by HFSD + *Gl*-2 for 4 weeks; and HFSD + Met Tx: HFSD for 13 weeks followed by HFSD plus metformin (250 mg/kg) for 4 weeks. Data are presented as the mean ± SEM. Different letters on a column indicate significant differences, $P < .05$. Values (P) correspond to one-way ANOVA from Tukey's multiple comparison test. CAT, catalase; GPX1, glutathione peroxidase 1; SOD1, superoxide dismutase 1; SOD2, superoxide dismutase 2.

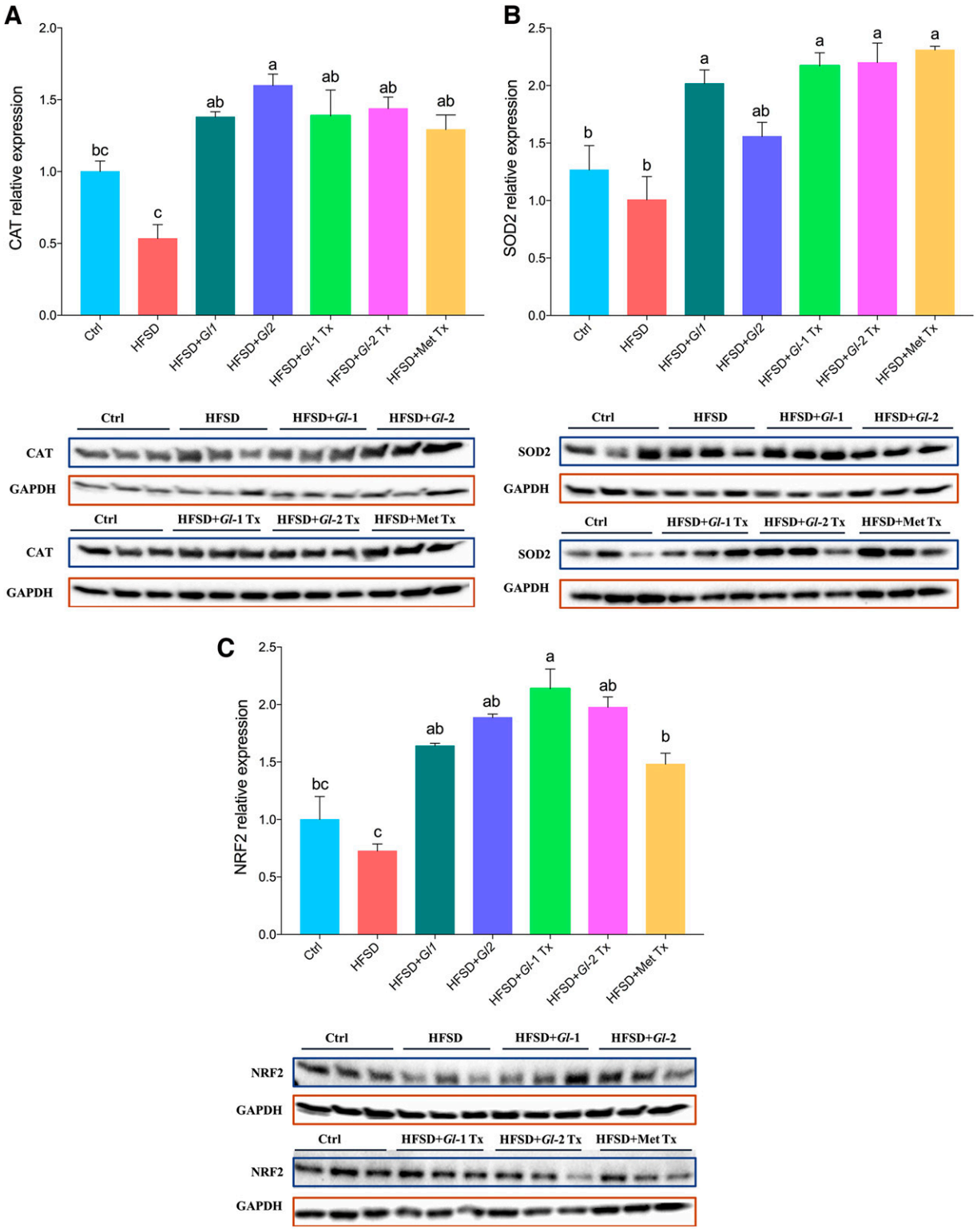

FIG. 5. Abundance of proteins by Western blot analyses and corresponding densitometry data, involved in the antioxidant defense of the liver from obese C57BL/6 mice fed with a high-fat and sugar diet during 17 weeks. **(A)** CAT. **(B)** SOD2. **(C)** NRF2. Ctrl: Control diet: AIN-93; HFSD: high-fat and sugar diet; HFSD + *Gl*-1: HFSD plus the *Gl*-1 extract (1% of the total diet); HFSD + *Gl*-2: HFSD plus the *Gl*-2 extract (1%); HFSD + *Gl*-1 Tx: HFSD for 13 weeks followed by HFSD + *Gl*-1 for 4 weeks; HFSD + *Gl*-2 Tx: HFSD for 13 weeks followed by HFSD + *Gl*-2 for 4 weeks; and HFSD + Met Tx: HFSD for 13 weeks followed by HFSD plus metformin (250 mg/kg) for 4 weeks. Data are presented as the mean ± SEM. Different letters on a column indicate significant differences, $P < .05$. Values (P) correspond to one-way ANOVA from Tukey's multiple comparison test. The expression of each protein analyzed was normalized to control GAPDH. GAPDH, glyceraldehyde-3-phosphate dehydrogenase; NRF2, nuclear factor erythroid 2-related factor 2.

group and showed the lowest weight increase of all experimental groups fed with an HFSD. Chang et al.[7] found that the aqueous extract of *Gl* decreased weight gain in mice due to its prebiotic properties, regulating dysbiosis induced by a high-fat diet, as well as maintaining intestinal integrity and reducing metabolic endotoxemia. Meneses et al.[11] and Romero-Córdoba et al.[12] also reported prebiotic effects of *Gl* extracts studied in C57BL/6 mice fed with a high-cholesterol diet.

In biochemical parameters, we found hypocholesterolemic effects in mice groups consuming the *Gl*-1 extract (HFSD + *Gl*-1, HFSD + *Gl*-1 Tx), or the *Gl*-2 extract (HFSD + *Gl*-2, HFSD + *Gl*-2 Tx), as well as the group administered with metformin (HFSD + Met Tx), in comparison with the HFSD group. This confirmed our previous results showing that *Gl* extracts reduce cholesterol levels through the modulation of reverse cholesterol transport genes (Abcg5, abcg8, Ldl-r), decreased expression of lipogenic genes (Acc, Fas), and the inhibition of HMGCoAr.[11] These hypocholesterolemic properties of the medicinal mushroom *Gl* have been shown in different *in vivo* models and are attributed to terpenes and glucans.[23,24] Although these effects have also been reported in clinical trials,[13] further studies are needed to determine the effective dose and administration in other types of *Gl* products.[25,26]

The *Gl* extracts decreased significantly TG levels in most mice groups (HFSD + *Gl*-1 Tx, HFSD + *Gl*-2, HFSD + *Gl*-2 Tx, HFSD + Met Tx), as well as LDL-c levels in all treated groups (HFSD + *Gl*-1, HFSD + *Gl*-1 Tx, HFSD + *Gl*-2, HFSD + *Gl*-2 Tx, HFSD + Met Tx). Chu et al.[27] reported a reduction of TG and LDL-c levels in patients with dyslipidemia, who consumed 1.44 g of *Gl* extract per day for 12 weeks, this effect was associated with decreased insulin resistance. Hu et al.[28] described mechanisms of action of metformin for lowering lipids through the decrease of PCSK9 (proprotein convertase subtilisin/kexin type 9) levels, which leads to increased LDLr (low-density lipoprotein receptor) in the liver.

Glucose levels were significantly reduced in the Ctrl, HFSD + *Gl*-2, HFSD + *Gl*-2 Tx, and HFSD + Met Tx mice groups, compared with the HFSD group. Huang et al.[29] reported that mice having induced diabetes showed decreased plasma glucose levels after *Gl* consumption, along with the modulation of digestion and carbohydrate metabolism. It has also been reported that the hypoglycemic effect of *Gl* polysaccharides is related to the suppression of important enzymes in gluconeogenesis and/or glycogenolysis.[30,31] A proteoglycan of *Gl* is capable of inhibiting the activity of the protein tyrosine phosphatase 1B, a promising therapeutic target for diabetes, as well as ameliorating plasma glucose in db/db mice.[32] Our results suggest that mice treated with the *Gl*-2 extract have a beneficial effect on the regulation of blood glucose levels, in a similar way to metformin. Metformin increases AMP-activated protein kinase levels, initiating the translocation of the GLUT-4 transporter, which increases muscle glucose uptake.[33]

ALT and AST levels were lower in mice groups fed with *Gl* extracts, being evident in the HFSD + *Gl*-2 Tx group compared with the HFSD group. Susilo et al.[34] reported that the administration of *Gl* decreases ALT and AST levels. Pan et al.[35] also reported an improvement in ALT levels in mice fed with *Gl*, as compared to metformin. *Gl* has accordingly significant hepatoprotective activity, along with its preventive effect on lipid accumulation in the liver.

The liver histology showed that the HFSD mice group developed clear steatosis, while those groups consuming *Gl* extracts showed a much lower content of lipid vacuoles. This is in agreement with our previous research work, in which *Gl* decreased the content of liver cholesterol and TG, while hypercholesterolemic mice had accumulation of lipid vacuoles.[11]

The antioxidant activity in the plasma of mice showed that the Ctrl group was significantly different from the rest of the treated groups. *Gl* extracts increased the antioxidant capacity of all treated groups, regulating oxidative homeostasis through their bioactive compounds, including polyphenols, polysaccharides, vitamins, carotenoids, and minerals.[13,36] It has also been shown that *Gl* polysaccharides have significant antioxidant activity by scavenging free radicals.[37,38] In this study, *Gl* extracts also increased the expression of GPX1, CAT, SOD1, and SOD2 genes, along with their protein expression confirmed by Western blot analysis. *Cao et al.*[39] reported that the administration of *Gl* increased liver *N*-acetylcysteinamide and pyruvate, which are ROS sequestrants, as well as levels of betaine, which can improve antioxidant activity by upregulating SOD and CAT gene expression. This synergistic effect of SOD and CAT genes eliminates free radicals and H_2O_2.

Shi et al.[40] proposed that the antioxidant activity of *Gl* is due to the supply of hydrogen by polysaccharides, forming stable radicals when combined with free radicals, as well as improving the activity of CAT, GSH, and SOD genes. Wu[41] studied the higher expression of SOD and GPX1 genes in *Gl* treatments, also attributed to the antioxidant activity of polysaccharides. Li et al.[42] reported that the administration of *Gl* increases the expression of Nfr2 and its HO-1 gene (haemoxygenase-1). Nrf2 is an important regulator of redox homeostasis and antioxidant defense in cells, regulating the expression of antioxidants and phase II detoxification enzymes by antioxidant response elements.[43]

The differential effect of HFSD + *Gl*-1 and HFSD + *Gl*-2 on antioxidant enzymes CAT, GPx, and SOD, in comparison with HFSD + *Gl*-1 Tx and HFSD + *Gl*-2 Tx, can be explained by considering that such endogenous antioxidant enzymes are the first line of defense against oxidative stress.[44] The antioxidant properties of *Gl*-1 and *Gl*-2 extracts reduced the oxidative stress induced by the HFSD diet administered to mice groups since the beginning of the study, leading to lower expression of genes and proteins studied. By contrast, the HFSD diet administered during 13 weeks induced oxidative stress in HFSD + *Gl*-1 Tx and HFSD + *Gl*-2 Tx groups, so there was a higher expression of CAT, GPx, and SOD enzymes, while *Gl*-1 and *Gl*-2 extracts were consumed during 4 weeks only.

Differences recorded between *Gl*-1 and *Gl*-2 (ASA 10 mM) extracts are due to the addition of ASA to the

cultivation substrate. ASA is capable of accelerating the metabolic activity of *Gl*, affecting substrate biodegradation.[13] Thus, the ASA promotes the synthesis or production of polysaccharides, *β*-methyl glucopyranoside, DL-arabinitol, ribitol, and reactive species in *Gl*-2 extracts, compared to the *Gl*-1 extract (no ASA).[45] Biological effects of *Gl* have been reported as directly related to the concentration of polysaccharides.[34,40]

The results of the present study revealed that bioactive compounds of *Gl* extracts exert significant beneficial antioxidant activity in obese mice fed with an HFSD, through molecular mechanisms involving the expression of antioxidant genes and proteins (GPX1, CAT, SOD1, SOD2). Existing evidence shows that oxidative stress plays a crucial role in cardiac and vascular abnormalities in different types of cardiovascular diseases, so an antioxidant therapy using *Gl* extracts may prove beneficial for preventing and treating these problems of public health.

AUTHORS' CONTRIBUTIONS

Z.B.-M.: Data curation, formal analysis, investigation, validation, and writing—original draft. M.E.M.: Conceptualization, data curation, formal analysis, investigation, methodology, project administration, supervision, validation, writing—original draft, and writing—review and editing. D.M.-C.: Conceptualization, funding acquisition, investigation, methodology, project administration, resources, supervision, and writing—original draft. M.S.-T.: Data curation, formal analysis, investigation, and writing—review and editing. J.H.-A. and D.C.-H.: Investigation. N.T.: Methodology and resources. M.B., I.C., B.P., N.F., A.M., W.M., M.A., and M.S.: Resources. A.R.T.: Methodology and resources. A.P.-H.: Conceptualization, formal analysis, methodology, supervision, validation, and writing—review and editing.

AUTHOR DISCLOSURE STATEMENT

The authors have declared that no competing interests exist.

FUNDING INFORMATION

This research was supported by the National Council of Humanities, Sciences and Technologies (www.conahcyt.mx) in Mexico, through the Research Projects Cátedras 105 and FORDECYT-273647, directed by D.M.-C.

SUPPLEMENTARY MATERIAL

Supplementary Table S1
Supplementary Table S2

REFERENCES

1. NCD Risk Factor Collaboration (NCD-RisC). Worldwide trends in body-mass index, underweight, overweight, and obesity from 1975 to 2016: A pooled analysis of 2416 population-based measurement studies in 128.9 million children, adolescents, and adults. Lancet 2017;390(10113):2627–2642; doi: 10.1016/S0140-6736(17)32129-3
2. De Lorenzo A, Gratteri S, Gualtieri P, et al. Why primary obesity is a disease? J Transl Med 2019;17(1):169; doi: 10.1186/s12967-019-1919-y
3. Dubois-Deruy E, Peugnet V, Turkieh A, et al. Oxidative stress in cardiovascular diseases. Antioxidants (Basel) 2020;9(9):864; doi: 10.3390/antiox9090864
4. Herranz-López M, Barrajón-Catalán E, Segura-Carretero A, et al. Lemon verbena (*Lippia citriodora*) polyphenols alleviate obesity-related disturbances in hypertrophic adipocytes through AMPK-dependent mechanisms. Phytomedicine 2015;22(6):605–614; doi: 10.1016/j.phymed.2015.03.015
5. Liang Z, Yuan Z, Li G, et al. Hypolipidemic, antioxidant, and antiapoptotic effects of polysaccharides extracted from reishi mushroom, *Gl* (Leysser: Fr) Karst, in mice fed a high-fat diet. J Med Food 2018;21(12):1218–1227; doi: 10.1089/jmf.2018.4182
6. Smina TP, Mathew J, Janardhanan KK, et al. Antioxidant activity and toxicity profile of total triterpenes isolated from *Gl* (Fr.) P. Karst occurring in South India. Environ Toxicol Pharmacol 2011;32(3):438–446; doi: 10.1016/j.etap.2011.08.011
7. Chang CJ, Lin CS, Lu CC, et al. *Gl* reduces obesity in mice by modulating the composition of the gut microbiota. Nat Commun 2015;6:7489; doi: 10.1038/ncomms8489
8. Xu F, Li X, Li Q, et al. Effect of *Gl* polysaccharide on antioxidative ability of rats with myocardial injury induced by doxorubicin. J Biosci Med 2022;10(4):14–19; doi: 10.4236/jbm.2022.104002
9. Sang T, Guo C, Guo D, et al. Suppression of obesity and inflammation by polysaccharide from sporoderm-broken spore of *Gl* via gut microbiota regulation. Carbohydr Polym 2021;256:117594; doi: 10.1016/j.carbpol.2020.117594
10. Cör D, Knez Ž, Knez Hrnčič M. Antitumour, antimicrobial, antioxidant and antiacetylcholinesterase effect of Gl terpenoids and polysaccharides: A review. Molecules 2018;23(3):649; doi: 10.3390/molecules23030649
11. Meneses ME, Martínez-Carrera D, Torres N, et al. Hypocholesterolemic properties and prebiotic effects of Mexican *Gl* in C57BL/6 mice. PLoS One 2016;11(7):e0159631; doi: 10.1371/journal.pone.0159631
12. Romero-Córdoba SL, Salido-Guadarrama I, Meneses ME, et al. Mexican *Gl* extracts decrease lipogenesis modulating transcriptional metabolic networks and gut microbiota in C57BL/6 mice fed with a high-cholesterol diet. Nutrients 2020;13(1):38; doi: 10.3390/nu13010038
13. Martínez-Carrera D, Larqué-Saavedra A, Tovar Palacio A, et al. Contribución de los hongos comestibles, funcionales y medicinales a la construcción de un paradigma sobre la producción, la dieta, la salud y la cultura en el sistema agroalimentario de México. In: Ciencia, Tecnología e Innovación en el Sistema Agroalimentario de México (Martínez-Carrera D, Ramírez Juárez J, eds.). Editorial del Colegio de Postgraduados-AMC-CONACYT-UPAEP-IMINAP: San Luis Huexotla: Texcoco, México; 2016; pp. 581–640.
14. Meneses ME, Martínez-Carrera D, González-Ibáñez L, et al. Effects of Mexican *Gl* extracts on liver, kidney, and the gut microbiota of Wistar rats: A repeated dose oral toxicity study. PLoS One 2023;18(4):e0283605; doi: 10.1371/journal.pone.0283605
15. Abenavoli L, Greco M, Milic N, et al. Effect of Mediterranean diet and antioxidant formulation in non-alcoholic fatty liver

disease: A randomized study. Nutrients 2017;9(8):870; doi: 10.3390/nu9080870
16. Martínez-Carrera D, Pérez Armendáriz B, Mayett Y, et al. Propiedades funcionales agregadas al tequila, otros mezcales y destilados de Agave convencionales, derivadas del extracto de un hongo comestible de uso tradicional en México (*Lentinula boryana*). IMPI patent no. 322035, July 8, 2014, Mexico City; pp. 1–21.
17. Reeves PG. Components of the AIN-93 diets as improvements in the AIN-76A diet. J Nutr 1997;127(5 Suppl):838S–841S; doi: 10.1093/jn/127.5.838S
18. Bowling JL, Katayev A. An evaluation of the Roche Cobas c111. Lab Med 2010;41(7):398–402; doi: 10.1309/LM6T8D1LKQXVNCAC
19. Coutiño-Hernández D, Sánchez-Tapia M, Leal-Vega F, et al. Modulation of gut microbiota by Mantequilla and Melipona honeys decrease low-grade inflammation caused by high fructose corn syrup or saccharose in rats. Food Res Int 2022;151:110856; doi: 10.1016/j.foodres.2021.110856
20. Li Y, Li N, Yu X, et al. Hematoxylin and eosin staining of intact tissues via delipidation and ultrasound. Sci Rep 2018;8(1):12259; doi: 10.1038/s41598-018-30755-5
21. Boon-Bee Goh G, Qiang Leow W, Liang S, et al. Quantification of hepatic steatosis in chronic liver disease using novel automated method of second harmonic generation and two-photon excited fluorescence. Sci Rep 2019;9(1):2975; doi: 10.1038/s41598-019-39783-1
22. Domingues CC, Kundu N, Kropotova Y, et al. Antioxidant-upregulated mesenchymal stem cells reduce inflammation and improve fatty liver disease in diet-induced obesity. Stem Cell Res Ther 2019;10(1):280; doi: 10.1186/s13287-019-1393-8
23. Berger A, Rein D, Kratky E, et al. Cholesterol-lowering properties of *Gl in vitro*, ex vivo, and in hamsters and minipigs. Lipids Health Dis 2004;3:2; doi: 10.1186/1476-511X-3-2
24. Hajjaj H, Macé C, Roberts M, et al. Effect of 26-oxygenosterols from *Gl* and their activity as cholesterol synthesis inhibitors. Appl Environ Microbiol 2005;71(7):3653–3658; doi: 10.1128/AEM.71.7.3653-3658.2005
25. Chan SW, Tomlinson B, Chan P, et al. The beneficial effects of *Gl* on cardiovascular and metabolic disease risk. Pharm Biol 2021;59(1):1161–1171; doi: 10.1080/13880209.2021.1969413
26. Klupp NL, Kiat H, Bensoussan A, et al. A double-blind, randomised, placebo-controlled trial of Gl for the treatment of cardiovascular risk factors of metabolic syndrome. Sci Rep 2016;6:29540; doi: 10.1038/srep29540
27. Chu T, Benzie I, Lam C, et al. Study of potential cardioprotective effects of *Ganoderma lucidum* (Lingzhi): Results of a controlled human intervention trial. Br J Nutr 2012;107(7):1017–1027; doi: 10.1017/S0007114511003795
28. Hu D, Guo Y, Wu R, et al. New insight into metformin-induced cholesterol-lowering effect crosstalk between glucose and cholesterol homeostasis via ChREBP (carbohydrate-responsive element-binding protein)-mediated PCSK9 (proprotein convertase subtilisin/kexin type 9) regulation. Arterioscler Thromb Vasc Biol 2021;41(4):e208–e223; doi: 10.1161/ATVBAHA.120.315708
29. Huang CH, Lin WK, Chang S, et al. Evaluation of the hypoglycaemic and antioxidant effects of submerged *Gl* cultures in type 2 diabetic rats. Mycology 2020;12(2):82–93; doi: 10.1080/21501203.2020.1733119
30. Xiao C, Wu QP, Cai W, et al. Hypoglycemic effects of *Gl* polysaccharides in type 2 diabetic mice. Arch Pharm Res 2012;35(10):1793–1801; doi: 10.1007/s12272-012-1012-z
31. Wang F, Zhou Z, Ren X, et al. Effect of *Gl* spores intervention on glucose and lipid metabolism gene expression profiles in type 2 diabetic rats. Lipids Health Dis 2015;14:49; doi: 10.1186/s12944-015-0045-y
32. Wang CD, Teng BS, He YM, et al. Effect of a novel proteoglycan PTP1B inhibitor from Gl on the amelioration of hyperglycaemia and dyslipidaemia in db/db mice. Br J Nutr 2012;108(11):2014–2025; doi: 10.1017/S0007114512000153
33. Jung OL, Soo KL, Ji HK, et al. Metformin regulates glucose transporter 4 (GLUT4) translocation through AMP-activated protein kinase (AMPK)-mediated Cbl/CAP signaling in 3T3-L1 preadipocyte cells. J Biol Chem 2012;287(53):44121–44129; doi: 10.1074/jbc.M112.361386
34. Susilo RJK, Winarni D, Husen SA, et al. Hepatoprotective effect of crude polysaccharides extracted from *Gl* against carbon tetrachloride-induced liver injury in mice. Vet World 2019;12(12):1987–1991; doi: 10.14202/vetworld.2019.1987-1991
35. Pan R, Lou J, Wei L. Significant effects of *Gl* polysaccharide on lipid metabolism in diabetes may be associated with the activation of the FAM3C-HSF1-CAM signaling pathway. Exp Ther Med 2021;22(2):820–830; doi: 10.3892/etm.2021.10252
36. Kozarski M, Klaus A, Jakovljevic D, et al. Antioxidants of edible mushrooms. Molecules 2015;20(10):19489–19525; doi: 10.3390/molecules201019489
37. Liu W, Wang H, Pang X, et al. Characterization and antioxidant activity of two low-molecular-weight polysaccharides purified from the fruiting bodies of *Gl*. Int J Biol Macromol 2010;46(4):451–457; doi: 10.1016/j.ijbiomac.2010.02.006
38. Hapuarachchi K, Wen T, Jeewon R, et al. *Ganoderma lucidum*—Are the beneficial medical properties substantiated? Mycosphere Essays 2016;7(6):687–715; doi: 10.5943/mycosphere/7/6/1
39. Cao YJ, Huang ZR, You SZ, et al. The protective effects of ganoderic acids from *Gl* fruiting body on alcoholic liver injury and intestinal microflora disturbance in mice with excessive alcohol intake. Foods 2022;11(7):949; doi: 10.3390/foods11070949
40. Shi M, Zhang Z, Yang Y. Antioxidant and immunoregulatory activity of *Gl* polysaccharide (GLP). Carbohydr Polym 2013;95(1):200–206; doi: 10.1016/j.carbpol.2013.02.081
41. Wu S. Hypolipidaemic and anti-lipidperoxidant activities of *Gl* polysaccharide. Int J Biol Macromol 2018;118(Pt B):2001–2005; doi: 10.1016/j.ijbiomac.2018.07.082
42. Li HN, Zhao LL, Zhou DY, et al. *Gl* polysaccharides ameliorates hepatic steatosis and oxidative stress in db/db mice via targeting nuclear factor E2 (erythroid-derived 2)-related factor-2/heme oxygenase-1 (HO-1) pathway. Med Sci Monit 2020;26(26):e921905; doi: 10.12659/MSM.921905
43. He F, Ru X, Wen T. NRF2, a transcription factor for stress response and beyond. Int J Mol Sci 2020;21(13):4777; doi: 10.3390/ijms21134777
44. Prior RL, Wu X. Diet antioxidant capacity: Relationships to oxidative stress and health. Am J Biomed Sci 2013;5(2):126–139; doi: 10.5099/aj130200126
45. Castañeda-Antonio MD, Martínez-Carrera DC, Rivera-Tapia JA, et al. Detection of polysaccharides in *Gl* extracts. Nova Sci 2019;10(21):247–257; doi: 10.21640/ns.v10i21.1538