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Ganoderma Lucidum 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

Authors' Contribution:
Study Design A
Data Collection B
Statistical Analysis C
Data Interpretation D
Manuscript Preparation E
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Background: Type 2 diabetes mellitus (T2DM) and its comorbidities, including obesity, hypertension, and hyperlipidemia, are commonly associated with non-alcoholic fatty liver disease (NAFLD). Ganoderma lucidum polysaccharide (GDLP) is one of the central bioactive components in *Ganoderma lucidum* with anti-inflammatory, antioxidant, and hepatoprotective properties. However, the effect and mechanisms of GDLP in hepatic steatosis remain largely unknown. In the present study, we aimed to investigate the function of GDLP in hepatic steatosis and the underlying mechanism.

Material/Methods: In this study, male *db/db* mice were received with a high-fat diet (HFD) to investigate the effect of GDLP in T2DM-induced hepatic steatosis. The biological characteristics of the hepatic steatosis were evaluated through the detection of clinical indicators, including biochemical parameters, histopathology, and related cytokine levels. Additionally, the protein expression levels of Nrf2 (nuclear factor E2 (erythroid-derived 2)-related factor-2) signaling pathway were investigated by using western blotting and immunohistochemical staining.

Results: The levels of food/water intake, body weight, fasting blood glucose, plasma lipids, urinary biomarkers, hepatic lipid accumulation, and tumor necrosis factor (TNF)-α were observably decreased in GDLP-treated *db/db* mice. Additionally, administration of GDLP increased the expression of various antioxidases, including superoxide dismutase (SOD), catalase (CAT), and glutathione peroxidase (GSH-Px), whereas it reduced the level of malonaldehyde (MDA). Furthermore, GDLP was significantly promoted protein expression level of Nrf2 and its downstream target gene HO-1 (heme oxygenase-1) while decreased TNF-α expression.

Conclusions: These results indicate that GDLP against T2DM-induced hepatic steatosis, oxidative stress, and inflammation by improving the Nrf2/HO-1 signaling pathway in *db/db* mice, suggesting the GDLP may serve as an effective strategy for in fatty liver treatment.

MeSH Keywords: Diabetes Mellitus, Type 2 • Fatty Liver • Reishi

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Background

Type 2 diabetes mellitus (T2DM), a chronic endocrine, metabolic disorder, is characterized by continuous hyperglycemia resulting from beta-cell dysfunction and impairment of insulin secretion [1]. The global diabetes prevalence in adults will be increased from 6.4% (285 million in 2010) to 7.7% (439 million in 2030) in 20 years with a rising health burden, particularly in developing countries [2]. Non-alcoholic fatty liver disease (NAFLD) is the most common chronic liver disease, especially in western world, histologically arranging from simplistic hepatic steatosis to serious non-alcoholic steatohepatitis (NASH) [3]. It has been estimated that the prevalence of NAFLD in T2DM patients is as high as 60% [4]. Diabetic comorbidities, including obesity, hypertension, hyperlipidemia, and metabolic syndrome, are also commonly associated with the progression of NAFLD [5].

Hepatic fat accumulation in NAFLD results in the overproduction of reactive oxygen species (ROS) and inflammatory cytokines, which can amplify liver damage, fibrosis, and hepatic lipid peroxidation [6]. Tumor necrosis factor-alpha (TNF-α) is a proinflammatory cytokine that plays an essential role in mediating inflammatory responses and oxidative stress in liver cells [7]. Significantly, Hui et al., reported that a higher TNF-α level had been observed in patients with NAFLD [8]. Nuclear factor E2 (erythroid-derived 2)-related factor-2 (Nrf2) is a leucine zipper transcription factor that exerts a protective effect against oxidative challenge [9]. Activated Nrf2 escapes from Keap1-mediated sequestration and translocates to the nucleus, activating the transcription of antioxidant genes, such as glutathione peroxidase (GSH-Px), heme oxygenase-1 (HO-1), and superoxide dismutase (SOD) [10]. A recent study also reported that augmented Nrf2 activity in the nucleus reduced oxidative stress and improved hepatic steatosis of an animal model [11].

GDLP is one of the major bioactive components in *Ganoderma lucidum* (*G. lucidum*) with multiple health-promoting effects, including antioxidation, anti-inflammation, anti-angiogenesis, and hepatoprotection [12]. A randomized and double-blind crossover trial with placebo-controlled showed that the consumption of GDLP substantially increased the total antioxidant capacity, antioxidase activity, and improved hepatic condition in healthy study participants [13]. Zhong et al. presented that GLPP alleviated hepatic steatosis of liver tissues in the *ob/ob* and ApoC3 mouse model via the regulation of bile acid synthesis and metabolism of fatty acid [14]. However, it remains unrevealed whether GDLP might affect the inflammatory and oxidative pathways in T2DM-induced hepatic steatosis.

In the current study, *db/db* mice were selected as a spontaneous T2DM model to assess the effect of GDLP on lipid accumulation, oxidative stress, inflammation, and mechanism in hepatic steatosis development and progression.

Material and Methods

Animals

Six-week-old C57BL/KsJ-*db/db* male mice (n=24) and age-matched wild-type C57BL6/J male mice (n=6) were purchased from Charles River Laboratories (Beijing Vital River Laboratory Animal Technology Co., Ltd., China; license number: SCXK (Beijing) 2017-0033). Animals were housed in standard pathogen-free surroundings with a 12-hour light-dark cycle, air temperature 20°C to 25°C, humidity 40% to 70% and with free access to food and water. All studies were approved by the Animal Care and Welfare Committee of Zhejiang Chinese Medical University (permit number: ZSLL-2018-36, Zhejiang, China), and performed following the Guide for the Care and Use of Laboratory Animals [15]. Male db/db mice were fed with a basic diet for 14 days to adapt to the laboratory condition and then fed on a high-fat diet (HFD, 60% of calories derived from fat, Research Diets, New Brunswick, NJ, USA) to induce hepatic steatosis. Male age-matched wild-type mice were maintained on a regular chow and were used as non-diabetic control mice. Meanwhile, the fasting blood glucose (FBG) concentration of db/db mice was measured from a tail vein blood sample using a OneTouch® Ultra blood glucose mete (LifeScan, Inc., USA). The establishment of the mice T2DM model was confirmed with the fasting blood glucose (FBG) level higher than 11.1 mmol/L.

GDLP administration

The purity of GDLP was above 95%, which purchased from Johncan International (Hangzhou, China). In this study, GDLP was dissolved in 0.5% sodium carboxyl methylcellulose (CMC-Na) for animal treatment. Metformin (Sigma-Aldrich, St. Louis, MI, USA) was dissolved in the drinking water. Male *db/db* mice were randomly divided into 4 groups (n=6 in each group) and received intragastric administration of different reagents once a day at 8: 30 am for 8 weeks. The 4 groups were: 1) diabetic mice group; 2) GDLP 100 mg/kg group was given GDLP at a dosage of 100 mg/kg/day; 3) GDLP 400 mg/kg mice were administered with GDLP at a dosage of 400 mg/kg/day; and 4) Met group received intragastric administration of metformin at the concentration of 300 mg/kg/day. The wild-type group (control) and diabetic mice group received the same volume of CMC-Na solution. The doses were adjusted weekly based on the body weight measurement. The body weight, food intake, water intake, and urine volume in each group were measured weekly.

Measurements of metabolic parameters

After 12-hour fasting, body weight and metabolic parameters were measured. At the end of the experiment, the whole blood

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from the tail vein was used for the analysis of hemoglobin A1c (HbAlc) level using the HbA1c analyzer (Roche Diagnostics, Basel, Switzerland). After that, mice were injected intraperitoneally with 1% pentobarbital sodium (50 mg/kg) anesthesia. Then, blood samples were collected and centrifuged at 3500 rpm for 15 minutes to acquire serum for further biochemical analyses. The levels of FBG, aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol (TC), triglyceride (TG), low-density lipoprotein cholesterol (LDL-C), blood urea nitrogen (BUN), high-density lipoprotein cholesterol (HDL-C), and serum creatinine (Scr) were analyzed using a 7020 full-automatic biochemical analyzer (Hitachi, Tokyo, Japan) according to the manufacturer's instructions. The levels of urinary creatinine (Ucr), uric acid (UA), and urine microalbumin (U-ALB) in the urine samples were detected with the diagnostic kits (Jiangcheng, China).

Enzyme-linked immunosorbent assay (ELISA)

Serum levels of superoxide dismutase (SOD), malonaldehyde (MDA), glutathione peroxidase (GSH-Px), catalase (CAT), TNF-α were detected by using enzyme-linked immunosorbent assay (ELISA) kits (MyBioSource Inc., San Diego, CA, USA) according to manufacturer's instructions. The absorbance at 450 nm was recorded by using a Power Wave Microplate Reader (Bio-TEK, USA).

Histopathological analyses and immunohistochemical staining

Mice were euthanized with carbon dioxide asphyxiation, and then liver tissues were removed immediately and washed with normal cold saline. The same portion of the liver samples in all mice was used for the histopathological examination. The rest fresh tissue samples were stored at –80°C for protein analysis. Livers were fixed overnight in a buffer solution containing 4% paraformaldehyde and then embedded in paraffin. The paraffin-embedded tissues were cut into 4 μm thickness sections and then stained with hematoxylin and eosin (H&E) or Oil Red O to assess hepatic steatosis. Additionally, to detect the protein expression of TNF-α and Nrf2 in liver tissues, immunohistochemistry (IHC) was applied. The primary antibodies anti-TNF-α (1: 100, #ab6671, Abcam, Cambridge, UK) and anti-Nrf2 (1: 200, #ab89443, Abcam), and IgG HRP-conjugated secondary antibodies, including goat anti-mouse (1: 100, HA1006) and goat anti-rabbit antibodies (1: 1000, HA1001, HuaBio, HangZhou, China) were applied for immunohistochemical staining. The preparations were photographed under an inverted microscope (Leica, Germany). The mean densitometry was quantitatively measured under a 400× magnification microscope for 6 microscopic fields randomly chosen by using Image-Pro Plus version 6.0 software (Media Cybernetics, Inc., Rockville, MD, USA), and results were displayed as average.

Western blot

Liver tissues were homogenized in RIPA buffer (Beyotime, China) with protease inhibitor cocktail (Bio-Rad Laboratories, Hercules, CA, USA) and quantified using bicinchoninic acid assay (Pierce, Rockford, IL, USA). Then the equivalent amount of protein samples was separated on a 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE), then transferred onto polyvinylidene fluoride (PVDF) membrane (Roche, Mannheim, Germany). The PVDF membranes were incubated with the following primary antibodies against TNF-α (1: 1000), Nrf2 (1: 1000), HO-1 (1: 2000, #ab13243, Abcam) and β-catenin (1: 2000, #ab32572, Abcam) at 4°C for 12 hours. After 3 washes with Tris-buffered saline with 0.1% Tween-20 (TBST) buffer, membranes were incubated with the goat anti-rabbit secondary antibody (1: 2000, #ab6721, Abcam) at room temperature for another 1 hour. Protein bands were photographed with Alphalmager™ 2000 Imaging System (Alpha Innotech, San Leandro, CA, USA), and the fluorescence intensity of bands was quantified by ImageJ software (v1.8.0, National Institutes of Health, USA).

Statistical analysis

All the data are presented as mean±standard deviation (SD). One-way ANOVA was analyzed by software SPSS (version 24.0, SPSS, Inc., Chicago, IL, USA), and $P<0.05$ was considered statistically significant.

Results

Effect of GDLP on energy expenditure, body weight, FBG, and HbA1c in *db/db* mice

Adult male *db/db* mice (n=6 in each group) were fed on HFD alone, HFD with GDLP (100 or 400 mg/kg/day) or HFD with metformin (Met, 300 mg/kg/day) for 8 weeks, whereas age-matched wild-type mice (n=6) were maintained on regular chow. Metformin is an oral anti-hyperglycemic agent that has been used successfully as a first-line treatment for T2DM patients [16,17]. It lowers the blood glucose level by inhibiting glucose production in the liver [18]. In this study, the food intake, water intake, and urine volume were significantly increased in the *db/db* mice compared with the wild-type group. Supplementing the HFD with GDLP at both doses and metformin significantly decreased the food intake, water intake, and urine volume in T2DM *db/db* mice (Figure 1A–1C). Compared to wild-type counterparts, *db/db* mice exhibited significantly elevated body weight, FBG level, and HbA1c concentration, which was suppressed by the treatment of GDLP and metformin (Figure 1D–1F).

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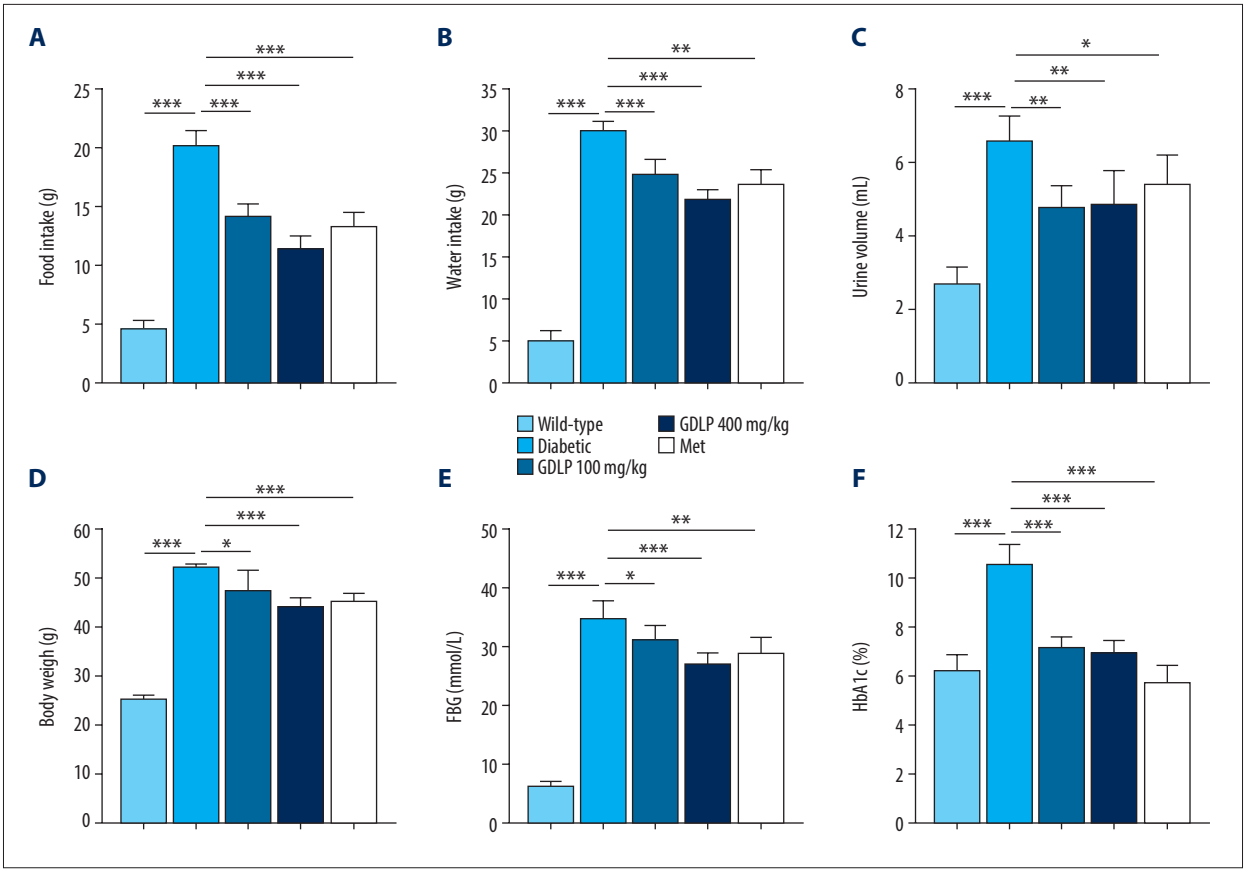

Figure 1. Effect of GDLP on energy expenditure, body weight, fasting blood glucose level and HbA1c concentration. Male *db/db* mice were fed on an HFD alone (Diabetic ctrl), or with GDLP (100 or 400 mg/kg/day) or with metformin (Met, 300 mg/kg/day) for 8 weeks. Wild-type mice on a regular chow were used as non-diabetic control (NC). (**A–D**) The metabolic parameters, including food intake, water intake, and urine volume, together with their body weight, were shown at the end of the experiment. (**E**) Blood samples were drawn from the eyeballs removed for the analysis of biochemical parameters. (**F**) Blood collected from the tail vein was used for the evaluation of HbA1c concentration. * $P<0.05$, ** $P<0.01$, *** $P<0.001$. GDLP – Ganoderma lucidum polysaccharide; HbA1c – hemoglobin A1c; HFD – high-fat diet.

Effect of GDLP on plasma lipids and urinary biomarkers in *db/db* mice

To evaluate the impact of GDLP on the regulation of lipid metabolism and urinary excretion, we first measured the lipid levels in plasma samples. Compared to wild-type mice, the concentrations of ALT, AST, TC, TG, LDL-C, Scr, and BUN were significantly upregulated in *db/db* mice. With the intervention of GDLP and metformin, the aberrant lipid metabolism was efficiently inhibited in the T2DM mouse model. The level of HDL-C, oppositely, was significantly decreased in *db/db* mice but recovered with the supplementation of metformin and GDLP at both doses (Figure 2A–2H). The levels of the urinary biomarkers measured in this study, Ucr, UA, and U-ALB, were significantly higher in *db/db* mice compared to those in the wild-type group. GDLP and metformin treatment sufficiently impeded the elevation of these urinary parameters (Figure 2I–2K).

Effect of GDLP on the accumulation of lipid droplets in *db/db* mice

Investigating the impact of GDLP on hepatic morphology, histological analysis on liver sections was performed using H&E and Oil Red O staining. Hepatocytes in wild-type mice were fat, large, and tightly bound together. Altered hepatic morphology was observed in T2DM mice, which showed enlarged hepatocytes and an increased number of lipid vacuoles. The administration of low and high doses of GDLP partially prevented the accumulation of lipids in the liver. Metformin intervention also reduced the number of lipid vacuoles (Figure 3). It suggested that GDLP reduced the level of circulating lipids and the accumulation of fat in the liver tissues.

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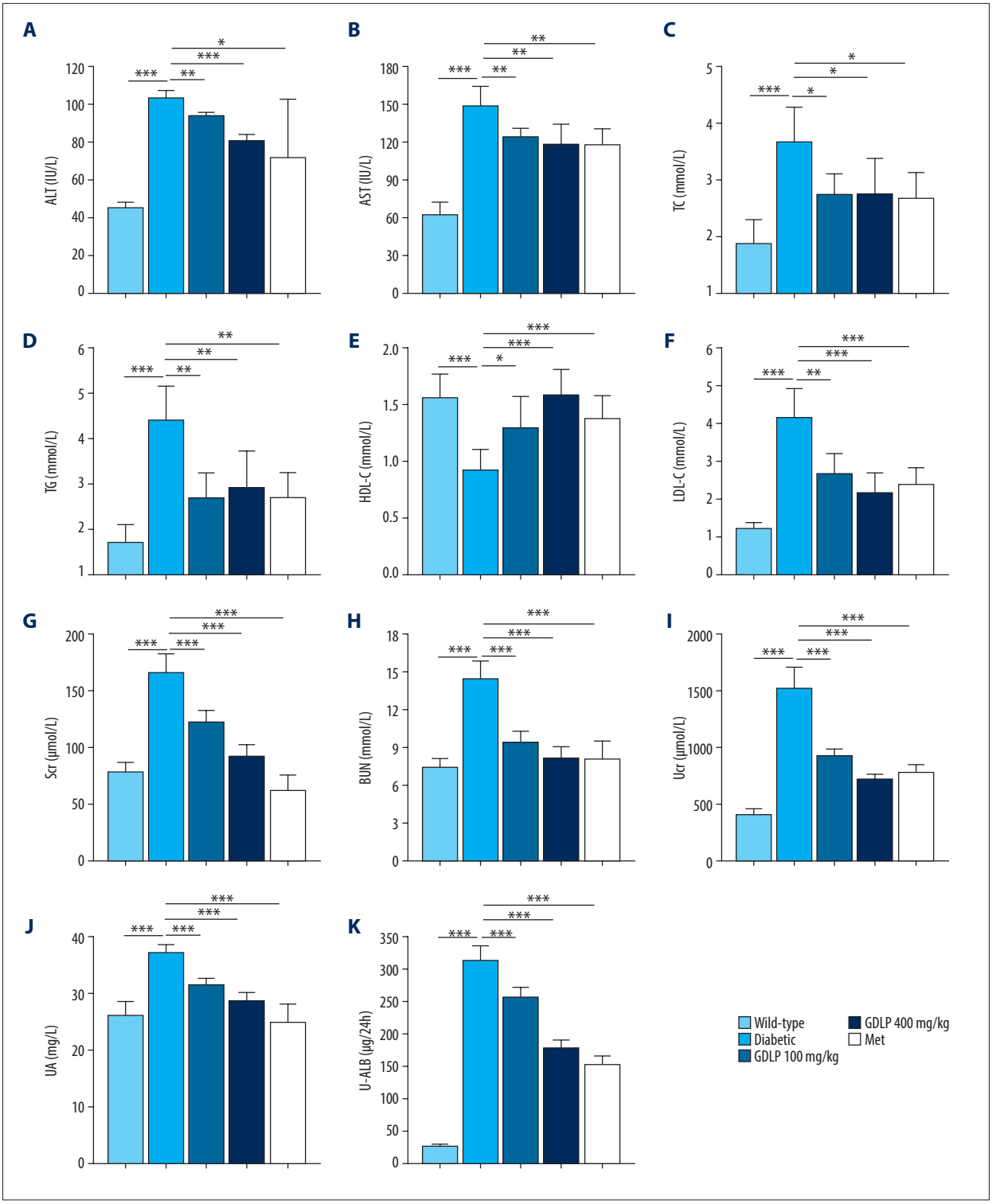

Figure 2. GDLP regulates the levels of plasma lipids and urinary parameters. Eight weeks after the intervention, blood samples were collected from the abdominal aorta in all mice. (**A–H**) The levels of ALT, AST, TC, TG, HDL-C, LDL-C, Scr, and BUN were measured using an automatic biochemical analyzer. (**I–K**) The urinary excretion of UA, Ucr, and U-ALB were detected in all groups of mice. * $P<0.05$, ** $P<0.01$, *** $P<0.001$. ALT – alanine aminotransferase; AST – aspartate aminotransferase; TC – total cholesterol; TG – triglyceride; HDL-C – high-density lipoprotein cholesterol; LDL-C – low-density lipoprotein cholesterol; Scr – serum creatinine; BUN – blood urea nitrogen; UA – uric acid; Ucr – urinary creatinine; U-ALB – urine microalbumin.

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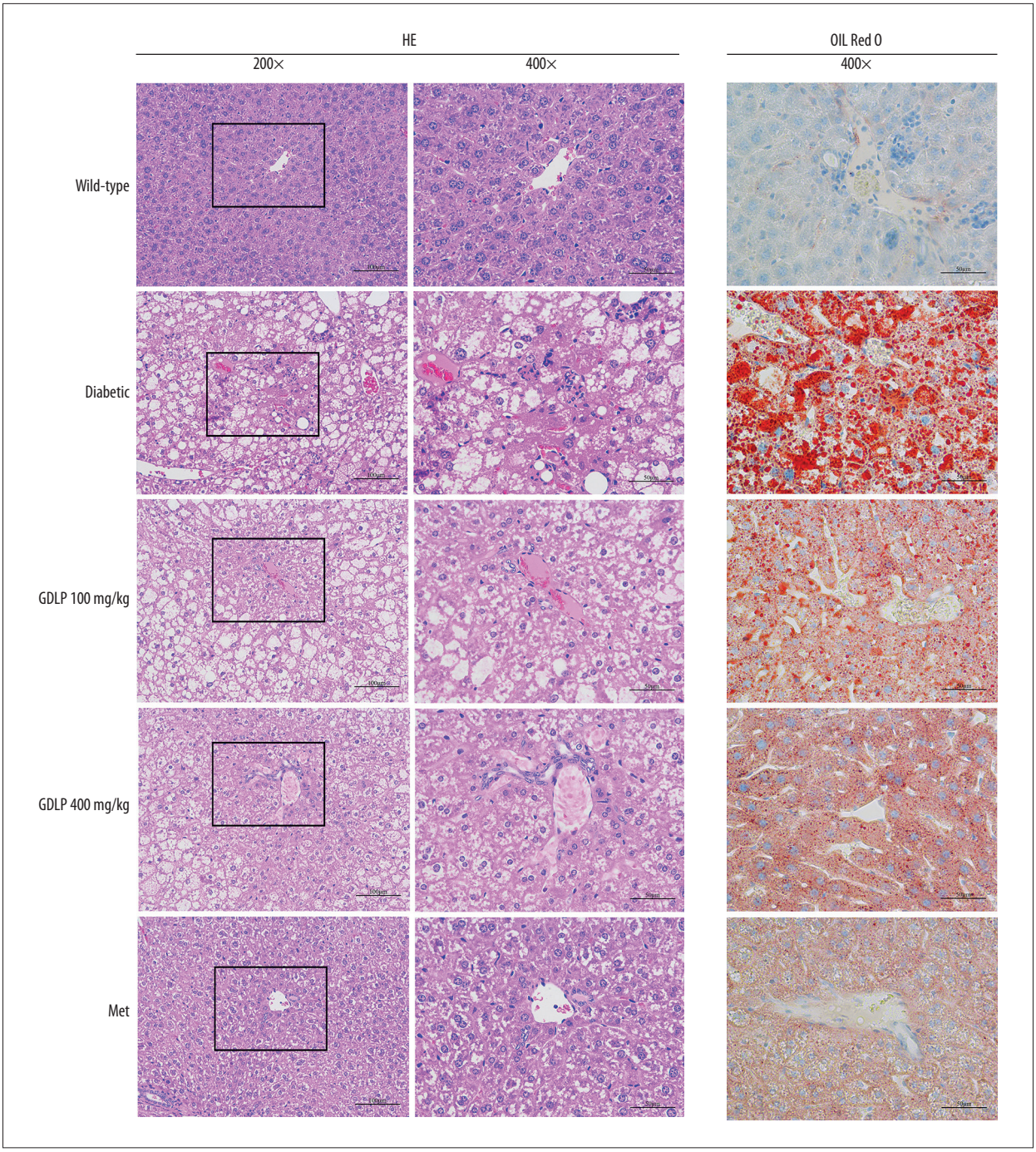

Figure 3. Effect of GDLP on hepatic morphology in *db/db* mice. Paraffin-embedded liver tissues were cut into sections (4 μm) and stained with H&E (200× and 400×) or Oil Red O (400×) to show the accumulation of hepatic lipid droplet. GDLP – Ganoderma lucidum polysaccharide; H&E – hematoxylin and eosin.

Effect of GDLP on oxidative stress in *db/db* mice

We further explored the effect of GPE on the antioxidant system. MDA is a product of polyunsaturated fatty acids peroxidation in cells and has been used as a reliable marker of oxidative stress [19,20]. By measuring the plasma level of MDA, we found that *db/db* mice suffered from significantly severer oxidative stress compared to wild-type group. The metformin and GDLP treatment were remarkably suppressed the upregulation of MDA in the T2DM model (Figure 4A). We also examined the expressions of several antioxidases, including SOD, CAT, and GSH-Px [21]. T2DM *db/db* mice showed significantly lower

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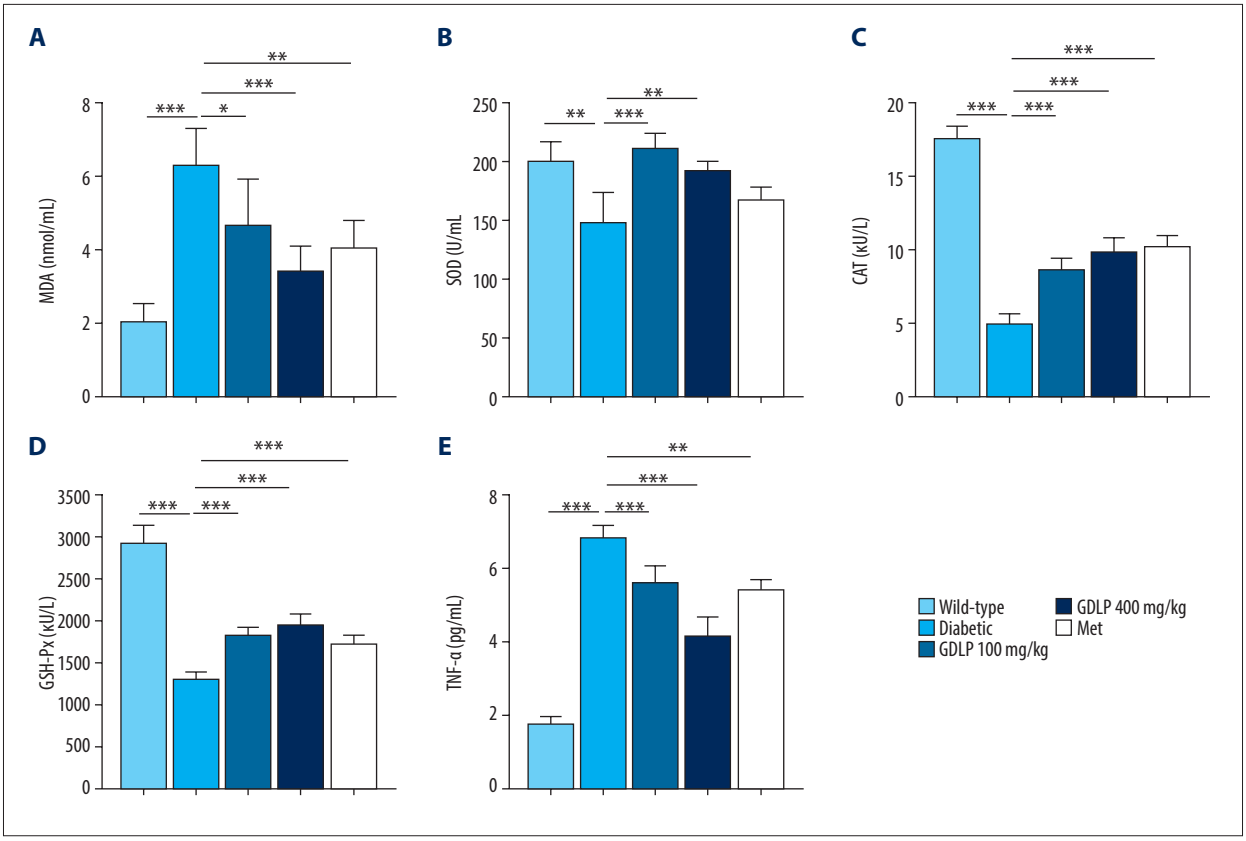

Figure 4. Effect of GDLP on oxidative stress and the expression levels of pro-inflammatory cytokine. Serum levels of (**A**) MDA, (**B**) SOD, (**C**) CAT, (**D**) GSH-Px, and (**E**) TNF-α were detected using ELISA kits. * $P<0.05$, ** $P<0.01$, *** $P<0.001$. GDLP – Ganoderma lucidum polysaccharide; MDA – malonaldehyde; SOD – superoxide dismutase; CAT – catalase; GSH-Px – glutathione peroxidase; TNF – tumor necrosis factor; ELISA – enzyme-linked immunosorbent assay.

expressions of antioxidant enzymes compared to controls, while the downregulation of antioxidases was recovered by GDLP and metformin interventions (Figure 4B–4D). Aberrant expression of pro-inflammatory cytokines is also significantly involved in T2DM-induced liver damage [22]. In the current study, *db/db* mice showed significantly promoted the plasma level of TNF-α compared to wild-type group. The administration of metformin and GDLP at both doses significantly suppressed TNF-α level in *db/db* mice (Figure 4E). These results indicated the antioxidative and anti-inflammatory effects of GDLP in the T2DM model.

Effect of GDLP on pro-inflammatory cytokine and Nrf2/HO-1 signaling pathway

To further investigate the underlying mechanism of GDLP, preventing the development of T2DM-induced liver tissue injury, we first examined the protein level of TNF-α in the liver. We observed that a high dose of GDLP at 400 mg/kg/day and metformin significantly decreased T2DM-induced TNF-α expression, whereas the low dose of GDLP at 100 mg/kg/day showed no significant effect (Figure 5A, 5B). Recent studies have revealed that the activation of the Nrf2/HO-1 signaling pathway could improve NASH via ameliorating oxidative stress [23]. Next, we also have assessed the protein expression levels of Nrf2 and HO-1 in liver samples. Compared to wild-type mice, the protein levels of Nrf2 and HO-1 were dramatically inhibited in the *db/db* group. With the intervention of a high dose of GDLP and metformin, the protein expression level of Nrf2 and HO-1 were significantly increased in *db/db* mice, whereas the low dose of GDLP had no significant regulatory impact on this pathway (Figure 5C, 5D). The results of the immunohistochemical analysis in liver tissues also point out that GDLP at high concentration observably decreased the protein expression levels of TNF-α levels (Figure 5E, 5F) and promoted the expression level of Nrf2 (Figure 5E, 5G). Taken together, a high dose of GDLP exhibited anti-inflammatory property and attenuated oxidative stress by activating the Nrf2/HO-1 pathway in the liver tissue of T2DM mice.

Discussion

With the increasing prevalence of chronic metabolic disorders, hepatic steatosis as the first step in NAFLD progression has

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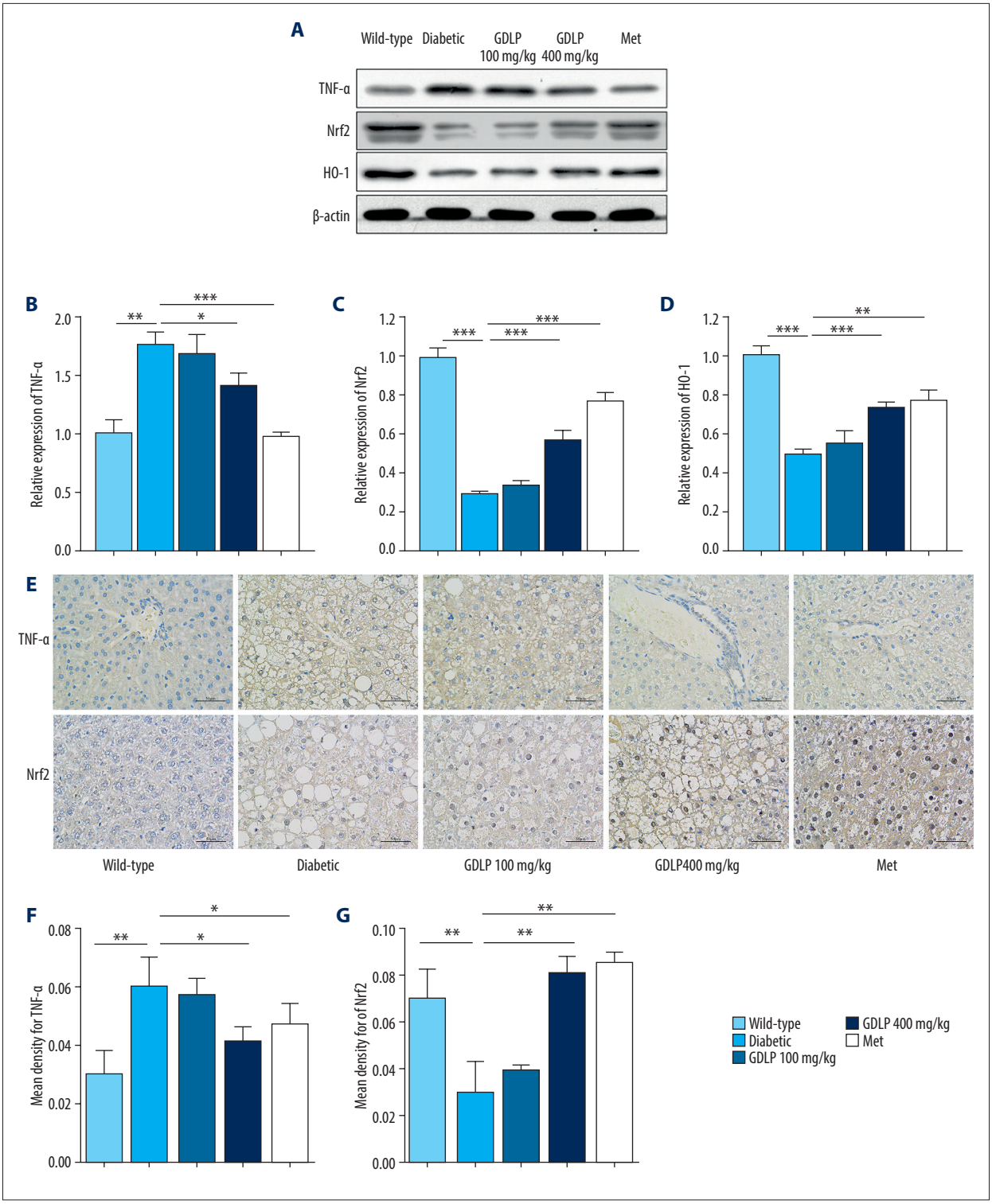

Figure 5. GDLP regulates the Nrf2/HO-1 pathway. Protein expressions of TNF-α, Nrf2, and HO-1 in liver tissues were analyzed using western blot. (**A**) Representative western blot images show the targeted bands of TNF-α, Nrf2, and HO-1, and the relative quantitation of band intensity is summarized (**B–D**). (**E**) Representative images of immunohistochemical staining of TNF-α and Nrf2 in liver tissues, and semi-quantitative analysis of immunohistochemical staining of TNF-α and Nrf2 (**F, G**). Magnification, 400×. * $P<0.05$, ** $P<0.01$, *** $P<0.001$. GDLP – Ganoderma lucidum polysaccharide; Nrf2 – nuclear factor E2 (erythroid-derived 2)-related factor-2; HO-1 – heme oxygenase-1; TNF – tumor necrosis factor; IOD – integral optical density.

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reached epidemic proportions [24]. Besides lifestyle modifications, the availability of pharmacotherapies for NAFLD patients with diabetes is limited to PPARγ agonists and other antidiabetic drugs [25]. Thus, novel therapeutic options targeting steatosis and inflammation are urgently needed. In the current study, we observed that GDLP ameliorated hepatic steatosis, dyslipidemia, oxidative stress status, and inflammatory response in the *db/db* mice with T2DM. The protective effect of GDLP against lipid accumulation in the liver involves the regulation of the Nrf2/HO-1 pathway. The *db/db* mouse harbors a mutation on the gene for the leptin receptor, resulting in a diabetic phenotype with hyperglycemia, increased ROS, and imbalanced energy expenditure [26]. Diet-stimulated hepatic fatty acid oxidation and necroinflammation have been reported in this animal model [27]. In the current study, C57BL/KsJ-*db/db* mice fed an HFD for 8 weeks showed significantly higher levels of food/water intake, urine volume, body weight, and blood glucose compared to wild-type mice placed on regular chow. *Db/db* mice also showed significantly elevated plasma levels of lipids, augmented lipid accumulation in the liver, stimulated oxidative stress, and the production of TNF-α, indicating the development of NAFLD.

G. lucidum is a medicinal polypore mushroom widely used in traditional Chinese medicine with various pharmacological properties [12]. Xiao et al. found that 1-week treatment of polysaccharides in *G. lucidum* significantly attenuated hyperglycemia and the weight gain of adipose tissue in diabetic mice [28]. Consistently, we found that GDLP at both 100 and 400 mg/kg/day suppressed T2DM-induced body weight gain and the upregulation of blood glucose level in *db/db* mice. The food/water intake and urine volume were also reduced by GDLP intervention at both doses. Another study in the *db/db* mouse model demonstrated that a proteoglycan extracted from *G. lucidum* with 78% glucose in polysaccharide controlled T2DM-accompanied hyperlipidemia in serum [29]. In this study, we showed that the plasma concentrations of ALT, AST, TC, TG, LDL-C, Scr, and BUN, and urinary markers were significantly downregulated in *db/db* mice supplemented with GDLP at both doses, whereas the level of HDL-C was restored considerably by GDLP at a dose-dependent manner. Emerging evidence has shown the hepatoprotective capacity of GDLP in animal models. Sprague-Dawley rats administered with GDLP exhibited reduced hepatocellular steatosis and enhanced activities of antioxidant enzymes, including CAT and SOD in ethanol-induced acute liver injury [30]. In mice with carbon tetrachloride-induced acute liver injury, GDLP remarkably decreased the release of proinflammatory cytokines in plasma, impeded necrosis, and vacuole formation in the liver [31]. Our histological examination in liver tissues showed that GDLP suppressed the accumulation of lipid droplets in *db/db* mice. The plasma levels of antioxidases SOD, CAT, and GSH-Px, and proinflammatory cytokine TNF-α in diabetic mice were also efficiently decreased by GDLP at both doses. A recent study in the *ob/ob* and ApoC3 mouse model showed that GLPP exerted a therapeutic effect on NAFLD via the regulation of fatty acid metabolism and bile acid synthesis [14]. However, little was known on whether GDLP alleviated the progression of NAFLD through the management of oxidative or inflammatory pathways.

Nrf2 is a master regulator against tissue oxidative stress via the upregulation of antioxidant genes [32]. The knockout of Nrf2 in mice led to more rapid steatohepatitis development compared to wild-type ones placed on HFD [33]. Reduced production and activation of Nrf2 were found in the liver with nonalcoholic steatohepatitis [34]. Overexpression of Nrf2 in mice, on the contrary, showed less lipid peroxidation and downregulated transcription of lipogenic enzymes in the liver [35]. Besides, the protein analyses from western blot and immunohistochemistry demonstrated that GDLP significantly promoted the hepatic protein expression of Nrf2 and its downstream target gene HO-1, indicating the activation of Nrf2/HO-1 signaling after GDLP-administered in *db/db* mice. In addition, there are not existing adverse events of GDLP across the study groups in this study, indicating that it has certain security in treatment of hepatic steatosis. Therefore, further experiments are still needed. Generally, our data showed that GDLP ameliorates oxidative stress on T2DM-induced hepatic steatosis by activating the Nrf2/HO-1 signaling pathway, suggesting that Nrf2 may serve as a potential therapeutic target for NAFLD treatment.

Conclusions

In summary, the study reported that GDLP supplementation has a hepatoprotective effect under T2DM-induced hepatic steatosis, oxidative stress, and inflammation through the activation of the Nrf2/HO-1 signaling pathway in *db/db* mice, suggesting that the GDLP therapy may become an ideal avenue for patients with hepatic steatosis under T2DM.

Availability of data and materials

The analyzed data sets generated during the present study are available from the corresponding author on reasonable request.

Conflict of interests

None.

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