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An assessment of the potential of defatted walnut powder extract against hyperlipidemia-intensified L-arginine-induced acute pancreatitis

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Abstract

Walnut kernel, the mature seed of *Juglans regia* L. recorded in the 2020 edition of the Chinese Pharmacopoeia, is a functional food riched in nutrients. Defatted walnut powder extract (DWPE), the residues of walnut kernel extracted for oil, has the effect on hypertriglyceridemia. However, the extract role and mechanism of it in hyperlipidemic acute pancreatitis research have not been elucidated. Thus, this paper aimed at exploring whether it could alleviate hyperlipidemic acute pancreatitis and further demonstrating the underlying mechanisms. The model was established by high-fat diet and intraperitoneal injection of L-arginine. After treatment with DWPE, the levels of total cholesterol, triglyceride, low-density lipoprotein cholesterol and high-density lipoprotein cholesterol of rats were significantly decreased when compared with model group. In addition, on the basis of hyperlipidemia-intensified L-arginine-induced accelerated blood lipid levels in rats, DWPE notably attenuated the pancreatic and hepatic pathological lesions. Furthermore, the levels of amylase, lipase and TNF-α were markedly decreased in the medication administration group. Therefore, DWPE would have a preventive effect on the progress of hyperlipidemic acute pancreatitis to a certain extent through reducing pancreatic cell injury, pancreatic oxidative stress level, and the levels of pancreatic digestive enzymes and inflammatory factors.

Keywords: defatted walnut powder extract; hyperlipidemic acute pancreatitis; pancreatic digestive enzyme; oxidative stress.

Practical Application: Protection of hyperlipidemic acute pancreatitis disease by defatted walnut powder extract.

1 Introduction

Pancreatitis can be referred as inflamed pancreas, which is a kind of progressive systemic inflammatory reaction where the pancreas is inflamed within a short period of time. This condition is referred as acute pancreatitis (AP) (Pasari et al., 2019; Huang et al., 2019). Hyperlipidemia (HLP) is the third most common cause of AP after alcohol and gallstones (Fei et al., 2020; Tarasiuk & Fichna, 2019). With the global economic level and dietary structure changes, the incidence of hyperlipidemic acute pancreatitis (HLAP) induced by high fat diet is on the rise from year to year (Wang et al., 2022; Choi et al., 2019; Benz et al., 2002), and presents more serious clinical symptoms, with the characteristics of easy recurrence, rapid disease change and high mortality (Garg & Singh, 2019; Zhu et al., 2017), which is a hot spot and difficulty in current clinical research. The most accepted mechanism is hydrolysis of plasma TGs by pancreatic lipase into free fatty acids (Yan et al., 2020), which triggers an inflammatory cascade with subsequent acinar cell and pancreatic capillary injury (Siriviriyakul et al., 2019). Thus, it is important to reduce TG levels in the management of HLAP. Current management of it includes dietary restrictions, analgesia, lipid-lowering agents (fenofibrate, gemfibrozil, ω-3 fatty acids, etc.) (Fei et al., 2020). However, current methods are rather disappointing (Li et al., 2020). Hence, there is a renewed interest in phytomedicines, which reduces severe adverse effects and may have benefits not only regarding the symptoms but also concerning the disease evolution (Li et al., 2020).

Walnut kernel, the mature seed of *Juglans regia* L. recorded in the 2020 edition of the Chinese Pharmacopoeia and it is a popular functional food riched in nutrients which cultivated all over the world (Cerit et al., 2017; Rusu et al., 2020; Liang et al., 2017). The seeds contain numerous fatty acids (linoleic and α-linolenic acids), hormone, melatonin and also are rich in phenolic compounds (Wang et al., 2021; Chauhan & Chauhan, 2020; Grace et al., 2014; Shimoda et al., 2009). It is reported to have the most diverse phenolic profile which is quite high compared to other tree nuts that are consumed (Ashraf et al., 2021; Medic et al., 2021; Bolling et al., 2011), and have a positive influence on human health and reduce the risks of lifestyle-related diseases such as cardiovascular disease, hypertriglyceridemia, and diabetes mellitus (Regueiro et al., 2014; Shimoda et al., 2009). It was found that the defatted walnut powder extract (DWPE), the residue of walnut kernel removal of walnut oil, was the most important source of walnut phenolics (Medic et al., 2021; Colaric et al., 2005). The preliminary study of this experiment found that DWPE had obvious antioxidant and lipid-lowering effects (Xu et al., 2022; Ren et al., 2021). Therefore, such results were supposed that DWPE had a protective effect on HLAP.

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Currently, accumulating evidence showed that DWPE significantly inhibited lipid levels (Shimoda et al., 2009; Yılmaz et al., 2016) and exhibited the abilities of anti-inflammation and antioxidant. However, the role of DWPE in the HLPA has been hardly investigated. In the present study, a pharmacologic study was experimented to indicate the preventing effect of DWPE against HLAP induced by high-fat diet complicated with L-arginine in SD rats.

2 Materials and methods

2.1 Chemicals and reagents

Petroleum ether and ethyl alcohol were obtained from Damao Chemical Reagent Factory (Tianjin, China). Fenofibrate and qingyanglidan granules were obtained from Shanghai Abbott Laboratories Limited and Jilin jurentang pharmaceutical limited company, respectively.

2.2 Preparation of Defatted Walnut Powder Extract (DWPE)

Walnut kernel was obtained from Xinjiang province, China, and identified as the seeds of *Juglans regia* L. by Dr. Yingni Pan, Shenyang Pharmaceutical University. Defatted walnut powder was the residue of walnut kernel after being soaked in petroleum ether (30-60 °C) with 5-fold volume for 1 h and sonicated 3 times for 30 min. We used the results from previous experiments to determine the optimal extraction process: 9.5 fold solvent to material ratio, 68% ethanol concentration, 55 min extraction period, and 3 times (Xu et al., 2022). And the filtrate was followed by being concentrated and dried to obtain DWPE, consistent with extract preparation in the previous study.

2.3 Animals and experimental design

Male Sprague-Dawley rats (weighing 200 ± 20 g; SPF) were purchased from the Experimental Animal Center of Shenyang Pharmaceutical University. The animals were placed in a temperature and humidity controlled room with a 12 h light/dark cycle and had a free access to water. All the animal experiments were carried out in accordance with the Guide for the Care and Use of the Laboratory Animals, and followed the Guidelines for Animal Experimentation of Shenyang Pharmaceutical University, Shenyang, China.

Twenty-four SD rats were divided into 4 groups randomly and were fed with basic feed (control group and AP group) and a high-fat diet (HLP group and HLAP group) for 2, 4, 6, 8 and 10 weeks, respectively. At the end of the 4th, 6th, 8th and 10th weeks, the rats were collected with orbital blood, so the HLP model was studied for 10 weeks. At the end of this 10-week controlled-diet period, acute pancreatitis and hyperlipidemic acute pancreatitis groups were induced with 2 × 2 g/kg body weight of arginine i.p.

Forty-two SD rats were randomly assigned to control group, HLAP group, high dose of DWPE group (DWPE-H) (0.72 g/kg), middle dose of DWPE group (DWPE-M) (0.36 g/kg), low dose of DWPE group (DWPE-L) (0.18 g/kg), Fenofibrate (31.5 mg/kg) and Qingyanglidan granules (QYT) (1.8 g/kg), randomly. The dosages of DWPE were 4 folds, 2 folds and 1 fold of the daily nutritional recommendations of nuts in human (30 g/day). The high fat diet contained 22% lard, 5% sucrose, 2.5% cholesterol, 0.2% sodium cholate, and the basal diet contained 17% crude protein, 15% crude fiber, 11% water, 9% crude ash and 3% crude fat. Besides, while control group rats were given a normal diet, the other six groups were given a high fat diet for 10 weeks. DWPE-H, DWPE-M, DWPE-L, Fenofibrate and QYT groups were given relative suspensions dissolved into saline solution by oral gavage daily for 10 weeks. By contrast, control and HLAP groups were given saline solution. At the end, model of acute pancreatitis induced by L-arginine was established in all rats through intraperitoneal injection. After 12-h fasting, all rats were sacrificed to collect blood, liver and pancreas tissues, and the samples were removed and stored at -80 °C (Figure 1).

2.4 Determination of pancreatic edema

The ratio of pancreatic weight (P.W.) to body weight (B.W.) was evaluated as an estimate of the degree of pancreatic edema. At the time of sacrifice, the pancreas and bodies of the rats were weighed. And P.W. was divided by B.W. and multiplied by 1000 to obtain the ratio as a natural number.

2.5 Lipid analysis

The concentrations of total cholesterol (T-CHO), triglyceride (TG), highdensity lipoprotein-cholesterol (HDL-C), low-density lipoprotein-cholesterol (LDL-C) in the blood were measured by the commercially available kits. The levels of T-CHO and TG (JianchengBio, Nanjing, China) in the liver were detected using their corresponding detection kits based on manufacturer instructions.

2.6 ELISA and plasma biochemical enzyme measurement

ELISA kits (JianchengBio, Nanjing, China) for tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) were used to check their protein concentrations in serum. The serum amylase (AMS) and lipase activities (LPS) were determined using biological reagent kits (JianchengBio, Nanjing, China).

2.7 Estimation of malondaialdehyde (MDA) and superoxide dismutase (SOD) estimation

Malondialdehyde (MDA) and superoxide dismutase (SOD) concentrations in the pancreas were measured using biological reagent kits (JianchengBio, Nanjing, China). The protein concentrations were detected by bicinchoninic acid (BCA) protein assay kit (Beyotime Co. Ltd. Shanghai, China).

2.8 Histopathology analysis

Sections of pancreas and liver were fixed into 4% paraformaldehyde solution for 24 h and embedded in paraffin. Then, the pancreatic tissue sections were stained with hematoxylin and eosin, and examined by light microscopy.

2.9 Statistical analysis

All the data was presented as mean ± standard deviation and all quantitative data of every experiment was obtained

from six animals or replicates in each group. Comparisons among multiple groups were analyzed using one-way ANOVA via GraphPad Prism 8 (GraphPad Software, San Diego, CA, USA), except for the differences of PAS and H&E staining scores between groups which were evaluated through Kruskal-Wallis test. *P* value less than 0.05 ($P < 0.05$) was considered to be statistically significant.

3 Results

3.1 Severity of hyperlipidemia acute pancreatitis

After 10 weeks of high-fat diet, T-CHO and TG levels were significantly increased in the HLP group and HLAP group compared with the normal diet group (Table 1). After the successful establishment of HLAP model, the animals with high-fat diet in HLP group did not modify the serum amylase and lipase activities or the ratio pancreatic weight/body weight as compared with those of the rats on control group, and did not cause any histological alteration in the pancreas. In marked contrast, the serum amylase and lipase activities, the ratio pancreatic weight/body weight and the histological alteration were significantly increased in the animals with AP and HLAP groups as compared with the rates on control group (Figure 2).

3.2 DWPE prevented the increased pancreatic edema and digestive enzyme activity

Fenofibrate, as the first choice for adjuvant treatment of severe HTG, significantly reduced serum TG content (Sundar et al., 2020); Qingyilidan granules have been the Chinese traditional medicine protective variety, which could significantly relieve the symptoms of AP.

At the end of the 10-week controlled-diet period, the animals weighed 400-500 g. The high-fat diet increased the body weight without significance than control group. The pancreatic weight to body weight ratio was calculated for each rats in each group to assess the effect of DWPE on HLAP rats. As shown in Figure 3, compared with control group, the P.W./B.W. ratios were lower in control rats than those in HLAP rats. Furthermore, DWPE-treated group was ameliorated the P.W./B.W. ratio. The activities of serum AMS and LPS levels were increased significantly in the HLAP group compared with the control group, while DWPE treatment decreased the activities of these markers. In addition, the AMS level in fenofibrate group was significantly reduced, without significance about LPS level. QYT only significantly reduced LPS level.

3.3 DWPE improved the effects of lipid levels in HLAP rats

Since blood lipid levels were important indicators for HLAP detection, the corresponding concentrations of them in serum were measured among groups. As shown in Table 2, the 10-week high-fat diet significantly increased T-CHO, TG and LDL-C levels from 2.14 ± 0.18, 0.47 ± 0.09 and 0.68 ± 0.37 mmol/L to 3.84 ± 0.66 ($P < 0.001$), 1.78 ± 0.99 ($P < 0.001$) and 1.74 ± 0.53 mmol/L ($P < 0.001$). Additionally, the level of HDL-C dropped from 1.02 ± 0.24 mmol/L to 0.38 ± 0.06 mmol/L ($P < 0.001$). And DWPE intragastric administration showed opposite alterations of these evaluated indicators which reduced T-CHO, TG and LDL-C,

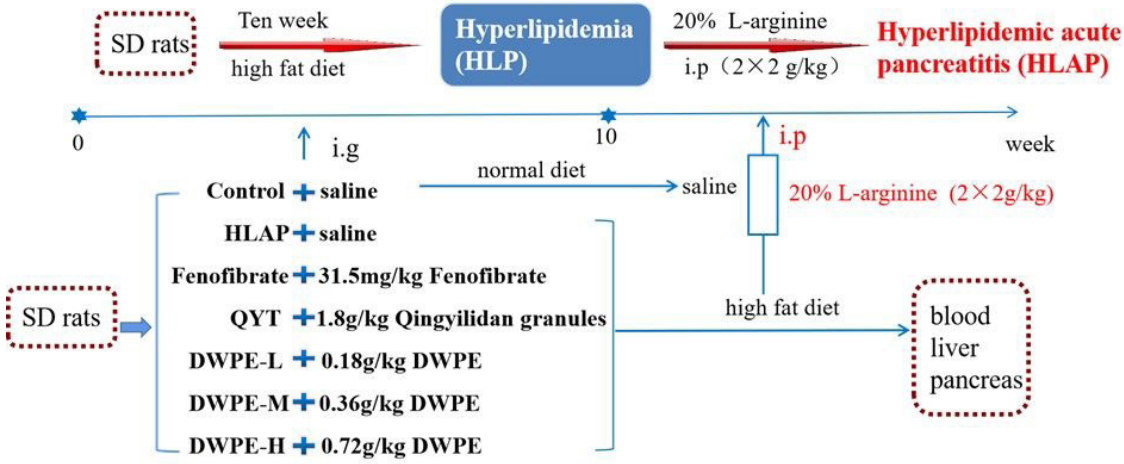

Figure 1. The overlook of the animal experiment.

Table 1. The effects of DWPE on the lipid levels.

	2 week		4 week		6 week		8 week		10 week	
	T-CHO	TG	T-CHO	TG	T-CHO	TG	T-CHO	TG	T-CHO	TG
Control	2.33 ± 0.14	0.62 ± 0.13	2.32 ± 0.18	0.43 ± 0.13	2.06 ± 0.33	0.50 ± 0.20	2.06 ± 0.17	0.62 ± 0.11	2.32 ± 0.18	0.65 ± 0.15
AP	2.37 ± 0.34	0.60 ± 0.11	2.13 ± 0.49	0.57 ± 0.62	2.12 ± 0.49	0.54 ± 0.20	2.39 ± 0.22	0.64 ± 0.25	2.12 ± 0.48	0.82 ± 0.17
HLP	2.73 ± 0.16	0.86 ± 0.15	2.35 ± 0.17	0.57 ± 0.17	2.57 ± 0.33	0.72 ± 0.16*	2.71 ± 0.15*	0.95 ± 0.20*	3.19 ± 0.37*	0.87 ± 0.15*
HLAP	2.66 ± 0.15	0.87 ± 0.13	2.92 ± 0.13	0.67 ± 0.15	2.63 ± 0.28	0.71 ± 0.12	2.37 ± 0.22	0.99 ± 0.17**	3.07 ± 0.31*	1.01 ± 0.06***

*$P < 0.05$. **$P < 0.01$. ***$P < 0.001$ vs control group.

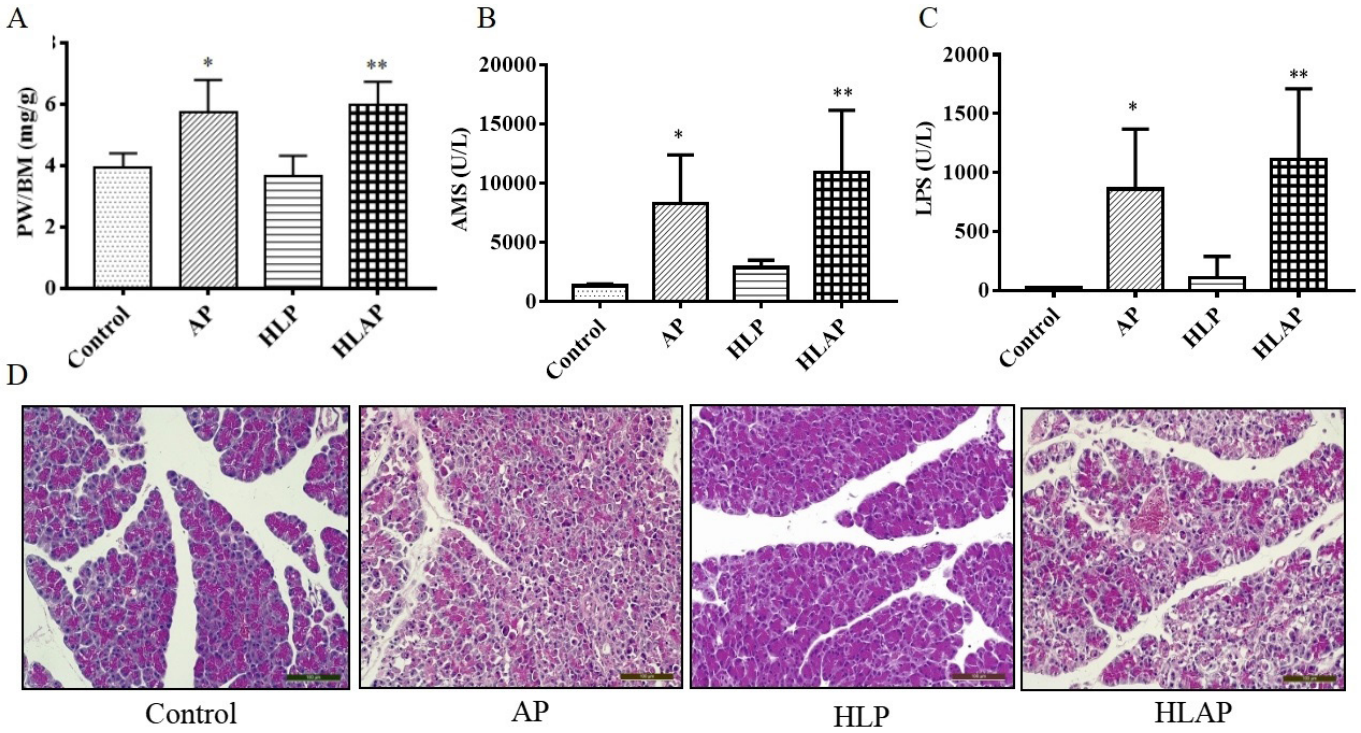

Figure 2. Toe effects of DWPE on (A) pancreatic organ index; (B) amylase; (C) lipase; (D) hematoxylin - eosin (H&E) images of the pancreas. Data are represented as mean ± SD (n = 6/group); *$P < 0.05$; **P < 0.01 vs control group.

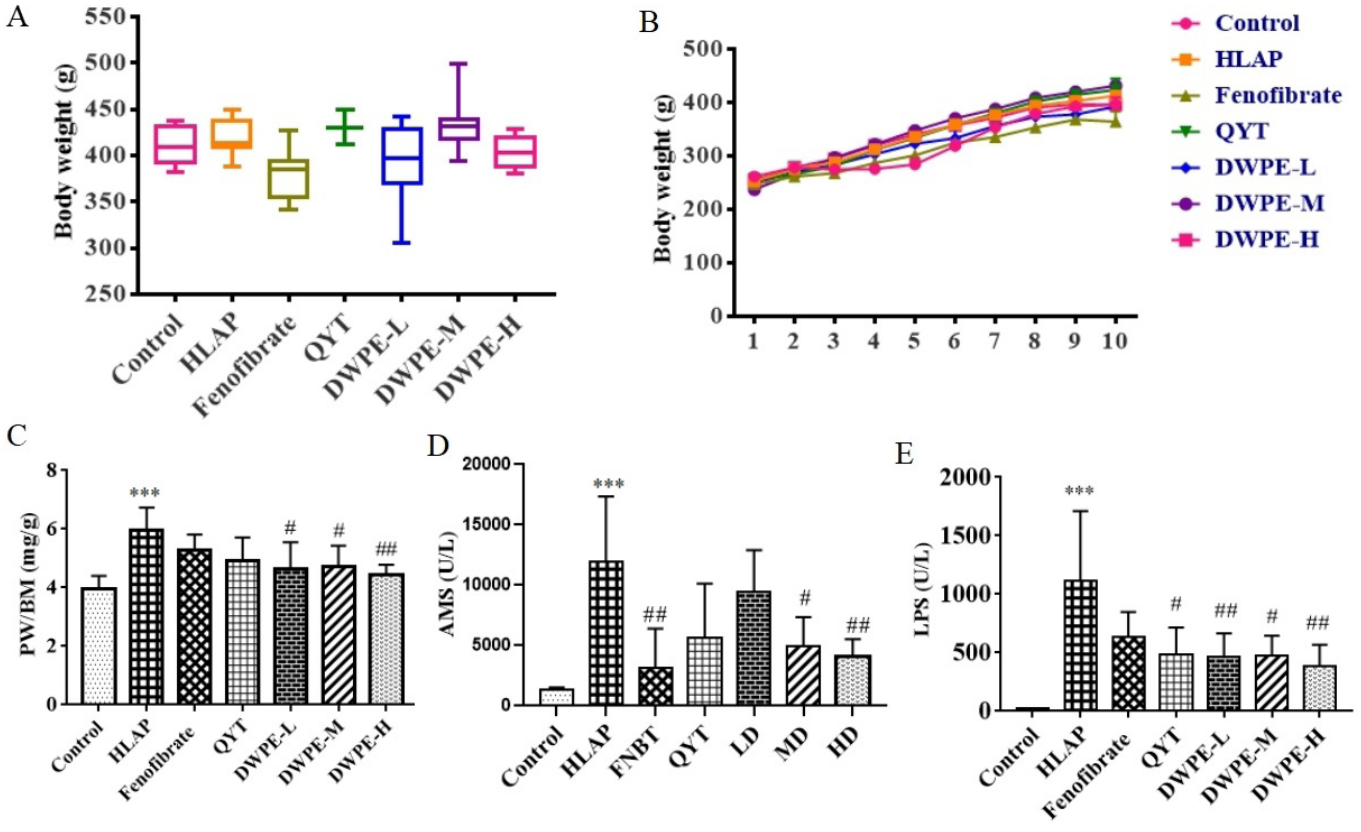

Figure 3. The effects of DWPE on the edema, digestive enzyme levels. (A) The final body weight; (B) the dynamic changes of body weight; (C) the pancreatic organ index; (D) amylase; (E) lipase. Data are represented as mean ± SD (n = 6/group); *$P < 0.05$; ***$P < 0.001$ vs control group; #$P < 0.05$; ##$P < 0.01$ vs HLAP group.

Table 2. The effects of DWPE on the serum lipid levels.

Groups	T-CHO (mmol/L)	TG (mmol/L)	HDL-C (mmol/L)	LDL-C (mmol/L)
Control	2.14 ± 0.18	0.47 ± 0.09	1.02 ± 0.24	0.68 ± 0.37
HLAP	3.84 ± 0.66***	1.78 ± 0.99***	0.38 ± 0.06***	1.74 ± 0.53***
Fenofibrate	2.32 ± 0.16##	0.86 ± 0.19#	0.38 ± 0.18	0.96 ± 0.46#
QYT	2.61 ± 0.39#	0.84 ± 0.18#	0.76 ± 0.24#	1.32 ± 0.38
DWPE-L	2.73 ± 0.94#	0.70 ± 0.17##	0.47 ± 0.18	1.53 ± 0.62
DWPE-M	2.28 ± 0.84###	1.10 ± 0.25	0.66 ± 0.19	1.23 ± 0.15
DWPE-H	2.12 ± 0.67###	0.57 ± 0.11###	0.89 ± 0.30##	0.80 ± 0.46##

*$P < 0.05$. ***$P < 0.001$ vs control group. #$P < 0.05$. ##$P < 0.01$. ###$P < 0.001$ vs HLAP group.

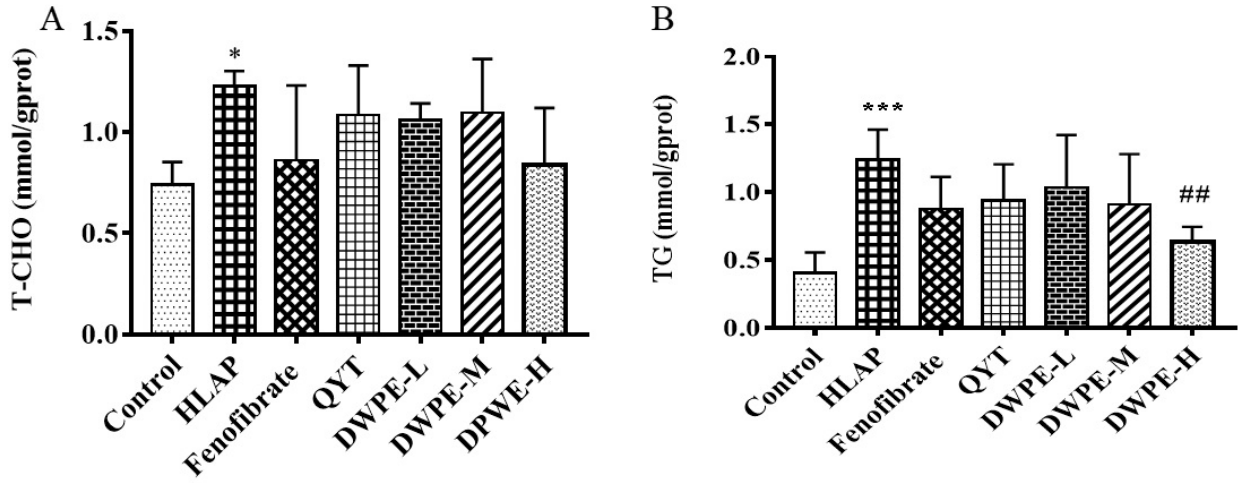

Figure 4. The effects of DWPE on the hepatic oxidative damage including T-CHO (A) and TG (B). Data are represented as mean ± SD (n = 6/group); *$P < 0.05$; ***$P < 0.001$ vs control group; ##$P < 0.01$ vs HLAP group.

with increased HDL-C compared with HLAP group (Table 2). Fenofibrate significantly reduced T-CHO, TG and LDL-C levels. QYT significantly reduced T-CHO and TG levels, with increased HDL-C level.

Furthermore, the effects of DWPE on liver lipid profile were elucidated (Figure 4). The HLAP group showed significantly difference compared with control group. And DWPE administration decreased markedly the liver levels of T-CHO and TG and there was significant difference in high dose group. There was no significant difference in fenofibrate and QYT groups.

3.4 DWPE relieved histological changes during hyperlipidemia pancreatitis

The severity of HLAP was assessed by examining morphological evidence of the extent of acinar cell injury and histological characteristics. We observed a change in the histological architecture of the pancreas in the model group, including severe pancreatic interstitial edema, vacuolization, prominent neutrophil infiltration, and acinar cell injury and necrosis. DWPE pretreatment attenuated the severity of the pancreatitis, as indicated by decreased acinar cell injury and interstitial edema and reduced inflammatory cell infiltration (Figure 5).

The hepatic tissue was assessed after L-arginine injection and high-fat diet feeding. In HLAP group, liver injury was evidenced by an abundance of in-flammatory cell infiltrates, nuclear concentration, ballooning degeneration, vacuolation, and focal necrosis. Compared with HLAP group, there were less lipid droplets and no hepatocyte ballooning in DWPE-H group. In DWPE-L and DWPE-H groups, lipid droplets and inflammatory infiltration were visible, but the state of hepatocytes was better than HLAP group.

3.5 DWPE reduced oxidative stress and inflammation in HLAP rats

To analyze the mechanism by which DWPE improved the course of hyperlipidemic acute pancreatitis, the paper studied whether it decreased the extent of lipid peroxidation due to oxidative stress in the pancreatic tissue. The pancreatic MDA concentration was significantly increased and SOD activity was significantly decreased in HLAP group as compared with the control rats with pancreatitis. In contrast, animals pretreated with DWPE showed dose dependant reduction in MDA concentration ($p < 0.05$ at 0.36 g/kg and $p < 0.05$ at 0.72 g/kg; Figure 6A). DWPE-H treatment led to increased SOD levels in a dose dependant manner ($p < 0.05$; Figure 6B).

Pro-inflammatory cytokines, TNF-α plays critical roles in the pathogenesis of pancreatitis (Pasari et al., 2019). As shown in Figure 6, serum level of TNF-α was increased during hyperlipidemic acute pancreatitis, however, they was reduced by DWPE treatment ($p < 0.05$ at 0.36 g/kg and $p < 0.01$ at 0.72 g/kg; Figure 6C).

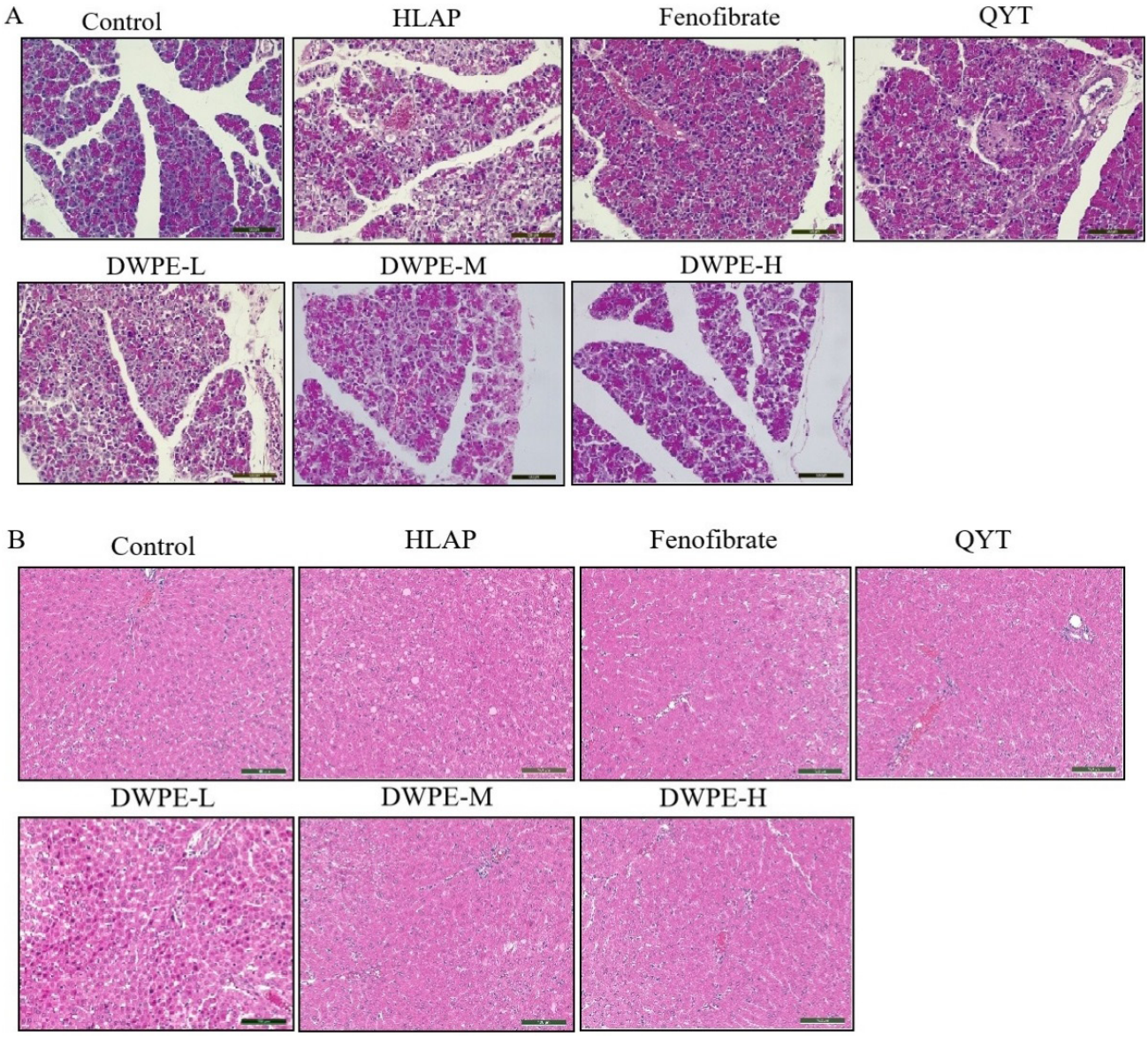

Figure 5. H&E images of the pancreas (A) and liver (B) tissue sections observed ata magnification of 100x for all tissues.

4 Discussions

The HLAP rats were established by high-fat diet and injected with L-arginine intraperitoneally. The results showed that both pancreas and liver were damaged at the same time, which was manifested as pancreatic injury with inflammatory cell infiltration, edema and vacuolization, prominent neutrophil infiltration, and acinar cell injury and necrosis. And liver injury showed extensive infiltration of inflammatory cells, nuclear concentration, balloon degeneration, vacuolation, and focal necrosis. The HLAP group increased the level of blood lipid, amylase and lipase in rats and aggravated the progression of pancreatic injury compared to those in the AP group. The data suggested that moderately elevated triglyceride levels might exacerbate the progression of acute pancreatitis. The findings in the present study were in line with the conclusion of Lindkvist et al. (2012).

Walnut kernel is considered as traditional Chinese medicine. People usually remove grease from it and use the residue as medicine (Ren et al., 2021). The results showed that the levels of blood lipid, AMS, LPS, TNF-α and oxidative stress in the rats were notably decreased after treatment with DWPE. Furthermore, the results of H&E staining also showed that DWPE significantly improved the pancreatic and hepatic pathological changes in rats. According to the literature, DWPE contained many active substances mainly including phenolic acids and dicarboxylic acid glycosides, such as gallic acid, ellagic acid, glansreginin A, glansreginin B and so on (Ren et al., 2021). Phenolic compounds such as ellagic acid which were the main components of DWPE have also been reported as potential drugs to improve the efficacy of acute pancreatitis (Yılmaz et al., 2016).

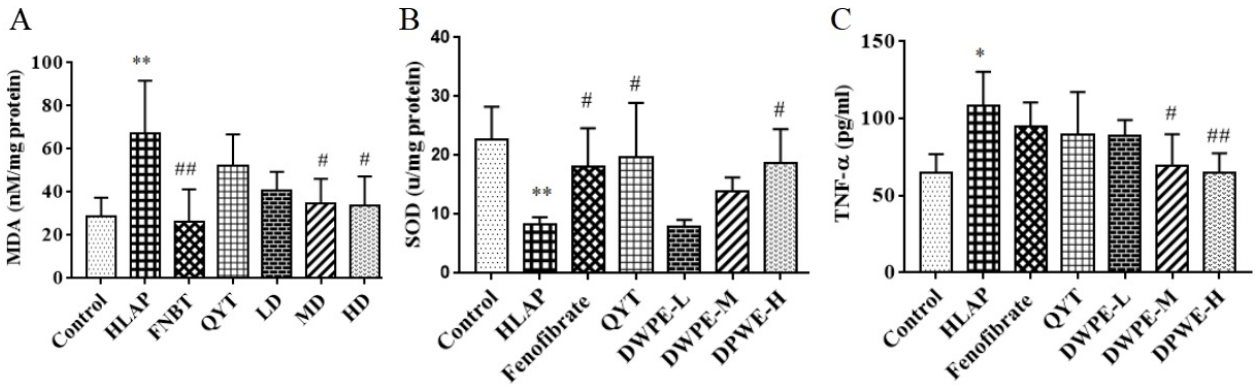

Figure 6. The effects of DWPE on oxidative stress and inflammation in HLAP rats including MDA (A), SOD (B) and TNF-α (C). Data are represented as mean ± SD (n = 6/group), $*P < 0.05$, $**P < 0.01$ vs control group; $\#P < 0.05$, $\#\#P < 0.01$ vs HLAP group.

5 Conclusions

In this studies, the results showed that DWPE might have a therapeutic effect on HLAP rats to ameliorate serum lipid spectrum and reduce liver tissue damage. Meanwhile, DWPE intervention would significantly reduce pancreatic cell injury, edema and inflammatory cell infiltration, improve pancreatic oxidative stress level, and reduce the levels of pancreatic digestive enzymes and the inflammatory factors. The research can provide a reliable new method for early clinical treatment and basic research of HLAP. The mechanism of inhibition HLAP development by reducing the oxidative stress and inflammatory response needs further research.

Conflict of interest

The authors declared no conflict of interest.

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