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Research Article

Hovenia Dulcis Thunb Polysaccharides Regulate Hyperglycemia in Diabetes Mice

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Abstract

This paper studied the hypoglycemic effect of *Hovenia dulcis* Thunb polysaccharides on mice. The alloxan was administrated by intraperitoneal injection in mice. The mice were divided into five groups, including control group, alloxan group, metformin group, low-dose HDP (HDP-L) and high dose HDP (HDP-H) groups. The mice were administrated by oral gavage for 28 days under the indicated treatment. Blood glucose and lipid biomarkers, histopathological changes in liver and pancreas, mitochondrial enzyme activity and antioxidant-related indicators as well as the associated genes mRNA changes were determined. The results showed that the weight loss was ameliorated by *HAP* in diabetic mice. Significant decreases were observed in the levels of glycated serum protein (GSP), fasting blood glucose (FBG), triglycerides (TG), total cholesterol (TC), and low-density lipoprotein cholesterol (LDL-C) of HDP and metformin treated mice. Significant increases were observed in the levels of serum insulin (SI), hepatic glycogen (HG), high-density lipoprotein cholesterol (HDL-C). Similarly, promotes mitochondrial enzyme activity, making TNF-α、IL-6 was significantly decreased and the level of antioxidant substances was increased in HDP and metformin treated mice when compared with the alloxan treated mice. Further, the mRNA levels of glucokinase (GK), pyruvate kinase (PK), glucose transporter 2 (GLUT2) and adenosine monophosphate dependent protein kinase (AMPK) were significantly increased, while the mRNA levels of glucose-6-phosphatase (G6Pase) and phosphoenolpyruvate carboxylase (PEPCK) were significantly decreased. Similar significant changes of the previous biomarkers and genes were observed when compared between HDP-L and HDP-H groups. These results, indicate *HAP* can effectively regulate hyperglycemia, which is related to the activation and regulation of hepatic glucose metabolism genes, especially AMPK signal pathway.

Keywords: *Hovenia dulcis* Thunb polysaccharides, Liver, Pancreas, Blood glucose, Glucose metabolism gene

Introduction

Diabetes mellitus (DM) is a chronic metabolic disorder characterized by insufficient insulin secretion and/or impaired insulin utilization, leading to elevated blood glucose concentrations above normal levels and disruption of glucose homeostasis. The research shows that there are 529 million people worldwide indicated DM, and the patients are expected to increase to 1.31 billion in 2050. More than 118 million people, which is account for 22 percent of the global diabetic population, are distributed in China [1]. DM is divided into three forms: type 1 diabetes (T1DM), type 2 diabetes (T2DM), gestational diabetes mellitus (GDM), while T2DM accounts for 90–95% of all DM [2]. Chemical therapeutics for T2DM are main single-target drugs that act on specific single pathways. For example, the level of blood glucose was decreased by inhibiting hepatic gluconeogenesis [3]. Glucagon-like peptide-1 (GLP-1) receptor agonists stimulate insulin secretion and suppress glucagon release by mimicking the action of endogenous GLP-1[4]. Simultaneously, long-term use of antidiabetic drugs may lead to adverse effects such as hypoglycemia, gastrointestinal disturbances and anemia [5,6]. Consequently, there is an urgent need to identify effective, low-toxicity, and natural drug alternatives.

Medicine food homology plants (MFHPs) have garnered significant attention due to their safety and low toxicity [7]. Their bioactive polysaccharides can ameliorate T2DM through multi-target mechanisms [8]. Studies indicate that polysaccharides isolated from MFHPs such as *Angelica sinensis* [9], *Dendrobium officinale* [10], *Pueraria lobate* [11], and *Astragalus membranaceus* [12] alleviate insulin resistance, enhance insulin sensitivity, and regulate glucose homeostasis. *Hovenia dulcis* Thunb is a deciduous tree of the Rhamnaceae family. It is indicated for its fleshy, twisted fruit stalks that exhibit a sweet jujube-like flavor. It is widely cultivated in China, such as in Shaanxi, Gansu and Jiangxi provinces. *Hovenia dulcis* Thunb is rich in carbohydrates, lipids, proteins, vitamins, and mineral elements. Notably, it is rich in amino acids with low concentrations of fatty acids, making it an ideal raw material for "high-carbohydrate, low-fat" functional foods. Studies have indicated that *Hovenia dulcis* Thunb exhibits marked bioactivities, including alcohol-sobriety and liver protection, hypotensive and diuretic effects, anti-lipid peroxidation, anti-aging, antitumor, sedative, and analgesic properties [13-15]. *Hovenia dulcis* Thunb polysaccharides (HDP) are one of its primary active components. Crude extracts from *Hovenia dulcis* Thunb inhibit α-amylase and α- glucosidase activities, which is correlated with the contained polysaccharide content [16]. Studies have revealed that HDP may enhance both non-specific and specific immunity in cyclophosphamide-induced mice by reducing epinephrine-induced hyperglycemia, fasting blood glucose (FBG), glycated hemoglobin (HbA1c), and increasing lipid metabolism. However, current research on HDP's hypoglycemic and hypolipidemic effects underlying the mechanisms are poorly elucidated. Thus, further investigation into the pharmacological actions and mechanisms of HDP are warranted.

In this study, the effects of HDP on blood sugar were investigated in an alloxan-induced diabetic mice. The blood glucose and lipid biomarkers, and histopathological changes, mitochondrial enzyme activity and antioxidant-related indicators, tumor necrosis factor- alpha (TNF-α) and interleukin 6 (IL-6) levels were assayed. Also, the mRNA levels of glucose-6-phosphatase (G6Pase), phosphoenolpyruvate carboxykinase (PEPCK), glucose transporter 2 (GLUT2), AMP-activated protein kinase (AMPK), glucokinase (GCK), pyruvate kinase (PK) were further assayed. The findings of this study will provide both scientific and applied evidence for the therapeutic potential of HDP in DM.

Materials and Methods

Extraction of *Hovenia Dulcis* Thunb Polysaccharides

Hovenia dulcis Thunb was obtained from Xunyang Taiyiyuan Biotechnology Co., Ltd., Shaanxi, China. *Hovenia dulcis* Thunb polysaccharides were extracted as the following: *Hovenia dulcis* Thunb was dried at 50°C, pulverized, and defatted with anhydrous ethanol. The filtrate (obtained via vacuum filtration) was concentrated and mixed with Sevag reagent at a 5:1 (v/v) ratio. The mixture was vortexed and centrifuged to remove denatured proteins. Then the supernatant was precipitated with 95% ethanol, followed by centrifugation at 5,000 rpm for 10 min. The precipitate was dissolved in 40°C hot water, treated with Fehling's reagent to form copper-polysaccharide complexes, and centrifuged. The complexes were washed with distilled water, dissolved in 0.5 mol/L HCl, and precipitated with 95% ethanol. The flocculent precipitate was subsequentially washed with ethanol, acetone, and diethyl ether, then freeze-dried to obtain white HDP powder.

Animals and Experimental Design

Kunming (KM) mice were purchased from the Experimental Animal Center of Xi'an Jiaotong University, China. Sixty male KM mice were feed in an environment at 25 ± 2°C, 50%~55% humidity with a 12 h light-dark cycle, and free access to food and water. Ten mice were randomly selected as the control group. Other mice were fasted 12 h and then intraperitoneally injected 150 mg/kg alloxan (Sigma Aldrich). FBG was measured after 72 h. The mice with fasting blood glucose ≥11.1 mmol·L−1 were selected and randomly divided into the four groups: alloxan group, metformin group, low-dose group of HDP (HDP-L), and high-dose group of HDP (HDP-H). The control group and alloxan group were orally administered with 0.1 ml of 0.9% NaCl, the metformin group was orally administered with 0.1 ml at a dose of 250 mg/kg metformin hydrochloride (Shiyao Group Ouyi Pharmaceutical Co., Ltd., China), and the low dose and high dose groups of HDP were orally administered with 0.1 mL of HDP solution prepared at doses of 200 mg/kg and 400 mg/kg, respectively. All experiments on animals were carried out by the guidelines of the Institutional Animal Ethics Committee.

Body weight of mice were weighed once a week during the experiment. After four weeks, mice were fasted for 12 h, blood samples were obtained and centrifuged at 4,000×g for 15 min. Serum was stored at -80°C for further analysis. Livers and pancreas were rapidly excised, weighed, and photographed. Liver and pancreas index were calculated. A part of the liver and pancreas were immersed in 4% paraformaldehyde. Hematoxylin- eosin (HE) staining

were used to analysis the changes in liver and pancreas tissues by light microscope. The other part of the liver was stored at -80°C for further in the experiment.

Biochemical Analysis

FBG was measured by using a Yuwell blood glucose meter (Jinan Qiansi Biotechnology Co., Ltd. China). The levels of glycated serum protein (GSP), serum insulin (SI), hepatic glycogen (HG), triglycerides (TG), total cholesterol (TC), and low- density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C) levels, tumor necrosis factor-alpha (TNF-α) and interleukin 6 (IL-6) assay kits were determined using the corresponding kits (Nanjing Jiancheng Bioengineering Institute, China) according to the instructions. Antioxidant biomarkers of glutathione peroxidase (GSH-Px), superoxide dismutase (SOD), glutathione (GSH), catalase (CAT), malondialdehyde (MDA), total antioxidant capacity (T-AOC), succinate dehydrogenase (SDH) and malate dehydrogenase (MDH) were estimated according to our previous protocol [17].

RT-PCR Analysis

Total RNA was extracted from liver and pancreas tissues. The RNA was extracted using the method provided by Qiagen and then reverse-transcribed to obtain cDNA (pre-denaturation at 95°C for 30 s; denaturation at 95°C for 5 s, annealing at 60°C for 20 s, extension at 72°C for 50 s, for 45 cycles). The concentration of RNA was determined using an ultraviolet spectrophotometer. With β-actin as the internal reference, the relative expression of each gene was calculated using the 2−ΔCt method. The primers of the genes are shown in (Table 1).

Table 1: Primer sequences used for RT-PCR.

Gene	Primer sequence (5'—3')	
	Upstream	Downstream
GK	TCCCTGTAAGGCACGAAGA	GAGAAGTCCCACGATGTTGTT
AMPK	CGGGGTCATTCTCTATGCTT	TTTAAACCACTCGTGTTCCCT
PEPCK	GACAGACTCGCCCTATGTG	GGTTGCAGGCCCAGTTGTTGGTG
G6Pase	GGCTCACTTTCCCCATCAGGT	CCAAGTGCGAAACCAAACAGGT
GLUT2	CCAGCACATACGACACCAGACG	CCAAAGAACGAGGCGACCATACG
PK	ATGATGTGGATCGAAGGGTC	TGGGTTGAAAGAAATAGGGT
β-actin	ACGTCAGGTCATCACTATCG	GGCATAGAGGTCTTTACGGATG

Statistical Analysis

Statistical analysis was performed using the SPSS 23.0 software. All data were expressed as mean ± SD. The t-test was used for significance analysis. When $P < 0.05$, there was a significant difference, which was statistically significant.

Results

Effects of *Hovenia Dulcis* Thunb Polysaccharides on Body Weight in Mice

The changes of mice body weight were analyzed and shown in (Table 2). When compared to the control group, the alloxan group, metformin group, and HDP-treated groups exhibited continuous weight loss and lethargy during the first three weeks, followed by gradual recovery from the fourth week. On day 7, the weight of alloxan group showed a significant reduction, while the HAP-H indicated a significant increase in body weight when compared to the alloxan group. The body weight of control group increased throughout the experiment, whereas the alloxan and HDP treated groups exhibited weight losses. The alloxan group exhibited the most significant weight loss, while the metformin group showed less weight reduction compared to HAP groups.

Table 2: Changes in body weights of mice.

Group	0 Day	7 Days	14 Days	21 Days	28 Days
Control	19.91±0.64	20.52±0.99	22.18±0.35	24.73±1.62	26.15±0.84
Alloxan	19.83±0.07	19.05±0.21*	18.23±0.21*	18.15±0.57*	17.30±0.07*
Metformin	19.77±0.85	19.68±0.71	19.41±0.35#	19.37±0.64#	19.67±0.35#
HDP-H	19.70±0.28	19.38±0.49	19.17±0.64#	18.53±0.35	18.76±0.71#
HDP-L	19.55±0.21	19.37±0.21#	19.10±0.07#	18.85±0. 07#	19.23±0.01#

Note*: *$p<0.05$ compared with the control; #$p<0.05$ compared with the alloxan group.

Moreover, HAP-H group demonstrated a more pronounced effect in attenuating weight elevation than the HDP-L group. These results indicate that both the metformin and the HAP significantly mitigated weight loss in mice. The HAP-H group displayed better effect in weight reduction than the HDP-L group. The findings suggest the beneficial role of HDP in improving effect on body weight loss in diabetic mice.

Effects of *Hovenia Dulcis* Thunb polysaccharides on Blood Glucose and Lipid Biomarkers

The effects of HDP on blood glucose-related biomarkers and on lipid-related biomarkers were shown in (Figure 1). When compared to the normal group, the alloxan group exhibited significantly increased in GSP, FBG, TG, TC, and LDL-C levels. HG, SI and HDL-C levels were significantly decreased. In contrast, when compared to the alloxan group, the metformin, HDP-L, and HDP-H groups indicated significantly lower levels of GSP, FBG, TG, TC and LDL-C, while HG, SI and HDL-C levels were significantly increased. Notably, significant changes of GSP, HG, SI, TG, TC, LDL-C and HDL-C were observed when compared between HDP-L and HDP-H groups (Figure 1).

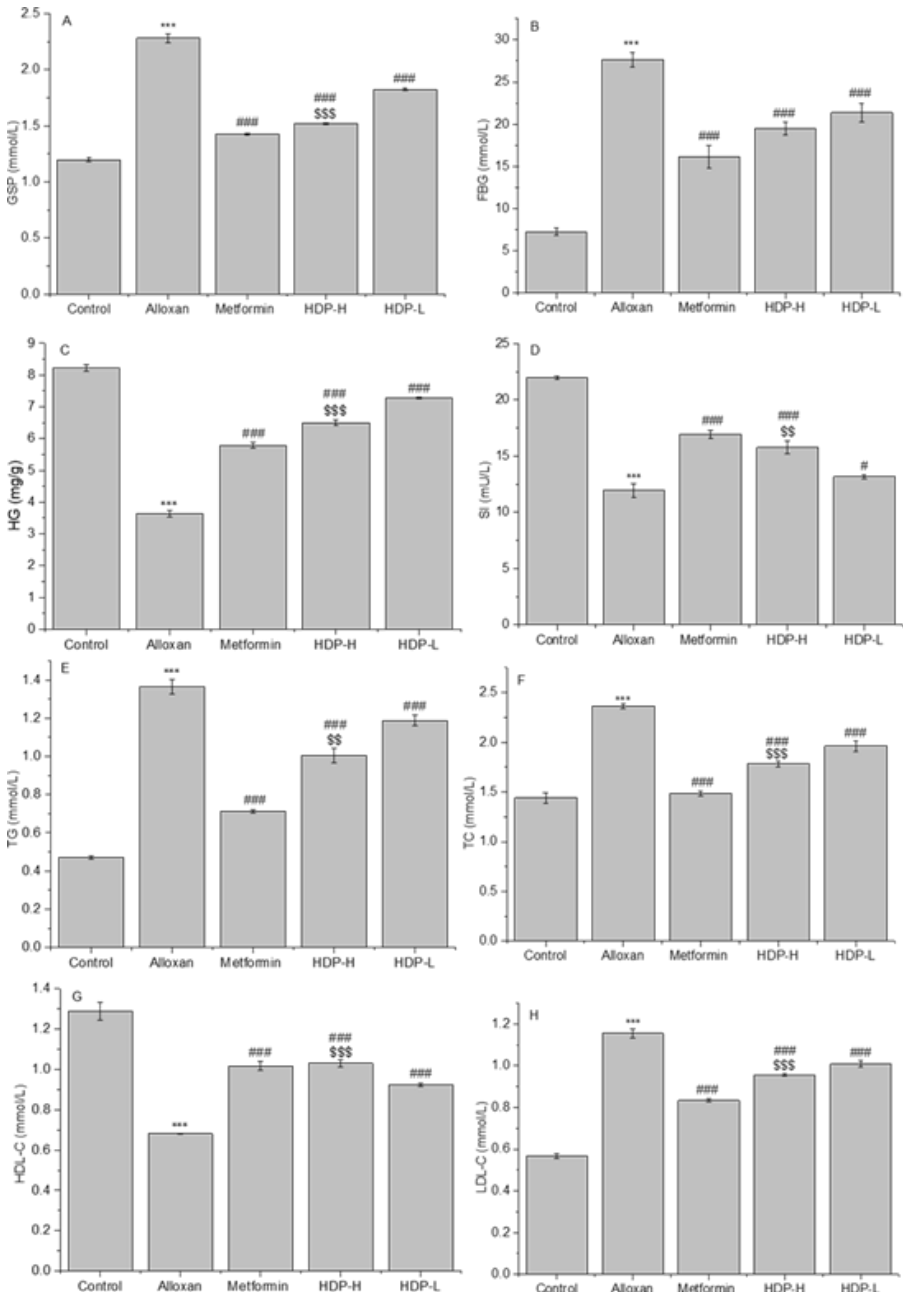

Figure 1: Effects on blood glucose and lipid related biomarkers under the indicated treatment. ***P<0.001 compared with the control, #P<0.05, ###P<0.05 compared with the alloxan group, $$P<0.01, $$$P<0.001 compared between HDP-L and HDP-H groups.

Effects of *Hovenia Dulcis* Thunb Polysaccharides on the Histopathological Changes in Liver and Pancreas

As shown in (Figure 2), hepatic tissues in the control mice exhibited normal hepatocyte distribution and intact hepatic sinusoids. Hepatic pathological changes were observed by the disordered hepatic cellular arrangement and structural abnormalities in the sinusoids in alloxan mice. Treatment with metformin substantially restored hepatocyte alignment and improved sinusoidal structural integrity. While the low-dose HDP treatment mice showed the sinusoidal irregularities and suboptimal cellular organization, the high-dose HDP group displayed partial restoration in sinusoid and perisinusoidal cells, suggesting the better hepatoprotective effect of HDP-H.

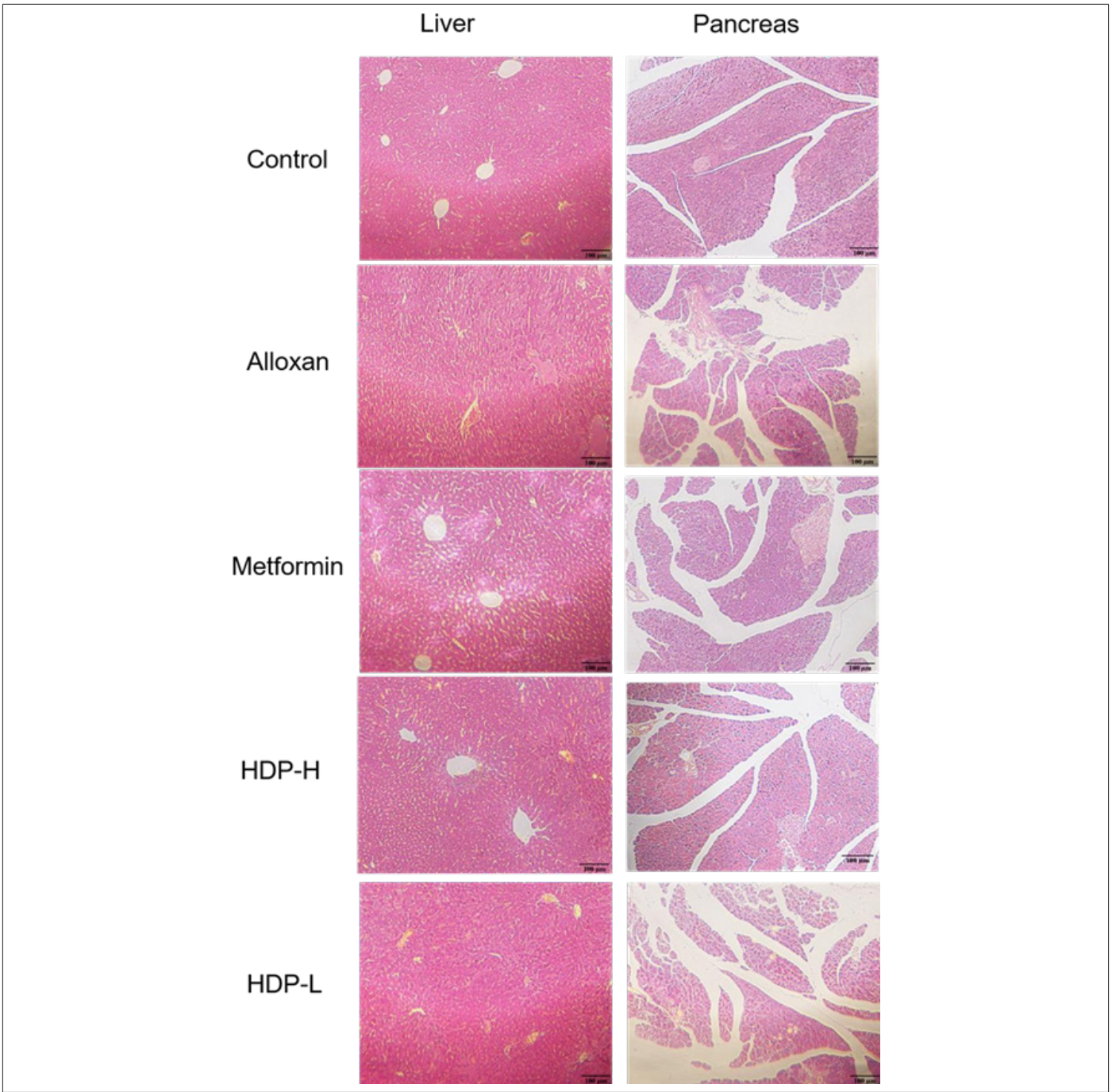

Figure 2: Pathological changes in liver and pancreas under the indicated treatment. HE staining was used and observed.

Simultaneously, the pancreatic tissues of control mice were distributed in an orderly manner, with clear veins and visible pancreatic islet structures. The alloxan mice of pancreatic tissue was destroyed, which the cells were enlarged and the pancreatic islets were significantly atrophied and almost invisible, indicating that alloxan caused serious damage in pancreas. After metformin treatment, the pancreatic tissues of mice were clear, the cell morphology was basically normal, and the pancreatic islet structure was visi-

ble. The pancreatic tissue of the mice in the low-dose HDP group were clear, the pancreatic islets were partially restored, and the cell morphology was improved. The pancreatic cells of the high-dose HDP group were well distributed and arranged. The pancreatic islet structures were visible, and the cells notably returned to normal morphology. The results further confirmed that the high dose of HDP exhibited an obvious effect on pancreas (Figure 2).

Effect of *Hovenia Dulcis* Thunb Polysaccharides on Mitochondrial Enzyme Activity and Antioxidant-Related Indicators

The activity of SDH and MDH in pancreatic was analyzed (Figure 3). The activities of SDH and MDH in the alloxan group were significantly decreased when compared with the control group, while the activities of SDH and MDH in the metformin and HDP treatment groups were significantly increased when compared to the alloxan group. The results of the mitochondrial enzyme study indicated that the antioxidant system of the mice was affected. To further explore the hypoglycaemic mechanism of HDP, the effects on antioxidant-related indexes were analysed, and the results were shown in (Figure 3). The levels of GSH-Px, T-AOC, SOD, GSH, and CAT in alloxan mice were significantly reduced as compared to the control mice, while the levels of the markers in the metformin, low-dose and high-dose HDP groups were significantly elevated when compared with the alloxan mice. The alloxan mice exhibited a significant increase in MDA levels when compared with the control mice; however, the metformin and HDP treated groups demonstrated a significant decrease in these levels when compared to the alloxan mice. Similar significant changes of the previous biomarkers were observed when compared between HDP-L and HDP-H groups.

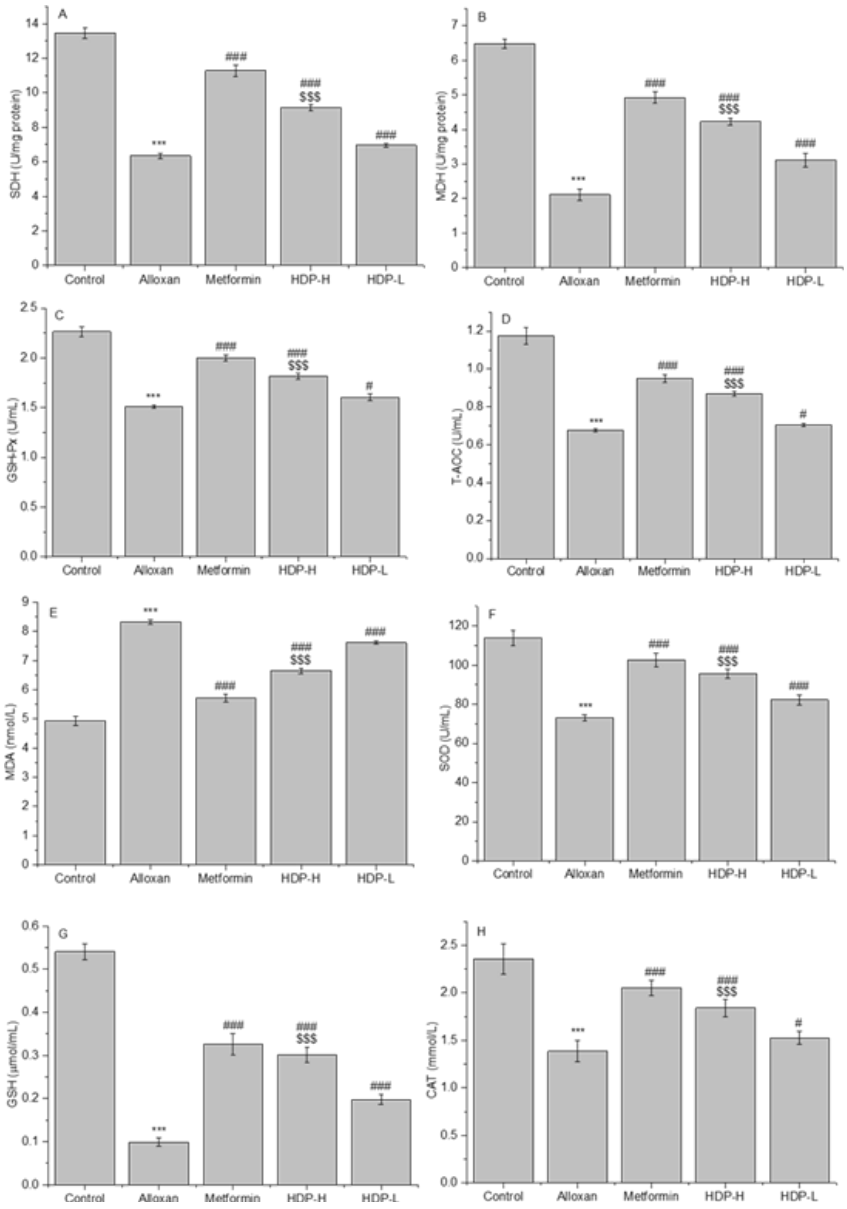

Figure 3: Effects on mitochondrial enzyme activity and antioxidant-related indicators under the indicated treatment. ***P<0.001 compared with the control, #P<0.05, ###P<0.05 compared with the alloxan group, $$$P<0.001 compared between HDP-L and HDP-H groups.

Effects Of *Hovenia Dulcis* Thunb Polysaccharides on Immune Function in Mice

As shown in (Figure 4), the levels of TNF-α and IL-6 in the alloxan mice were significantly higher than those in the control group, indicating that the inflammatory response in alloxan mice was aggravated. However, the levels of TNF-α and IL-6 in mice were significantly lower than those in the alloxan group after low-dose and high- dose HDP as well as metformin treatment. Significant decreases were observed in HDP- H mice when compared with the HDP-L mice.

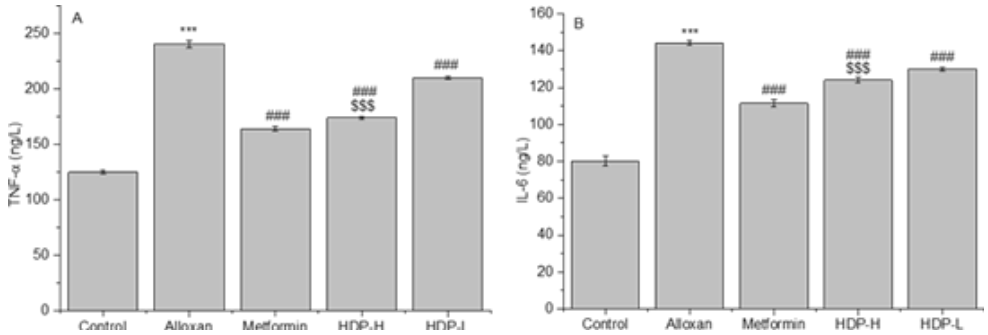

Figure 4: Effects on TNF-α and IL-6 indicators under the indicated treatment. ***P<0.001 compared with the control, ###P<0.05 compared with the alloxan group, $$$P<0.001 compared between HDP-L and HDP-H groups.

Effect of *Hovenia Dulcis* Thunb Polysaccharides on Hepatic Glucose Metabolism Genes

To further investigate the hypoglycemic mechanism of *Hovenia dulcis* Thunb polysaccharides, we analyzed their effects on hepatic glucose metabolism-related genes of GK, PK, GLUT2, G6Pase, PEPCK, AMPK, as shown in (Figure 5). In alloxan mice, the mRNA levels of GK, PK, GLUT2, and AMPK were significantly decreased as compared to the control mice, whereas in metformin, low-dose HDP, and high-dose HDP groups showed significant increases in these mRNA levels when compared with the alloxan mice. Conversely, mRNA levels of G6Pase and PEPCK were significantly elevated in alloxan mice but substantially reduced in metformin and HDP treatment groups. Notably, high-dose HDP indicated significant changes than those in low-dose HDP mice except PEPCK levels.

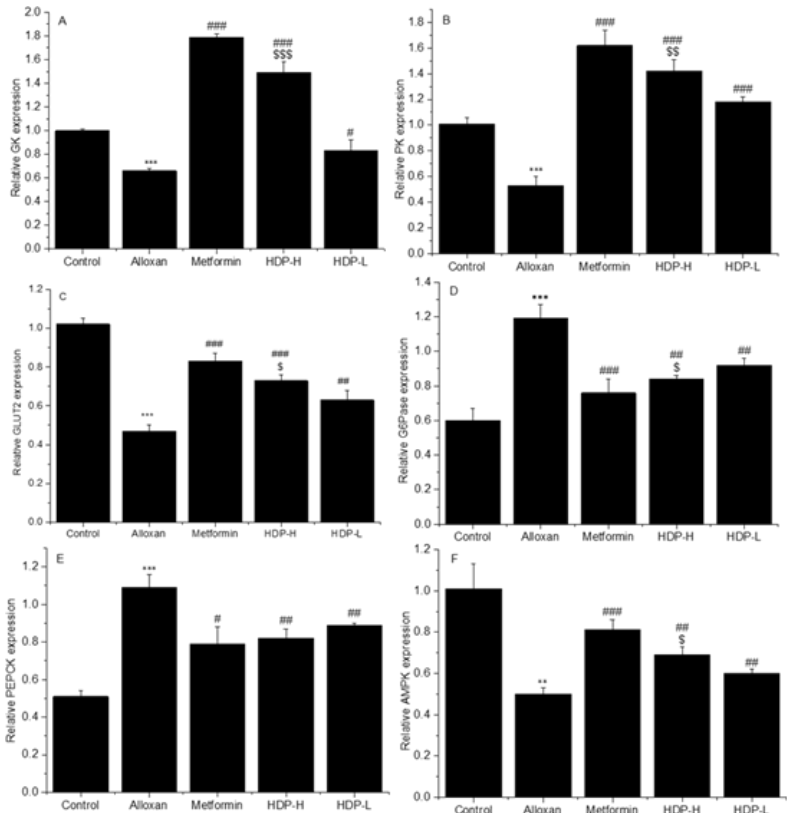

Figure 5: Effects on hepatic glucose metabolism genes under the indicated treatment. ***P<0.001 compared with the control, #P<0.05, ##P<0.01, ###P<0.001compared with the alloxan group, $P<0.05, $$P<0.01, $$$P<0.001 compared between HDP-L and HDP-H groups.

Discussion

Plant polysaccharides exhibit the characteristics such as biocompatibility, biodegradability, chemical modifiability, minimal side effects, and so on. To date, over 100 natural plants contain polysaccharides with bidirectional regulation and low toxicity, demonstrating functions including antioxidant activity, immune modulation, antitumor effects, hepatoprotection, hypoglycemic action, anti-aging properties, and gastrointestinal protection [18]. Alloxan-monohydrate was used to induce diabetes in animals, which results in the destruction of the β-cells [19]. In this study, when a diabetic model was established via intraperitoneal injection of alloxan (150 mg/kg) [20]. Diabetic model mice were confirmed by intraperitoneal injection of alloxan, as evidenced by the model group's FBG levels exceeding 11.1 mmol/L. Body weight is a critical biomarker of physiological changes in experimental animals. The most severe weight loss and marked lethargy were observed in alloxan mice, while HDP intervention attenuated weight reduction. The findings suggesting the beneficial role of HDP in improving effect on body weight loss in diabetic mice.

The level of abnormal elevation of postprandial or FBG is one of the prominent biomarker for diabetic patients. Therefore, reducing blood glucose in diabetic patients remains a major role in diabetes management [20]. GSP reflects the average blood glucose concentration over the past 1–3 weeks in diabetic patients, serving as a sensitive marker for short-term glycemic control in diabetic patients. Hepatic glycogen, a polysaccharide stored in liver cells as an energy reserve, plays a pivotal role in stabilizing blood glucose levels by breaking down into glucose to supply energy. Insulin, secreted by pancreatic β-cells, is the sole hormone capable of lowering blood glucose levels while promoting the synthesis of glycogen, lipids, and proteins. Consequently, SI levels are vital for the diagnosis, therapeutic evaluation, and prognosis in diabetes.

Thus, the findings of the present study indicate that alloxan significantly elevated GSP and FBG levels while reducing HG and SI levels in mice. Conversely, the metformin and HDP significantly reduced GSP and FBG levels and increased hepatic glycogen and serum insulin levels, with the high- dose HDP group showing better effects than that in HDP-L group. The results are consistent with previous studies [21]. Research has shown that GSP correlates with other indicators of glycemic control in diabetic patients [22]. Therefore, the findings suggest that both metformin and HDP play a crucial role in regulating blood glucose levels. Additionally, the results of lipid-related biomarkers indicate that metformin and HDP significantly ameliorated dyslipidemia, this may reduce the risk of cardiovascular complications in diabetic conditions. Hyperglycemic conditions result in metabolic disturbances and functional impairments in body, notably in liver and pancreas. The liver orchestrates carbohydrate metabolism through dual regulatory mechanisms of glycogen synthesis and gluconeogenesis to establish glucose homeostasis. The pancreas is a key regulator of blood glucose level through the insulin secreted by islet β-cells, which plays a vital role in maintaining the balance of glucose metabolism [23,24]. In this study, alloxan mice exhibited histopathological alterations in hepatic and pancreatic tissues, marked by cellular hypertrophy, inflammatory infiltration, islet atrophy, and extensive β-cell destruction. The findings provide the evidence supporting the potential application of HDP in diabetes management.

SDH and MDH, as the marker enzymes of mitochondrial, cooperate with each other in mitochondrial metabolism to maintain the cell's energy supply and metabolic balance. Elevated levels of antioxidant substances may positively influence glycemic control. Excessive free radicals are closely associated with the pathogenesis and progression of diabetes, and antioxidants could potentially mitigate disease progression by neutralizing these free radicals. Furthermore, antioxidants may enhance the insulin sensitivity, thereby contributing to the stabilization of blood glucose levels. GSH-Px serves as a key role in antioxidant capacity. SOD plays a pivotal role in maintaining oxidative-antioxidative equilibrium [25,26]. GSH exhibits direct antioxidant properties. CAT, accounting for approximately 40% of peroxisomal enzyme content, acts as a marker enzyme for peroxisomes [27]. The results of the present study reflect HDP enhance antioxidant defenses to facilitate glycemic control.

Diabetes can cause immune system disorders, which can increase the risk of infections or other diseases. TNF-α plays an important role in the pathological damage of certain autoimmune diseases. Elevated TNF-α is due to the development of an inflammatory response and the possible presence of tumor markers. High levels of IL- 6 suggest possible inflammation or immunodeficiency [28]. These results showed that metformin and HDP could significantly reduce the levels of TNF-α and IL-6, suggesting that the inflammatory response in mice was effectively alleviated. This finding provides an experimental basis for the potential role of HDP in regulating diabetes-related immune disorders and inflammatory responses.

Diabetes is primarily characterized by disorders of glucose metabolism and decreased insulin secretion. As the central organ regulating blood glucose homeostasis, the liver modulates the expression of numerous hepatic genes through insulin signaling pathways [29]. GK is predominantly localized in pancreatic β-cells, hepatocytes, hypothalamus, and gastrointestinal tract, which catalyzes the phosphorylation of hexoses to hexose-6-phosphate. By converting glucose to glycogen, GK critically governs hepatic glucose utilization, with its enzymatic activity directly determining glucose conversion rates [30]. As a rate-limiting enzyme in glycolysis, PK drives glucose oxidation by catalyzing the conversion of phosphoenolpyruvate to pyruvate [31]. GLUT2 participates in glucose metabolism, and its inhibitory effect can lead to abnormal glucose output, affecting insulin secretion. GLUT2 plays a pivotal role in glucose homeostasis by mediating glucose efflux into the bloodstream during hyperglycemia, thereby exacerbating elevated blood glucose levels [32]. The observed downregulation of GK, PK and GLUT2 in diabetic conditions aligns with their roles in promoting hyperglycemia, consistent with our findings in alloxan mice. The significant upregulation of these markers following metformin and HDP treatments suggests the potential activation of GK, PK, and GLUT2 activities.

AMPK plays a pivotal role in maintaining systemic energy homeostasis [33]. AMPK reduces peripheral insulin resistance through modulation of glucose and lipid metabolism. Substantial evidence confirms its capacity to suppress hepatic glucose output and reduce fasting blood glucose [34]. Mechanistically, AMPK activation downregulates PEPCK and G6Pase expression, thereby inhibiting hepatic gluconeogenesis [35,36]. In alloxan induced mice, a significant reduction in AMPK levels accompanied by elevated PEPCK and G6Pase expression, collectively contributing to hyperglycemia. Both metformin and HDP treatments reversed the similar changes, suggesting AMPK signaling pathway activation as a potential mechanism for glycemic regulation. Analogously, catalpol from *Rehmannia glutinosa* and ethyl acetate extracts of *Morus alba* fruits exhibit hypoglycemic effects via AMPK activation [37,38], underscoring AMPK's pivotal role in glucose homeostasis and its therapeutic potential as a molecular target.

Notably, high-dose HDP indicated superior efficacy to its low-dose counterpart in modulating diabetic hyperglycemia. As a first-line therapy for T2DM, metformin reduces hepatic glucose production and lowers FBG [39], consistent with its observed ameliorative effects on hyperglycemia in alloxan induced mice in this study. This study demonstrates that HAP significantly ameliorated weight loss, glycemic parameters, lipid metabolism biomarkers, antioxidant markers, immune markers of TNF-α and IL-6 in alloxan induced mice. Molecular analyses revealed upregulated mRNA expression of GK, PK, GLUT2, and AMPK, coupled with downregulated G6Pase and PEPCK, implicating AMPK signaling pathway activation in glucose regulation. These findings establish HDP as a potent modulator of glucose homeostasis via AMPK activation, providing scientific and translational evidence for its therapeutic potential in diabetes management.

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Conflict of Interest

The authors declare that they have no conflicts of interest.

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