

# Treatment with astragaloside IV reduced blood glucose, regulated blood lipids, and protected liver function in diabetic rats

Journal of International Medical Research

49(3) 1–14

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DOI: 10.1177/0300060519841165

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## Abstract

**Objectives:** To investigate the effects of astragaloside IV on blood glucose, blood lipids, and liver function in diabetic rats.

**Methods:** Fifty diabetic rats were randomly placed into five groups ( $n = 10$  each): the diabetes mellitus (DM) group received intragastric saline, the metformin hydrochloride group received intragastric metformin hydrochloride, and the astragaloside-30, -60, and -120 groups received intragastric astragaloside 30 mg/kg, 60 mg/kg, and 120 mg/kg for 28 days, respectively. Ten non-diabetic rats received intragastric saline as controls.

**Results:** Relative to the DM group, fasting blood glucose, triglyceride, total cholesterol, serum alanine transaminase, and serum aspartate aminotransferase levels decreased in the astragaloside-60 and astragaloside-120 groups; serum alkaline phosphatase decreased solely in the astragaloside-120 group. Serum superoxide dismutase (SOD), glutathione (GSH-Px), and catalase (CAT) levels were elevated, while maleic dialdehyde (MDA) decreased in the astragaloside-120 group, relative to the DM group. Relative to the DM group, the liver index and liver cell apoptosis rate were reduced, while histopathological changes in liver tissue were ameliorated in the astragaloside groups; moreover, liver tissue SOD, GSH-Px, and CAT levels were increased, while liver tissue MDA was reduced.

**Conclusions:** Astragaloside IV can lower blood glucose, regulate blood lipids, and protect liver function in diabetic rats.

## Keywords

Astragaloside IV, diabetes mellitus, blood glucose, blood lipids, oxidative stress, liver function protection, rat model

Date received: 20 September 2018; accepted: 11 March 2019

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## Introduction

Diabetes mellitus (DM) is a worldwide threat to public health: in 2015, there were an estimated 415 million DM patients; this number is predicted to increase to 642 million by 2040.<sup>1,2</sup> DM is characterized by pathological features that include hyperinsulinemia, insulin resistance, and pancreatic  $\beta$ -cell dysfunction. Additionally, DM patients have increased risks of severe and fatal complications, such as diabetic nephropathy and coronary heart disease.<sup>1,3</sup> Hyperglycemia-induced complications in DM patients include abnormal endothelial function, increased glycation end-product formation, hypercoagulability, elevated platelet reactivity, and sodium-glucose cotransporter-2 (SGLT-2) hyperexpression; these changes result in micro and macrovascular diseases.<sup>2</sup> In addition, liver damage due to oxidative stress can critically endanger the lives of DM patients.<sup>4-8</sup> Although treatment of DM has progressed in recent years because of the discovery of novel potential treatment targets, there remain many difficulties.<sup>9-11</sup> For instance, metformin is commonly used for treatment of DM; however, it has been associated with vitamin B deficiency and cannot be used in DM patients with poor renal function.<sup>12</sup>

Traditional Chinese medicine has been used for treatment of DM for thousands of years. Astragaloside is a major component of *Astragalus membranaceus*, a common ingredient in the formula for DM treatment in traditional Chinese medicine; notably, astragaloside is effective in reducing inflammation and enhancing immunity.<sup>13</sup> There is preliminary evidence to support the use of astragaloside in the treatment of DM because it has shown an ability to reduce cellular injuries and apoptosis caused by high glucose exposure in vitro, and has been shown to influence podocyte apoptosis in an animal model of diabetic nephropathy.<sup>14-16</sup> However, in

vivo evidence to support the use of astragaloside in treatment of DM remains limited. Therefore, our study aimed to investigate the effect of astragaloside IV on blood glucose reduction, blood lipid regulation, and liver function protection in a rat model of DM.

## Methods

### DM rat model construction

Eight-week-old male Wistar rats (weight: 180–220 g) were purchased from the Experimental Animal Center of Hebei Province (Hebei, China) with license No. SCXK 2008-1-003. The type 2 DM rat model was generated as follows: rats were fed a high-glucose and high-lipid diet (containing 10% lard, 20% sucrose, 1% cholate, 2.5% cholesterol, and 66.5% normal rat diet) for 8 weeks; they were then injected with streptozotocin (STZ) 60 mg/kg. Fasting blood glucose (FBG) in each rat was measured in triplicate at 72 h post-STZ injection; a sustained FBG level  $> 16.7$  mmol/L was considered indicative of successful induction of DM.

### Ethics approval

The study was approved by the Animal Ethics Committee of the Cangzhou Central Hospital with approval number (2016)-Ethical Approved-(008); all related experiments were conducted in accordance with the “Code for the Care and Use of Animals for Scientific Purposes” and under the principles of replacement, refinement, and reduction.

### Treatments

Fifty DM rats were randomly placed into five groups ( $n = 10$  each): DM group, metformin hydrochloride (HCL) group, astragaloside-30 group, astragaloside-60 group, and astragaloside-120 group.

Astragaloside was first dissolved in 5% dimethyl sulfoxide, then dissolved in normal saline; metformin HCL was dissolved directly in normal saline. In the DM group, saline was administered through an intragastric route, once per day for 28 days; in the metformin HCL group, metformin HCL 200 mg/kg was administered through an intragastric route, once per day for 28 days; in the astragaloside-30 group, astragaloside IV ( $C_{41}H_{68}O_{14}$ ) (Chengdu JinTaiHe Pharmaceutical Chemical Co., Ltd., Chengdu, China) 30 mg/kg was administered through an intragastric route, once per day for 28 days; in the astragaloside-60 group, astragaloside 60 mg/kg was administered through an intragastric route, once per day for 28 days; and in the astragaloside-120 group, astragaloside 120 mg/kg was administered through an intragastric route, once per day for 28 days. In addition, the control group comprised 10 normal rats that received normal saline via intragastric administration, once per day for 28 days.

### **Detection of blood FBG**

Blood samples were obtained from the tail vein at 0 (D0), 7 (D7), 14 (D14), 21 (D21), and 28 (D28) days after initiation of treatment, and FBG levels were determined in blood samples from all time points using ACCU-CHEK Performa Connect (Roche, Basel, Switzerland).

### **Detection of blood lipid concentrations and liver function indices**

For detection of blood lipid concentrations and liver function indices, serum was separated from the blood samples by centrifugation at  $620 \times g$  for 15 minutes at room temperature. Blood lipids and liver function indices were determined in blood samples from D28 only, using an automated

Biochemist Analyzer (Mairui Medical Company, Shenzhen, China). Lipids included triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C); liver function indices included alanine transaminase (ALT), aspartate aminotransferase (AST), and alkaline phosphatase (ALP)

### **Detection of serum oxidative-stress indices**

For the detection of superoxide dismutase (SOD), glutathione (GSH-Px), catalase (CAT), and maleic dialdehyde (MDA) levels, serum was separated from blood samples as above. SOD, GSH-Px, CAT, and MDA were detected in blood samples from D28 only, using an ultraviolet-visible spectrophotometer (Shanghai Tianshi Scientific Instrument, Shanghai, China) in accordance with the manufacturer's instructions. The kits used for detection of SOD, GSH-Px, CAT, and MDA were as follows: the Cu/Zn SOD Human ELISA Kit (Invitrogen, Carlsbad, CA, USA), Glutathione Colorimetric Detection Kit (Invitrogen), Human Catalase ELISA Kit (Abcam, Cambridge, UK), and Malondialdehyde (MDA) Assay Kit (Nanjing Jiancheng Bioengineering Institute, Nanjing, China), respectively.

### **Detection of liver index and liver hematoxylin-eosin (H&E) staining**

Rats were sacrificed by exsanguination at D28 and their livers were harvested and weighed. The liver index was calculated using the formula: liver index = (liver weight/body weight)  $\times$  100%. Liver tissue samples were fixed in 4% paraformaldehyde and dehydrated with alcohol, then cleared with xylene. Cleared liver tissue samples were embedded in paraffin and sliced into 5 to 8- $\mu$ m slices, which were

subsequently dewaxed in xylene. Liver slices then underwent conventional H&E staining. Stained samples were dehydrated in alcohol and cleared with xylene, then mounted with resin.

### Apoptosis detection

Liver tissue was cut into pieces of roughly 1 mm<sup>3</sup>, and apoptosis of liver cells was assayed using the One-Step TUNEL Apoptosis Assay Kit (Roche), in accordance with the manufacturer's instructions; the rate of apoptosis was calculated using ImageJ Software (National Institutes of Health, Bethesda, MD, USA).

### Detection of liver oxidative-stress indices

Liver tissue was cut into pieces of roughly 1 mm<sup>3</sup>, and 100 µl RIPA Lysis and Extraction Buffer (Thermo Fisher Scientific, Waltham, MA, USA) was added. The tube was then placed on ice for 5 minutes with occasional shaking. Subsequently, the lysed liver tissue was centrifuged at 10,000 × g for 5 minutes at 4°C, followed by acquisition of liquid supernatant containing intracellular proteins. Levels of SOD, GSH-Px, CAT, and MDA in liver tissue at D28 were determined using the kits previously described for analysis

of serum (Cu/Zn SOD Human ELISA Kit, Glutathione Colorimetric Detection Kit, Human Catalase ELISA Kit, MDA assay kit) with an ultraviolet-visible spectrophotometer.

### Statistics

Statistical analysis was performed using SPSS 21.0 software (IBM, Armonk, NY, USA) and graphs were generated by using GraphPad Prism 5.01 (GraphPad, La Jolla, CA, USA). Data are shown as mean ± standard deviation. Comparisons between two groups were performed by one-way analysis of variance. P < 0.05 was considered to be statistically significant.

## Results

### Effect of astragaloside IV on FBG

As shown in Table 1, FBG levels in the DM group were increased at D0, D7, D14, D21, and D28, compared with those of the control group (all P < 0.001), confirming successful generation of DM model rats. After treatment, FBG levels in the metformin HCL group were reduced at D0, D7, D14, D21, and D28, compared with those of the DM group (all P < 0.001). FBG levels in the astragaloside-30 group did not

**Table 1.** Comparison of fasting blood glucose at each time point among groups.

Group	Dose (mg/Kg)	D0 (mmol/L)	D7 (mmol/L)	D14 (mmol/L)	D21 (mmol/L)	D28 (mmol/L)
Control	–	6.07 ± 0.92	6.18 ± 0.86	6.14 ± 0.94	6.12 ± 0.98	6.10 ± 0.88
DM	–	17.3 ± 2.4***	17.2 ± 2.6***	17.0 ± 2.5***	17.2 ± 2.7***	17.1 ± 2.5***
Metformin HCL	200	17.3 ± 2.3	11.7 ± 2.4####	9.8 ± 2.2####	9.4 ± 2.1####	9.2 ± 2.3####
Astragaloside	30	17.2 ± 2.5	16.5 ± 2.7&&&	16.3 ± 2.8&&&	16.2 ± 3.0&&&	15.8 ± 2.9&&&
	60	17.2 ± 2.3	16.1 ± 2.8&&	15.2 ± 3.2&&&	14.6 ± 3.1&&&	12.8 ± 2.7###&&
	120	17.4 ± 2.4	13.5 ± 3.0###	11.3 ± 2.7####	10.4 ± 2.6####	9.3 ± 2.5####

Data shown are mean ± standard deviation. Comparison between two groups at each time point, as determined by one-way analysis of variance. \*\*\*, P < 0.001, comparison between DM and control groups; ###, P < 0.01, ####, P < 0.001, comparison between the metformin HCL group/groups with various doses of astragaloside and the DM group; &&, P < 0.01, &&&, P < 0.001, comparison between groups with various doses of astragaloside and the metformin HCL group. DM, diabetes mellitus; HCL, hydrochloride.

significantly differ at D0, D7, D14, D21, or D28, compared with those of the DM group. In the astragaloside-60 group, FBG level was only reduced at D28 ( $P < 0.01$ ), compared with that of the DM group. In the astragaloside-120 group, FBG levels were reduced at all time points (all  $P < 0.05$ ), compared with those of the DM group. Furthermore, FBG levels in the astragaloside-30 and astragaloside-60 groups significantly differed from those in the metformin HCL group, whereas FBG levels in the astragaloside-120 group did not.

### *Effect of astragaloside IV on serum lipids*

Compared with those in the control group, TG, TC, and LDL-C levels were elevated in the DM group ( $P < 0.001$ ), while HDL-C level was reduced ( $P < 0.05$ ) (Figure 1). Compared with that in the DM group, TG levels in the astragaloside-60 and astragaloside-120 groups were significantly reduced ( $P < 0.01$  and  $P < 0.001$ , respectively); moreover, TG level was reduced in the astragaloside-120 group, compared with that in the metformin HCL group ( $P < 0.05$ ) (Figure 1a). As shown in Figure 1b, TC levels in the metformin HCL, astragaloside-60, and astragaloside-120 groups were all lower than that in the DM group ( $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$ , respectively). LDL-C levels in the metformin HCL, astragaloside-30, astragaloside-60, and astragaloside-120 groups did not significantly differ from that of the DM group (Figure 1c). HDL-C levels in the metformin HCL, astragaloside-30, astragaloside-60, and astragaloside-120 groups did not significantly differ from that of the DM group (Figure 1d).

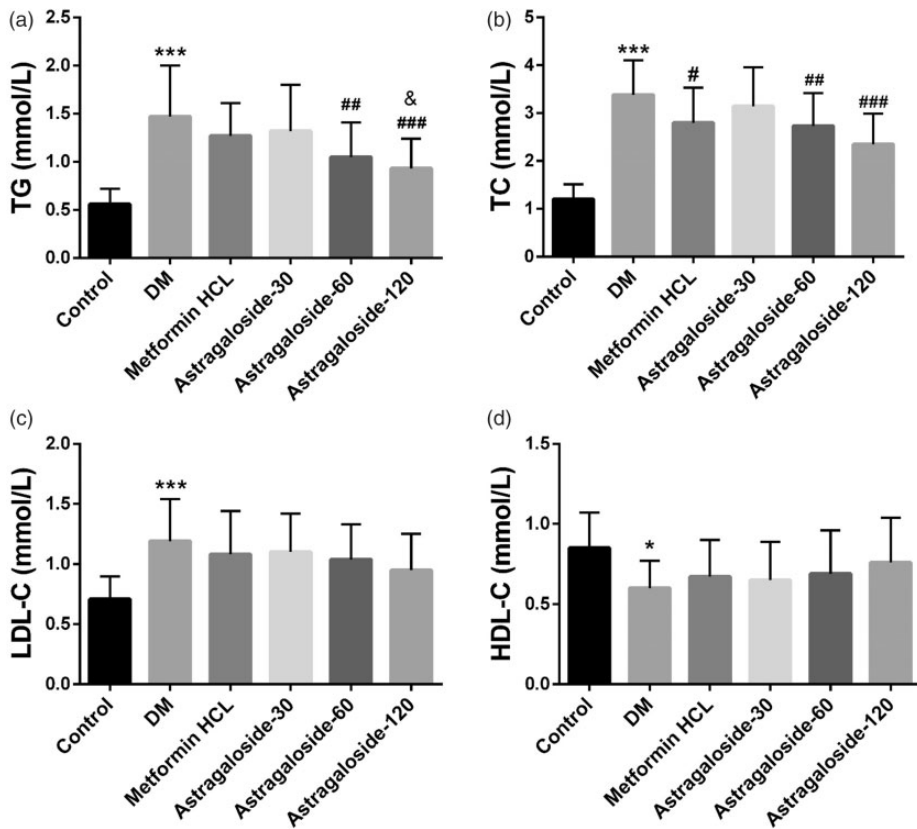
### *Effect of astragaloside IV on serum liver function indices*

Compared with those in the control group, serum ALT, AST, and ALP levels

all significantly increased in the DM group ( $P < 0.001$ ; Figure 2). As shown in Figure 2a, blood ALT levels in the metformin HCL, astragaloside-60, and astragaloside-120 groups were lower than those of the DM group ( $P < 0.01$ ,  $P < 0.05$ , and  $P < 0.001$ , respectively). AST levels were reduced in the metformin HCL, astragaloside-60, and astragaloside-120 groups, compared with that in the DM group ( $P < 0.05$ ,  $P < 0.01$ , and  $P < 0.001$ , respectively; Figure 2b). The ALP level was significantly reduced solely in the astragaloside-120 group, compared with that in the DM group ( $P < 0.01$ ; Figure 2c)

### *Effect of astragaloside IV on serum oxidative-stress indices*

As shown in Figure 3, serum levels of SOD, GSH-Px and CAT were all reduced in the DM group ( $P < 0.001$ ), compared with that in the control group; conversely, the MDA level was significantly increased in the DM group ( $P < 0.001$ ). As shown in Figure 3a, serum SOD levels were elevated in the astragaloside-60 and astragaloside-120 groups, compared with that in the DM group ( $P < 0.001$ ); moreover, serum SOD levels were increased in the astragaloside-60 and astragaloside-120 groups, compared with that in the metformin HCL group. The GSH-Px level was solely elevated in the astragaloside-120 group, compared with that in the DM group ( $P < 0.01$ ) (Figure 3b); additionally, the CAT level was increased in the astragaloside-120 group, compared with that in the DM group ( $P < 0.05$ ) (Figure 3c). MDA serum levels significantly decreased in the metformin HCL, astragaloside-30, astragaloside-60, and astragaloside-120 groups, compared with that in the DM group ( $P < 0.001$ ,  $P < 0.05$ ,  $P < 0.001$ , and  $P < 0.001$ , respectively; Figure 3d).



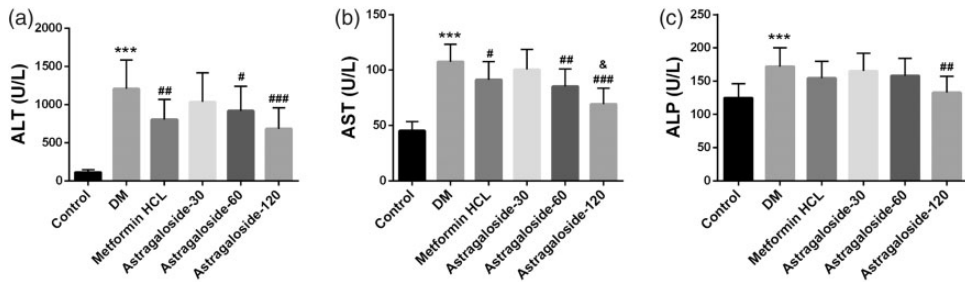
**Figure 1.** Triglyceride (TG), total cholesterol (TC), low-density lipoprotein cholesterol (LDL-C), and high-density lipoprotein cholesterol (HDL-C) levels after treatment. TG levels in astragaloside-60 and astragaloside-120 groups were lower than that in the control group; compared with that in the metformin hydrochloride (HCL) group, the TG level was lower in the astragaloside-120 group (a). TC levels in metformin HCL, astragaloside-60, and astragaloside-120 groups were all reduced, compared with that in the diabetes mellitus (DM) group (b). However, LDL-C levels in the metformin HCL, astragaloside-30, astragaloside-60, and astragaloside-120 groups were similar to that in the DM group (c). The HDL-C levels in the metformin HCL, astragaloside-30, astragaloside-60, and astragaloside-120 groups did not significantly differ from those in the DM group (d). Comparisons between two groups were determined by one-way analysis of variance.  $P < 0.05$  was considered to be statistically significant. \* $P < 0.05$ , \*\*\* $P < 0.001$ , comparison between the DM and control groups. # $P < 0.05$ , ### $P < 0.01$ , #### $P < 0.001$ , comparison between each treatment group and DM group. & $P < 0.05$ , comparison between each astragaloside treatment group and metformin HCL group.

TG, triglyceride; TC, total cholesterol; LDL-C, low-density lipoprotein cholesterol; HDL-C, high-density lipoprotein cholesterol; DM, diabetes mellitus; HCL, hydrochloride.

### Effect of astragaloside IV on the liver index and histopathological feature of liver tissue

As shown in Figure 4, body weight was reduced while the liver index was increased

in the DM group, compared with those measurements in the control group. As shown in Figure 4a, body weights in the metformin HCL, astragaloside-60, and astragaloside-120 groups were significantly higher than that in the DM group



**Figure 2.** Liver function indices in blood after treatment. Blood alanine transaminase (ALT) levels in metformin hydrochloride (HCL), astragaloside-60, and astragaloside-120 groups were reduced, compared with that in the diabetes mellitus (DM) group (a). Blood aspartate aminotransferase (AST) levels were reduced in the metformin HCL, astragaloside-60, and astragaloside-120 groups, compared with that in the DM group (b); blood AST level was lower in the astragaloside-120 group than in the metformin HCL group. Additionally, blood alkaline phosphatase (ALP) level was reduced in the astragaloside-120 group, compared with that in the DM group (c). Comparisons between two groups were determined by one-way analysis of variance.  $P < 0.05$  was considered to be statistically significant. \*\*\* $P < 0.001$ , comparison between DM and control groups. # $P < 0.05$ , ## $P < 0.01$ , ### $P < 0.001$ , comparison between each treatment group and the DM group. & $P < 0.05$ , comparison between each astragaloside treatment group and metformin HCL group. DM, diabetes mellitus; ALT, alanine transaminase; HCL, hydrochloride; AST, aspartate aminotransferase; ALP, alkaline phosphatase.

( $P < 0.001$ ). The liver index was significantly reduced solely in the astragaloside-120 group, compared with that in the DM group ( $P < 0.05$ ; Figure 4b). H&E staining revealed pathological features in liver tissue: compared with the control group, the DM group exhibited disoriented hepatic lobule, hepatic sinusoid hyperemic edema, vacuolar hepatocyte degeneration, nonuniform cytoplasmic staining, and hepatocyte nucleus pyknosis (Figure 4c). Compared with the DM group, histopathological changes of liver tissue were ameliorated in the metformin HCL, astragaloside-30, astragaloside-60, and astragaloside-120 groups.

### Effect of astragaloside IV on cellular apoptosis

Cellular apoptosis was assessed by TUNEL assay, and the results are shown in Figure 5a. The cellular apoptosis rate was increased in the DM group, compared with the control group ( $P < 0.001$ ; Figure 5b). In addition, cellular apoptosis rates

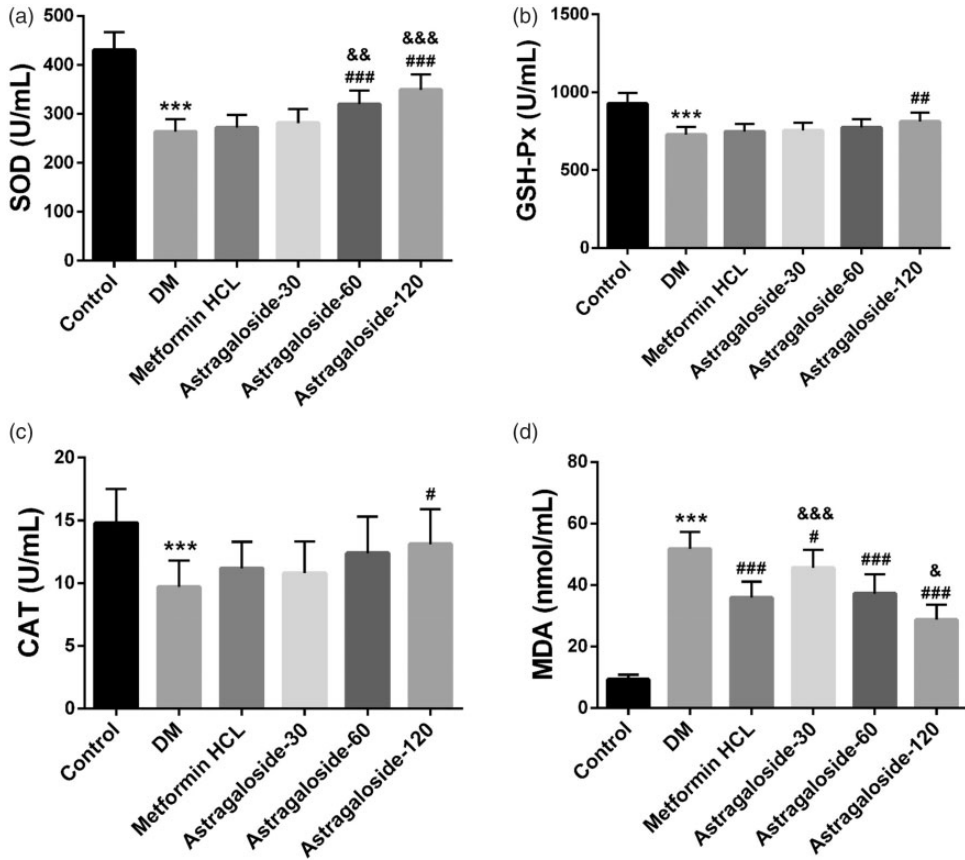
were reduced in the metformin HCL, astragaloside-30, astragaloside-60, and astragaloside-120 groups, compared with that in the DM group ( $P < 0.001$ ,  $P < 0.01$ ,  $P < 0.001$ , and  $P < 0.001$ , respectively).

### Effect of astragaloside IV on liver tissue oxidative-stress indices

As shown in Figure 6, liver tissue SOD, GSH-Px, and CAT levels were significantly reduced in the DM group, compared with the control group; in contrast, liver tissue MDA level was significantly elevated in the DM group. Liver tissue SOD, GSH-Px, and SOD levels were significantly elevated in the astragaloside-120 group, compared with the DM group (Figure 6a–c).

## Discussion

Astragaloside IV is a primary component of the well-known traditional Chinese medicine, *Astragalus membranaceus*; multiple studies have shown that administration of astragaloside IV leads to a reduction of

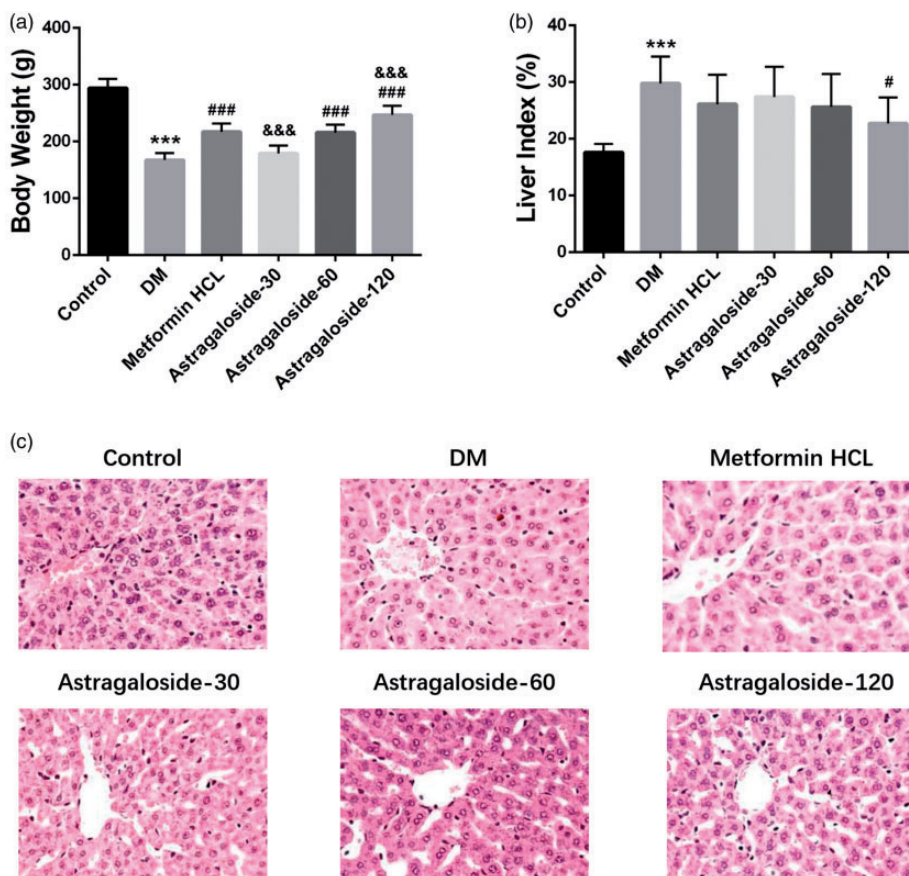


**Figure 3.** Blood oxidative-stress indices after treatment. Blood serum superoxide dismutase (SOD) levels were higher in the astragaloside-60 and astragaloside-120 groups than in the diabetes mellitus (DM) group; blood SOD levels also increased in the astragaloside-60 and astragaloside-120 groups, compared with that in the metformin hydrochloride (HCL) group (a). Blood glutathione peroxidase (GSH-Px) level was increased in the astragaloside-120 group, compared with that in the DM group (b). In addition, blood catalase (CAT) level was elevated in the astragaloside-120 group, compared with that in the DM group (c). Blood maleic dialdehyde (MDA) levels were reduced in the metformin HCL, astragaloside-30, astragaloside-60, and astragaloside-120 groups, compared with that in the DM group (d). The MDA level was lower in the astragaloside-120 group than in the metformin HCL group; conversely, MDA level was increased in the astragaloside-30 group, compared with that in the metformin HCL group. Comparisons between two groups were determined by one-way analysis of variance.  $P < 0.05$  was considered to be statistically significant. <sup>\*\*\*</sup> $P < 0.001$ , comparison between DM and control groups. <sup>##</sup> $P < 0.05$ , <sup>###</sup> $P < 0.01$ , <sup>####</sup> $P < 0.001$ , comparison between each treatment group and DM group. <sup>&</sup> $P < 0.05$ , <sup>&&</sup> $P < 0.01$ , <sup>&&&</sup> $P < 0.001$ , comparison between each astragaloside treatment group and metformin HCL group. DM, diabetes mellitus; SOD, serum superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; MDA, maleic dialdehyde; HCL, hydrochloride.

blood glucose and regulation of blood lipids in DM. A recent experiment demonstrated that astragaloside IV repressed adipose lipolysis and reduced hepatic glucose

production through Akt-dependent phosphodiesterase 3B expression in mice fed a high-fat diet; this prevented lipid accumulation and extrahepatic glucose production.<sup>17</sup>

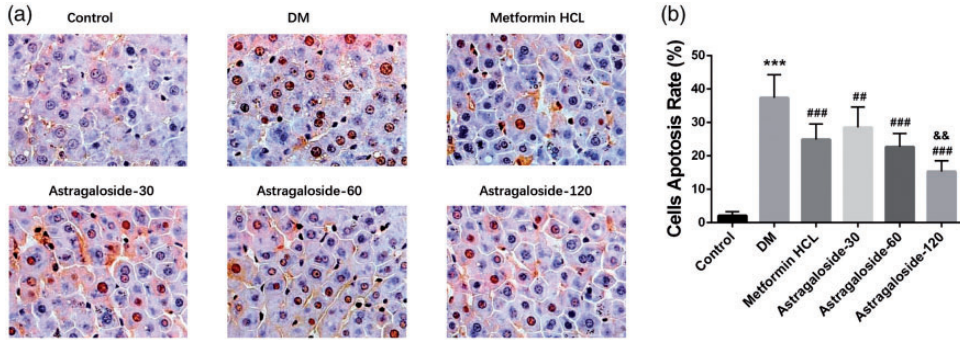




**Figure 4.** Body weight, liver index, and histopathological changes in liver tissue after treatment. Body weights in metformin hydrochloride (HCL), astragaloside-60, and astragaloside-120 groups were elevated, compared with that in the diabetes mellitus (DM) group (a). Compared with that in the metformin HCL group, body weight was reduced in the astragaloside-30 group, whereas it was increased in the astragaloside-120 group. Liver index was reduced in the astragaloside-120 group, compared with that in the DM group (b). Regarding histopathological changes in liver tissue, no pathological changes in liver tissue or hepatocytes were present in the control group; in contrast, the DM group showed disoriented hepatic lobule, hepatic sinusoid hyperemic edema, vacuolar hepatocyte degeneration, nonuniform cytoplasm staining, and hepatocyte nucleus pyknosis (c). Pathological changes in the metformin HCL, astragaloside-30, astragaloside-60, and astragaloside-120 groups were ameliorated, compared with those in the DM group. Comparisons between two groups were determined by one-way analysis of variance.  $P < 0.05$  was considered to be statistically significant. \*\*\* $P < 0.001$ , comparison between DM and control groups. # $P < 0.05$ , #### $P < 0.001$ , comparison between each treatment group and DM group. &&& $P < 0.001$ , comparison between each astragaloside treatment group and metformin HCL group. HCL, hydrochloride; DM, diabetes mellitus.

Furthermore, astragaloside IV promoted glucose transport through the insulin receptor substrate (IRS)1/protein kinase B (AKT) pathway and repressed the  $\kappa$ B

kinase (IKK)/inhibitor- $\kappa$ B $\alpha$  (I $\kappa$ B $\alpha$ ) pathway in C2C12 myotubes.<sup>18</sup> Lv et al.<sup>19</sup> showed that astragaloside IV could reduce blood glucose in DM mice, potentially

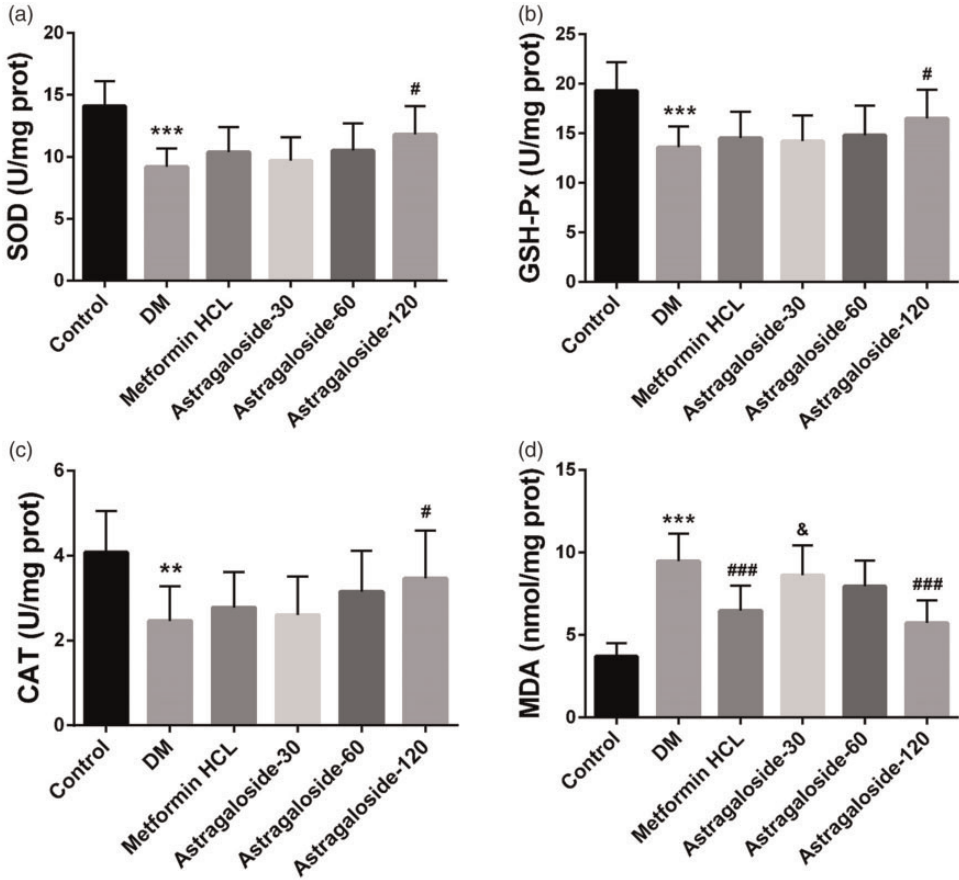


**Figure 5.** Cellular apoptosis after treatment. Cellular apoptosis was detected by One Step TUNEL Apoptosis Assay Kit (a), which showed that the rate of cellular apoptosis was increased in the diabetes mellitus (DM) group, compared with that in the control group; this suggested successful generation of the DM model (b). Rates of cellular apoptosis were lower in the metformin hydrochloride (HCL), astragaloside-30, astragaloside-60, and astragaloside-120 groups, compared with that in the DM group. Moreover, the rate of cellular apoptosis in the astragaloside-120 group was lower than that in the metformin HCL group. Comparisons between two groups were determined by one-way analysis of variance.  $P < 0.05$  was considered to be statistically significant. \*\*\* $P < 0.001$ , comparison between DM and control groups. ### $P < 0.01$ , ### $P < 0.001$ , comparison between each treatment group and DM group. && $P < 0.01$ , comparison between each astragaloside treatment group and metformin HCL group. DM, diabetes mellitus; HCL, hydrochloride.

through suppression of glycogen phosphorylase and glucose-6-phosphatase activities. Moreover, a previous study demonstrated the reduction of FBG level in diabetic nephropathy rat models upon administration of astragaloside IV.<sup>20</sup> In the present study, we found that astragaloside IV markedly reduced FBG and serum lipid levels in diabetic rats, and that its effects on blood lipids were more pronounced than those of metformin HCL. When considered in the context of the results of previous studies, our findings suggest that astragaloside IV could ameliorate hyperglycemia through multiple pathways (e.g., IKK/ $\text{I}\kappa\text{B}\alpha$  and IRS/AKT pathways) and alleviate hyperlipidemia through mediation of blood lipid metabolism-related enzymes (e.g., Akt-dependent phosphodiesterase 3B).<sup>17-19,21</sup>

Oxidative stress is a major pathological process associated with an increased risk of cardiovascular disease in DM patients, and astragaloside IV comprises a promising

regulatory treatment that may reduce damage caused by oxidative stress in various conditions.<sup>7,22</sup> A previous *in vitro* experiment revealed that astragaloside IV can protect apolipoprotein E-deficient mice from cardiac remodeling through the regulation of multiple pathways and pathological processes, including the reduction of oxidative stress through reversal of oxidant-antioxidant effects in heart tissue.<sup>23</sup> An additional *in vitro* experiment showed that astragaloside IV can protect retinal capillary endothelial cells against high glucose-induced oxidative stress through reduction of intracellular hydrogen peroxide, mitochondrial reactive oxygen species, MDA level, and nicotinamide adenine dinucleotide phosphate level; and through enhancement of total SOD (including manganese SOD), catalase, glutathione peroxidase, and glutathione content.<sup>24</sup> Astragaloside IV has also been shown to mitigate necrotizing enterocolitis through reduction of oxidative stress and inflammation through



**Figure 6.** Oxidative-stress indices in liver tissue after treatment. Liver tissue superoxide dismutase (SOD) (a), glutathione peroxidase (GSH-Px) (b), and catalase (CAT) levels (c) were elevated in the astragaloside-120 group, but not in other treatment groups, compared with those in the diabetes mellitus (DM) group. Liver tissue maleic dialdehyde (MDA) levels were reduced in the metformin hydrochloride (HCL) and astragaloside-120 groups, compared with that in the DM group; the MDA level was also lower in the astragaloside-30 group, compared with that in the metformin HCL group (d). Comparisons between two groups were determined by one-way analysis of variance.  $P < 0.05$  was considered to be statistically significant.  $**P < 0.01$ ,  $***P < 0.001$ , comparison between DM and control groups.  $\#P < 0.05$ ,  $###P < 0.001$ , comparison between each treatment group and DM group.  $\&P < 0.05$ , comparison between each astragaloside treatment group and metformin HCL group. SOD, serum superoxide dismutase; GSH-Px, glutathione peroxidase; CAT, catalase; MDA, maleic dialdehyde; DM, diabetes mellitus; HCL, hydrochloride.

the vitamin D3-upregulated protein 1/NF- $\kappa$ B signaling pathway.<sup>25</sup> The previous studies clearly demonstrated antioxidative functions of astragaloside IV. In addition, a prior animal study showed that a combination of ginsenoside and astragaloside IV

increased levels of CAT, GSH-Px, and total anti-oxidative capacity in rats with diabetic nephropathy, compared with the use of ginsenoside or astragaloside IV alone; moreover, the combined treatment resulted in more robust reduction of MDA level.<sup>26</sup>

Partially consistent with the findings of the previous study, we found that variations in doses of astragaloside IV led to marked elevations in serum levels of SOD, GSH-Px, and CAT in diabetic rats, while greatly reducing blood MDA level; the antioxidant effects of astragaloside IV in previous studies could at least partly explain our results.<sup>22,24,25,27,28</sup>

The liver is frequently affected in DM patients; importantly, it houses nearly all interactions related to lipid metabolism.<sup>29</sup> In the present study, we found that astragaloside IV exhibited protective effects with regard to liver function in diabetic rats; notably, 120 mg/kg astragaloside IV showed the strongest effect, which we suspect was caused by reduction of oxidative-stress damage in liver tissue and corresponding reduction in liver cell apoptosis. Several previous studies have shown that astragaloside IV is protective of liver function in various diseases. Notably, astragaloside IV suppressed abnormal proliferation of biliary epithelial cells by inhibition of the Notch signaling pathway, thereby blocking liver fibrosis.<sup>30</sup> Moreover, astragaloside IV alleviated free fatty acid production caused endoplasmic reticulum stress and lipid accumulation through the adenosine monophosphate-activated protein kinase (AMPK) pathway in hepatocytes.<sup>31</sup> Finally, in an experiment in diabetic rats, astragaloside IV relieved hepatic fibrosis through regulation of the protease-activated receptor-2 (PAR2) pathway.<sup>32</sup> These findings suggest that astragaloside IV prevents liver damage due to various etiologies (e.g., oxidative stress) by reducing liver fibrosis and abnormal hepatocyte activity via modulation of multiple pathways (e.g., the AMPK and PAR2 pathways).<sup>29–32</sup>

In conclusion, astragaloside IV showed a robust ability to lower blood glucose, regulate blood lipids, and protect liver function in diabetic rats.

## Declaration of conflicting interest

The authors declare that there is no conflict of interest.

## Funding

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

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