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**Hypoglycemic, hypolipidemic and antioxidant effects of  
*Sarcandra glabra* polysaccharide in type 2 diabetic mice**

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## Abstract

*Sarcandra glabra* (Thunb.) Nakai is a traditional Chinese herbal medicine and dietary supplement used for treating several diseases. The anti-diabetic activity of *S. glabra* polysaccharides was studied in this paper for the first time. *In vitro*  $\alpha$ -glucosidase inhibition assay indicated the acidic *S. glabra* polysaccharide (SGP-2) had an  $IC_{50}$  of  $87.06 \pm 11.76$   $\mu\text{g/mL}$ , which was much lower than that of acarbose at  $338.90 \pm 46.86$   $\mu\text{g/mL}$ . Moreover, high fat diet (HFD) with streptozotocin (STZ) induced diabetic mice were administered SGP-2 (150, 300, or 600 mg/kg/day, respectively) for 3 weeks. Postprandial blood glucose levels (PBGL), total cholesterol, triglyceride and free fatty acid levels in diabetic mice treated with SGP-2 were significantly decreased ( $p < 0.05$ ) compared to those of the model group. The results of oral glucose tolerance test (OGTT) and homeostasis model assessment-insulin resistance (HOMA-IR) index indicated SGP-2 could significantly improve ( $p < 0.05$ ) the insulin resistance and glucose tolerance in diabetic mice. Furthermore, the activities of antioxidant enzymes, hexokinase, and pyruvate kinase were significantly increased ( $p < 0.05$ ) in SGP-2 treated groups. Thus we proposed that SGP-2 exerted hypoglycemic activity via relieving insulin resistance, reducing postprandial blood glucose levels and ameliorating lipid metabolism, as well as alleviating oxidative stress. These data suggested that SGP-2 with anti-hyperglycemic activity could be used in medicinal preparations for diabetes mellitus and its complications.

## Keywords

*Sarcandra glabra* polysaccharide;  $\alpha$ -glucosidase; Type 2 Diabetes mellitus; Postprandial blood glucose; Hypoglycemic

Introduction

Diabetes mellitus (DM) is a hereditary, chronic metabolic disease that results from an absolute or relative deficiency of insulin secretion or impaired insulin action <sup>1</sup>. It is one of the most common chronic diseases in nearly all countries, has been considered as 1 of the 3 leading causes of death. In the next twenty years, there would be a 69% increase in numbers of adults with diabetes in developing countries and a 20% increase in developed countries <sup>2</sup>. Type 2 DM, known as non-insulin-dependent DM, leading to abnormalities in the metabolism of lipid and protein, is a serious metabolic disorder that affects more than 90% of the diabetic population. Although many different hypoglycemic drugs have been synthesized in the treatment of DM, adverse effects, contraindications and expensive prices limited their usage. Therefore, natural plants products with low toxicity and high efficiency from natural resources have been considered by researchers <sup>3</sup>. Polysaccharides as one of the active biomacromolecules obtained from many natural plants were proved to have many bioactivities including anticancer, anti-inflammatory, antioxidant, and antiviral <sup>4</sup>. Recently, polysaccharides from plants and medicinal fungi were found to possess anti-hyperglycemic activity. Wang *et al.* found a sulfated polysaccharide fucoidan extracted from *Saccharina japonica* could increase levels of serum insulin and reduce blood glucose level in alloxan diabetic mice <sup>1</sup>. A polysaccharide mainly composed of glucose from *Catathelasma ventricosum* was examined to have anti-hyperglycemic and antioxidant activity in streptozotocin (STZ)-induced diabetic mice reported by Liu *et al.* <sup>5</sup>. Wang *et al.* studied the hypoglycemic activity and possible mechanism of a water soluble  $\beta$ -D-fructan (MDG-1) from *Ophiopogon japonicas*. Their results indicated that MDG-1 had remarkable anti-diabetic activity through the Insulin receptor (InsR) / Insulin receptor substrate-1 (IRS-1) / Phosphoinositide 3-kinase (PI3K) / Glycogen synthase kinase 3 (GSK-3) / Glucose transport protein-4 (Glut-4) signaling pathway <sup>6</sup>. *Sarcandra glabra* (Thunb.) Nakai (family Chloranthaceae) is a natural source of traditional Chinese herbal medicine which grows in southern China, Japan and southeastern Asia. *S. glabra* has been proved to have effects for the treatment of cancer, inflammation, diarrhea, rheumatism and injuries <sup>7,8</sup>. Besides using as herbal medicine, *S. glabra* also was used as an herbal tea or dietary supplement for a long time <sup>7</sup>. In traditional Chinese medicine, diabetes was considered as “Xiao Ke” symptom, the herbals with anti-“Xiao Ke” effect and invigorating spleen functions could be used for diabetes treatment <sup>6</sup>. *S. glabra* was recorded to have the functions of invigorating spleen and anti-“Xiao Ke” effect according to the *Mindong of Materia Medica*, an ancient Chinese medical book, which meant it had potential to treat diabetes based on the traditional Chinese medicine theory <sup>9</sup>. However, until now there was no report either on the anti-hyperglycemic activity or the anti-diabetes active components of *S.*

*glabra*.

According to the principle that structure determines function and an example that acarbose with oligosaccharide structure is used as anti-diabetic drug by inhibiting  $\alpha$ -D-glucosidase activity<sup>10</sup>, a hypothesis is presented that the special structures of saccharides in *S. glabra* may contribute to its function. In our previous studies, we obtained two polysaccharides from *S. glabra*. coded as SGP-1 and SGP-2, respectively. SGP-1 was found to compose of glucose, galactose, and mannose with antioxidant activity determined *in vitro* while SGP-2 was determined to be an acidic polysaccharide that has five different monosaccharides in composition with anti-cancer activity determined *in vitro*<sup>8,11</sup>. As polysaccharides with complex structures may have different functions, therefore, the two polysaccharides were evaluated for their hypoglycemic activity in the present study.

## Materials and methods

### Materials and reagents

The materials of *S. glabra* were collected from Nanchang, Jiangxi Province, China and authenticated by Prof. Minjian Qin from China Pharmaceutical University (Nanjing, Jiangsu, China). Polysaccharides, SGP-1 and SGP-2, were extracted from the dried powder of *S. glabra* and purified as described previously<sup>8,11</sup>. Briefly, the dried powder (200 g) of *S. glabra* defatted and decolorized with ethanol was extracted with hot distilled water (6 L, 85°C for 3h). The filtrate was concentrated and precipitated by 4 volumes of 95% ethanol. The precipitates were freeze-dried to give the crude polysaccharide. The crude product was fractionated on DEAE-32 column eluted with distilled water and 1M NaCl solution, respectively. The major fractions from distilled water and NaCl solution were further purified using Sephacryl S-400 column eluted with water to obtain SGP-1 and SGP-2, respectively.

The kits for the assay of blood glucose (GLU), total cholesterol (TC), triglyceride (TG), low density lipoprotein cholesterin (LDL), high density lipoprotein cholesterin (HDL) and free fatty acid (FFA), the activities of superoxide dismutase (SOD), glutathione peroxidase (GSH-Px), catalase (CAT), total antioxidant capacity (TAOC), malondialdehyde (MDA) and activities of pyruvate kinase (PK) and hexokinase (HK) were purchased from Jiancheng Bioengineering Institute (Nanjing, Jiangsu, China). p-Nitrophenyl- $\alpha$ -glucopyranoside (pNPG),  $\alpha$ -glucosidase from *Saccharomyces cerevisiae*, and streptozotocin (STZ) were purchased from Sigma-Aldrich (St. Louis, MO, USA). Acarbose and Metformin (MET) were purchased from Bayer Schering Pharma (Wedding, Berlin, Germany) and Beyotime Institute of Biotechnology (Haimen, Jiangsu, China), respectively. All other chemicals used in this study were of analytical grade.

**$\alpha$ -D-glucosidase inhibitory activity and half inhibition concentration (IC<sub>50</sub>)**

The  $\alpha$ -D-glucosidase inhibitory activity was determined by measuring the release of p-nitrophenol (pNP) from pNPG according to Chapdelaine *et al.* with slight modification <sup>12</sup>. Briefly, a mixture of 160  $\mu$ L SGP-1, SGP-2 or acarbose in the concentrations range of 15.63-1000  $\mu$ g/mL and 20  $\mu$ L of 0.1 M phosphate buffer (pH 6.8) containing  $\alpha$ -D-glucosidase solution (1 U/mL) was incubated in 96 well plates at 37°C for 10 min. The enzymatic reaction was initiated by adding saturated pNPG and was incubated for another 30 min at 37°C and terminated by addition of 1 M sodium carbonate (20  $\mu$ L). Enzyme activity was determined by measuring the absorbance of the produced pNP at 405 nm in triplicate. The inhibitory rate of SGP-1, SGP-2 or acarbose on  $\alpha$ -glucosidase was calculated by the following formula:

$$\text{Inhibition percentage (\%)} = [1 - (A_{\text{sample}} - A_{\text{blank}}) / (A_{\text{control}} - A_{\text{blank}})] \times 100$$

A<sub>sample</sub>, and A<sub>control</sub> are the absorption values in the presence and absence of the SGP-1, SGP-2 or acarbose, respectively. A<sub>blank</sub> is the absorption value of the sample without the  $\alpha$ -glucosidase.

IC<sub>50</sub> values of samples were calculated by graphical method, respectively.

**Animals**

Six-week-old male C57BL/6J mice were purchased from Yangzhou University Medical Center. Animals were housed eight per cage in a controlled room temperature and humidity conditions with a 12 h light/dark cycle temperature (23-25°C). Mice were free access to diet and purified water. Regular pellet diet, consisting of 5% fat, 53% carbohydrate, 23% protein and with total calorific value 25.0 kJ/kg, while high fat diet (HFD) consisting of 22% fat, 48% carbohydrate, 20% protein with total calorific value 44.3 kJ/kg was ordered from Qinglongshan Farm in Nanjing Jiangning District, China. All animal procedures were performed in accordance with the guidelines of the institutional animal care and use of laboratory animals of China Pharmaceutical University. The experimental protocol was approved by the Animal Care and Use Committee of the School of Life Science and Technology, China Pharmaceutical University.

**Induction of diabetes in mice and study design**

The mice were allowed to adjust for one week before the experiments. For a development of type 2 diabetes, mice were fed with HFD for a period of 3 weeks. Then the mice were injected intraperitoneally with a single dose of 100 mg/kg STZ in 0.1 M citrate buffer (pH 4.5) after overnight fasting. After another 3 weeks HFD feeding, the mice with blood glucose > 11.1 mmol/L were used for further study <sup>13</sup>.

The HFD/STZ induced diabetic mice were randomly divided into six groups (8 mice per group), and normal mice were used as the control.

Group 1: non-diabetic control (Control), normal mice were fed with regular diet and intragastric administrated with 0.9% of saline (w/w).

Group 2: diabetic control (Model), diabetes mice were fed with HFD diet and intragastric administrated with 0.9% of saline (w/w).

Group 3, 4, 5: SGP-2-L, SGP-2-M, SGP-2-H, diabetes mice were fed with HFD diet and intragastric administrated with 150, 300, 600 mg/kg/day of SGP-2 dissolved in 0.9% of saline (w/w).

Group 6: Acarbose, diabetes mice were fed with HFD diet and intragastric administrated with 10 mg/kg/day of acarbose dissolved in 0.9% of saline (w/w).

Group 7: Metformin, diabetes mice were fed with HFD diet and intragastric administrated with 200 mg/kg/day of metformin dissolved in 0.9% of saline (w/w).

During 21 days of treatment, the postprandial blood glucose, body weight and food consumption were monitored weekly. On the last treatment day, the animals were fasted overnight, blood samples were collected from orbital sinus and immediately centrifuged at 3500 rpm for 15 min to obtain serum. Then all mice were sacrificed by cervical dislocation, pancreas and parts of livers excised from the animals were further studied for pathological histology by hematoxylin and eosin (HE) stain. Other parts of liver and small intestine tissues were removed promptly and stored at -70 °C.

#### Measurement of insulin sensitivity

Oral glucose tolerance test (OGTT) and homeostasis model assessment-insulin resistance (HOMA-IR) index were determined to evaluate insulin sensitivity. OGTT was performed after 12h overnight fast according to the method reported by Shirwaikar & Rajendran<sup>14</sup>. Glucose (2 g/kg) was administered by gastric perfusion and blood samples were collected from orbital sinus at 0, 15, 30, 60, 90 and 120 min, respectively. The serum obtained after centrifugation at 3500 rpm was measured for serum glucose levels using a glucose oxidase–peroxidase glucose assay kit (Jiancheng, Nanjing, China). At the end of 21 days treatment, the fasting plasma glucose and the fasting plasma insulin concentrations were measured using glucose assay kit and mouse insulin enzyme-linked immunosorbent assay (ELISA) kits (Jiancheng, Nanjing, China) respectively. HOMA-IR was calculated as follows:

$$\text{HOMA-IR} = \text{FPG (fasting plasma glucose, mmol/L)} \times \text{FINS (fasting insulin, mU/L)} / 22.5^{15}.$$

**Measurement of serum lipids**

Serum levels of TC, TG, LDL, HDL and FFA were determined according to the instructions of corresponding commercial Kits (Jiancheng, Nanjing, China).

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**Measurement of oxidative stress parameters in liver and serum**

Livers were homogenized in 0.1 g/mL of ice-cold isotonic physiological saline. The suspension was centrifuged (3000 rpm) at 4 °C for 10 min to afford the required supernatant. Total protein was determined by the BCA method. Then, the activities of SOD, GSH-Px, CAT, TAOC and MDA level of both blood serum and liver supernatant were measured following the instructions on the kits.

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**Analysis of activity of hexokinase and pyruvate kinase**

Pretreated liver supernatants from sacrificed mice were used for testing the activity of pyruvate kinase and hexokinase using the pyruvate kinase and hexokinase kit, respectively (Jiancheng, Nanjing, China).

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**Statistical analysis**

Data were expressed as mean ± SD (n = 3) and analyzed through one way analysis of variance (ANOVA) by Tukey's post-test for multiple comparisons (SPSS, 6.0V). Probability values < 0.05 and < 0.01 were regarded as statistically significant and highly significant, respectively.

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**Results and discussion**

**Inhibitory effects against α-glucosidase activities *in vitro***

Fig. 1a showed the inhibitory effects of SGP-2 and acarbose on α-glucosidase at different concentrations. Both SGP-2 and acarbose showed a dose-dependent inhibition effect on α-glucosidase. Obviously, SGP-2 exhibited stronger inhibition potential than acarbose in all tested concentrations. At a concentration of 1000 μg/mL, the inhibition effect of SGP-2 could reach to 79.30%, which was significantly higher than that of acarbose at the same concentration. The α-glucosidase inhibitory (IC<sub>50</sub>) of SGP-2 and acarbose was 87.06 ± 11.76 μg/mL and 338.90 ± 46.86 μg/mL, respectively. However, SGP-1 did not show any effect on α-glucosidase activity at all determined concentrations (data not shown). Therefore, SGP-2 was the main concerned subject in this study and was further evaluated in diabetic mice models.

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α-glucosidase is an important enzyme for carbohydrate hydrolysis, which is a membrane-bound enzyme at the



small intestine epithelia. The postprandial blood glucose level can be delayed and reduced rise by inhibition of this enzyme <sup>16</sup>. Up to now, some medicines, which have  $\alpha$ -glucosidase inhibitory effects have been developed for hypoglycemic, such as acarbose, miglitol, and voglibose. In addition, some polysaccharides from food/medicinal plant sources have been reported to have inhibitory effect on  $\alpha$ -glucosidase <sup>17,18</sup>. Bisht *et al.* reported an *Acacia tortilis* polysaccharide exhibited  $\alpha$ -glucosidase inhibitory activity both *in vitro* and *in vivo* <sup>10</sup>. Two polysaccharides extracted from *Ampelopsis grossedentata* leaves and stems presented inhibitory effects of 78.00% and 32.80% at 4 mg/mL on  $\alpha$ -glucosidase <sup>19</sup>. While Song *et al.* reported a pumpkin polysaccharide could reach to a 97.43% inhibitory rate on the concentration of 0.7-0.9 mg/mL <sup>20</sup>. SGP-2 with a low IC<sub>50</sub> value on inhibition of  $\alpha$ -glucosidase inferred that it could be one of the active components responding to hypoglycemic activity of *S. glabra*.

#### Effect of SGP-2 on blood glucose level, body weight and food intake in HFD/STZ-induced diabetic mice

The method of high-fat diet combined with low dosage of STZ injection (HFD/STZ) has usually been used to induce type 2 diabetic mice successfully <sup>21,22</sup>. SGP-2 exhibited a great  $\alpha$ -glucosidase inhibition activity from *in vitro* experiment. Thus, the anti-diabetes effect of SGP-2 was evaluated using HFD/STZ induced diabetic mice. In order to choose the right diabetic mice, we also did two groups of mice feeding with HFD alone and injecting with STZ alone, respectively. As shown in Table 1, compared with control group, HFD or STZ injection alone did not significantly affect blood glucose, whereas the HFD/STZ group appeared hyperglycemia but lower insulin concentrations than the control, suggesting the development of insulin resistance. Thus, these mice with postprandial blood glucose levels (PBGL) exceeding 11.1 mmol/L in HFD/STZ group were chosen as diabetic mice for further study.

Through *in vitro* assay, the possible anti-diabetic mechanism of SGP-2 was the inhibition of  $\alpha$ -glucosidase, thus a commonly used  $\alpha$ -glucosidase inhibitor, acarbose, was chosen to be our positive control. During the 21-d experimental period, the blood glucose levels of different experimental groups were shown in Table 2. The blood glucose concentrations of the mice in normal control group were mostly constant and were significantly lower than those of the diabetic mice in the other 5 groups. The model group exhibited a significant increase of blood glucose concentration, which was from  $15.49 \pm 2.43$  mmol/L to  $25.69 \pm 1.52$  mmol/L. The administration of SGP-2 or acarbose groups showed significant ( $p < 0.01$ ) decrease in PBGL when compared with that of model group. The PBGL in the SGP-2 groups (150, 300, 600 mg/kg/day) were 45.39%, 50.14%, and 53.72%, respectively, lower than those in the model group, while those in the acarbose control group was 51.38% lower than those in the model

group. Simultaneously, the body weight and food intake were also measured after administration of SGP-2 or acarbose, but there was no significant difference in body weight among all groups over the 21 days treatment (data not shown). The differences in food take among the 3 weeks were significant. Model group caused a remarkable increase compared to control group, while the food intake was significantly ( $p < 0.05$ ) decreased in SGP-2 groups and acarbose group than the model group (Fig. 1b). The results indicated that SGP-2 could potentially act as a blood glucose lowering agent. SGP-2 had a notable decrease in postprandial hyperglycemia, which may become an ingredient of functional foods in the future. On the other hand, we did not find any side effect in SGP-2 groups, which indicated the safety of SGP-2 administration.

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**Effects of SGP-2 on insulin resistance**

From Fig. 1c, model group presented higher HOMA-IR index ( $p < 0.01$ ) than other groups. Compared to the model group, SGP-2 groups (150, 300, 600 mg/kg/day) significantly improved the HOMA-IR index by 34.60% ( $p < 0.05$ ), 46.80% ( $p < 0.01$ ) and 54.30% ( $p < 0.01$ ), respectively, whereas acarbose significantly improved the HOMA-IR index by 58.50% ( $p < 0.01$ ). HOMA-IR is a most commonly used index of insulin resistance. HOMA-IR is more convenient for quantifying insulin resistance comparable to the gold standard glucose clamp technique<sup>23</sup>. In this study, we found that SGP-2 decreased the values of HOMA-IR to the normal levels, illustrating the ability of SGP-2 to improve the insulin resistance.

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**Effect of SGP-2 on oral glucose tolerance test**

To assess the effect of SGP-2 on glucose homeostasis in mice, OGTT was performed as an index for evaluation of islets' function. If insulin secretion is inadequate, blood glucose concentration could reach to a very high level after oral glucose in 2 hours, which illustrates the degree of glucose tolerance is decreased<sup>24</sup>. The OGTT results showed that the model group displayed significant impaired glucose tolerance while SGP-2 groups and acarbose group exerted significant decreases in blood glucose levels in diabetes mice at all the time points in 0–2 h after a single oral ingestion of glucose (2 g/kg). As shown in Fig. 1d, at 30 min, the model group presented an almost 4-fold increase of blood glucose concentration, and it was unable to return to the basal level (120 min). The SGP-2-H group decreased the postprandial glucose peak (30 min) and glucose level (120 min) by 50.70% and 60.90%, respectively, compared to that of the model group. While acarbose group only decreased the glucose peak by 40.00% and the glucose level by 59.80%. Therefore, SGP-2 could improve the glucose tolerance of diabetic mice.

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### Effects of SGP-2 on lipid metabolic parameters

Diabetes mellitus is one of the most common metabolic diseases, abnormality in lipid metabolism is often  
275 important determinant of the course and status of the disease<sup>25</sup>. To further investigate whether SGP-2 took any else  
action mechanism in the treatment of diabetes mellitus, lipid metabolic parameters were determined in all  
experimental groups. Serum TC, TG and FFA levels of model group were significantly higher than those of the  
control group, while the value of serum HDL/LDL was decreased as compared to the control group ( $p < 0.01$ )  
showing in Fig 2. As a conventional hypoglycemic drug to treat diabetes by inhibiting the activity of  $\alpha$ -glucosidase,  
280 acarbose may not show a very good hypolipidemic effect. Another positive anti-diabetic drug, metformin with  
identified effects on hypoglycemic and lipid metabolism in type 2 DM was chosen to compare with the effects of  
SGP-2<sup>28</sup>. As shown in Fig. 2a-d, SGP-2-H and metformin group highly significantly decreased the levels of TG,  
TC and FFA, increased the HDL/LDL value compared to that of the model group ( $p < 0.01$ ). While SGP-2-M  
group could significantly decrease TG, TC and FFA levels as well as increase the value of HDL/LDL compared to  
285 that of model group ( $p < 0.05$ ).

High concentrations of TG and/or TC and low ratio of HDL/LDL are considered as characteristic features of  
dyslipidemia<sup>9</sup>. According to previous reports, Hu *et al.* found the *Hedysarum polybotrys* polysaccharide could  
ameliorate hyperglycemia through altered lipid levels by lowering TC and TG concentrations while elevating the  
HDL level<sup>26</sup>. Gerald *et al.* indicated that TG decreased significantly ( $p < 0.05$ ), while TC and HDL-C contents did  
290 not change through therapy from acarbose<sup>27</sup>. In our experiments, after treatment with SGP-2 for 21 days, levels of  
TC and TG from diabetic mice were dose-dependently decreased, and the ratio of HDL/LDL increased in a  
dose-dependent manner. The results indicated that SGP-2 with beneficial effect on hyperglycemia and  
dyslipidemia differently from acarbose could also decrease the risk of coronary heart disease, atherosclerosis, and  
diabetic complications.

295 Insulin is an anti-lipolytic hormone and when the normal suppression of the release of FFA from adipose tissue is  
impaired by insulin resistance, an elevation in circulating FFA could be another characteristic marker of diabetic  
dyslipidemia occurring<sup>29</sup>. High FFA could induce atherogenic lipid profile by very-low-density lipoprotein  
over-production and alterations in glucose metabolism. Theoretically, insulin resistance of intracellular lipolysis  
may impair cellular FFA uptake and cause a higher FFA flow out of the adipocytes, which could explain in part the  
300 elevated FFA levels frequently seen in insulin resistance<sup>30</sup>. Boden has found elevated FFA levels can account for a  
large amount of insulin resistance in obese patients with type 2 DM<sup>31</sup>. Our results showed that FFA secretion was

inhibited in SGP-2 groups, especially in SGP-2 with high dosage, which was consistent with the result of HOMA-IR determination. Thus, we illustrated SGP-2 relieved insulin resistance in some extent.

305     **Effects of SGP-2 on activities of antioxidant enzymes and MDA levels in the serum and liver**

Many researchers have demonstrated that diabetes mellitus is along with oxidative damage caused by generation of reactive oxygen species. Thus, improving the activities of antioxidant enzymes including T-AOC, SOD, CAT and GSH-Px could increase the response of antioxidant defense systems to oxidative stress <sup>5</sup>. As shown in Fig. 3 a-e and Fig. 4 a-e, the activities of T-AOC, SOD, CAT and GSH-Px in liver and serum were significantly decreased in  
310     model group compared to that of the control group ( $p < 0.01$ ), indicating diabetic is associating with oxidative stress. MDA is a main product of lipid peroxidation, the level of MDA indicates the degree of lipid peroxidation. The MDA levels in both liver and serum of model group were significantly increased compared to that of control group ( $p < 0.01$ ). After administration of SGP-2 for 21 days, both middle and high dose of SGP-2 could increase the liver T-AOC and SOD activity significantly. SGP-2-H group also exhibited the significant increase of the liver  
315     CAT and GSH-Px activity compared to that of the model group. Moreover, SGP-2 could significantly decrease the liver MDA level in a dose-dependent manner. In the liver of positive control groups, acarbose group only significant decreased the MDA level, while metformin could significantly increase the activities of antioxidant enzymes and decrease the MDA level. Similar with the results in liver, metformin still had significant effects on activities of antioxidant enzymes and MDA level in serum (Fig. 4), while acarbose group only showed increased  
320     CAT activity in serum (Fig. 4c). But, SGP-2 could exhibit dose-dependently increased activity of CAT. High dose of SGP-2 also could significantly increase activities of other antioxidant enzymes. But unlike the effect of SGP-2 in liver, all three doses of SGP-2 did not exhibit any effect to the MDA level in serum. These results indicated SGP could improve the activities of antioxidant enzymes and partially reduce the MDA levels.

According to the results, the acarbose group did not show significant positive effect on these enzymes, implicating  
325     acarbose with the therapeutic dose could not be capable of increasing these enzymes' activity but SGP-2 could. To better comparison the effect of SGP-2, metformin was chosen as effectively positive control. The results showed that high dosage of SGP-2 could prevent decreases in antioxidant enzymes activity and suppress the increase in MDA level to a similar extent comparing with that of metformin. These results were consistent with previous published studies using acarbose or metformin as positive control by Kurt and Chidambaram *et al.*, respectively <sup>32</sup>,  
330     <sup>33</sup>. Kurt's results showed that acarbose could inhibit the postprandial blood glucose level, but without antioxidant activity <sup>32</sup>. Chidambaram *et al.*'s study indicated metformin could both decrease the blood level and increase the

antioxidant enzymes activity<sup>33</sup>. Thus, the effect of SGP-2 on alleviating oxidative stress also contributed to its hypoglycemic effect.

### 335 **Effects of SGP-2 on activities of pyruvate kinase and hexokinase in liver**

Previous studies reported insulin resistance is along with decreased glucose utilization and depressed activities of hepatic glycolytic enzymes<sup>34</sup>, such as hexokinase (HK) and pyruvate kinase (PK). Thus, increasing glucose utilization by increasing the activity of HK and PK could alleviate insulin resistance. As shown in Fig. 5a, SGP-2-H and metformin groups could significantly increase activity of HK compared to that of model group ( $p <$   
 340 0.05). While in the PK's activity determination, SGP-2 dose-dependently increased the activity of PK, the value of SGP-2-H even higher than that of metformin group (Fig. 5b). This result inferred that SGP-2 could decrease blood glucose through increasing glucose utilization.

HK and PK are key enzymes in glucose metabolism, and mainly are distributed in liver. HK is an important enzyme in maintaining glucose homeostasis and is considered to be a marker in regulating glucose hepatic  
 345 release/uptake. While PK is a rate-limiting enzyme for aerobic oxidation of glucose. In diabetic mice, decreased level of insulin could lead to impairment in the activity of HK and PK<sup>15, 35</sup>. Therefore, increasing glucose utilization to alleviate insulin resistance could be achieved by increasing the activity of HK and PK. Matsuda *et al.* studied the possible mechanism to be slightly up-regulate PK gene transcription of a dietary fructose<sup>36</sup>. The mechanism of SGP-2 in increasing HK and PK's activity need further deeply study.

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### **Effects of SGP-2 on pancreas and liver tissues**

As shown in Fig. 6 and Fig. 7, degenerative pathologic morphologies were observed in the pancreas and liver tissues from model groups. Especially, the number of islet cells was decreased and the diameters of pancreatic island were diminished from the HE stained pancreas tissue sections in model group. However, the control group  
 355 indicated no notable changes in pancreas histology after 3 weeks. SGP-2 treatment markedly alleviated these histopathological changes, and the damage of pancreas tissues was repaired in SGP-2 groups, acarbose and metformin group to some extent (Fig. 6). For liver tissue sections, there was clearly hepatomegaly in model group. The hepatic steatosis in the model group was characterized by ballooned hepatocytes. The administration of SGP-2, acarbose and metformin could effectively prevent the hepatic steatosis (Fig. 7).

360 Liu *et al.* reported *Catathelasma ventricosum* polysaccharides (CVPs) could protect and repair liver, kidney and pancreas tissues of STZ-induced diabetic mice<sup>5</sup>. Huang *et al.* reported the polysaccharide from *Pleurotus*

*tuber-regium* could protect pancreatic  $\beta$ -cells<sup>37</sup>. Consistent with their findings, our result showed that SGP-2 from *S. glabra* protected and repaired liver and pancreas tissues from HFD/STZ induced diabetic mice. The histopathology sliced images gave a perceptual intuition result that SGP-2 as a new purified polysaccharide could be beneficial to the management of diabetic organs damage and complications.

Conclusion

In conclusion, according to our present findings, the polysaccharide of *S. glabra* (SGP-2) exhibited high  $\alpha$ -glucosidase inhibitory activity *in vitro*. While in *in vivo* assays, SGP-2 possessed potent anti-hyperglycemic effect on HFD/STZ-induced diabetic mice for their effects of relieving insulin resistance, reducing postprandial blood glucose levels and ameliorating lipid metabolism, as well as alleviating oxidative stress. SGP-2 could be explored as the ingredient of functional foods in treatment of diabetes and complications.

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425 **Figure Captions**

**Fig. 1** Inhibitory rate of different concentrations of SGP-2 and acarbose on  $\alpha$ -glucosidase activity *in vitro* (a) , each bar represents as mean  $\pm$  SD, n=3 independent experiments. \* $p$  < 0.05, \*\* $p$  < 0.01. Effects of SGP-2 on food intake in HFD/STZ diabetic mice (b). HOMA-IR index of each group in the diabetic mice (c). An oral glucose tolerance test after 12-h fasting was performed after 3 weeks of treatment (d). Data are expressed as mean  $\pm$  SD (n=8).  
430 Model: diabetic model group; SGP-2-L: 150 mg/kg/day; SGP-2-M: 300 mg/kg/day; SGP-2-H: 600 mg/kg/day; Acarbose: 10 mg/kg/day. # $p$  < 0.01 vs. control group, \* $p$  < 0.05 vs. model group, \*\* $p$  < 0.01 vs. model group.

**Fig. 2** Effect of SGP-2 on dyslipidemia. HFD/STZ mice were treated with SGP-2 or acarbose or metformin for 3 weeks. Serum total cholesterol (TC) (a), the ratio of high density lipoprotein cholesterol and low density lipoprotein cholesterol (HDL/LDL) (b), serum triglyceride (TG) (c), and free fatty acid (FFA) (d) were determined  
435 at the end of the 3 weeks study. Data are expressed as mean  $\pm$  SD (n=8). Model: diabetic model group; SGP-2-L: 150 mg/kg/day; SGP-2-M: 300 mg/kg/day; SGP-2-H: 600 mg/kg/day; Acarbose: 10 mg/kg/day; Metformin: 200 mg/kg/day. # $p$  < 0.01 vs. control group, \* $p$  < 0.05 vs. model group, \*\* $p$  < 0.01 vs. model group.

**Fig. 3** Effect of SGP-2 on oxidative stress parameters in liver. HFD/STZ mice were treated with SGP-2 or acarbose or metformin for 3 weeks. Liver total antioxidant capacity (T-AOC) (a), liver catalase (CAT) (b), liver superoxide  
440 dismutase (SOD) (c), liver glutathione peroxidase (GSH-Px) (d), and liver malonaldehyde (MDA) (e). Data are expressed as mean  $\pm$  SD (n=8). Model: diabetic model group; SGP-2-L: 150 mg/kg/day; SGP-2-M: 300 mg/kg/day; SGP-2-H: 600 mg/kg/day; Acarbose: 10 mg/kg/day; Metformin: 200 mg/kg/day. # $p$  < 0.01 vs. control group, \* $p$  < 0.05 vs. model group, \*\* $p$  < 0.01 vs. model group.

**Fig. 4** Effect of SGP-2 on oxidative stress parameters in serum. HFD/STZ mice were treated with SGP-2 or  
445 acarbose or metformin for 3 weeks. Serum total antioxidant capacity (T-AOC) (a), serum catalase (CAT) (b), serum superoxide dismutase (SOD) (c), serum glutathione peroxidase (GSH-Px) (d), and serum malonaldehyde (MDA) (e). Data are expressed as mean  $\pm$  SD (n=8). Model: diabetic model group; SGP-2-L: 150 mg/kg/day; SGP-2-M: 300 mg/kg/day; SGP-2-H: 600 mg/kg/day; Acarbose: 10 mg/kg/day; Metformin: 200 mg/kg/day. # $p$  < 0.01 vs. control group, \* $p$  < 0.05 vs. model group, \*\* $p$  < 0.01 vs. model group.

**Fig. 5** Liver pyruvate kinase (PK) (a) and liver hexokinase (HK) (b) were determined at the end of the 3 weeks  
450 study. Data are expressed as mean  $\pm$  SD (n=8). Model: diabetic model group; SGP-2-L: 150 mg/kg/day; SGP-2-M:



300 mg/kg/day; SGP-2-H: 600 mg/kg/day; Acarbose: 10 mg/kg/day; Metformin: 200 mg/kg/day. <sup>#</sup> $p < 0.01$  vs. control group, <sup>\*</sup> $p < 0.05$  vs. model group, <sup>\*\*</sup> $p < 0.01$  vs. model group.

**Fig. 6** Effect of SGP-2 on the histological morphology of mice's pancreas by HE staining (100×). Control group showed normal pancreas (a). Model group elicited severe injury of pancreas and the islets were depletion in numbers severely (b). Treated with acarbose (10 mg/kg/day), metformin (200 mg/kg/day), and SGP-2 (150, 300 and 600 mg/kg/day) respectively reduced the degree of pancreas injuries (c-g).

**Fig. 7** Effect of SGP-2 on the histological morphology of mice's liver by HE staining (200×). Control group showed normal livers (a). Model group manifested hepatic steatosis and the hepatocytes were severe edema (b). Treated with acarbose (10 mg/kg/day), metformin (200 mg/kg/day), and SGP-2 (150, 300 and 600 mg/kg/day) respectively reduced the degree of livers damage (c-g).

Table 1

Characterization of the HFD/STZ mouse model

	Plasma glucose(mmol/L)	Plasma insulin(mU/L)
Control	9.30 ± 0.23	3.93 ± 0.75
STZ alone	12.46 ± 10.96	2.30 ± 0.15
HFD alone	12.21 ± 1.37	3.13 ± 0.18
HFD/STZ	17.54 ± 3.34*	2.03 ± 0.57*

485 Data are means ± SD (n=8). C57BL/6J mice were fed with regular diet and injected with saline (Control) or STZ  
(STZ alone) or fed with HFD and injected with saline (HFD alone) or STZ (HFD/STZ). Plasma glucose and  
plasma insulin were measured under the fed condition. \**p* < 0.05 vs. control.

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**Table 2**

Effect of SGP-2 on postprandial blood glucose level (mmol/L)

Group	0 day	7 days	14 days	21 days
Control	10.12 ± 0.62	9.88 ± 0.79	9.92 ± 0.83	10.01 ± 0.85
Model	15.49 ± 2.43 <sup>#</sup>	20.04 ± 6.39 <sup>#</sup>	24.13 ± 3.25 <sup>#</sup>	25.69 ± 1.52 <sup>#</sup>
SGP-2-L (150 mg/kg/day)	15.36 ± 3.08 <sup>#</sup>	18.90 ± 4.90 <sup>#</sup>	15.83 ± 3.38 <sup>#**</sup>	14.03 ± 1.58 <sup>**</sup>
SGP-2-M (300 mg/kg/day)	15.21 ± 1.25 <sup>#</sup>	16.54 ± 4.14 <sup>#*</sup>	13.23 ± 2.16 <sup>**</sup>	12.81 ± 1.15 <sup>**</sup>
SGP-2-H (600 mg/kg/day)	15.14 ± 1.53 <sup>#</sup>	14.61 ± 3.99 <sup>#*</sup>	12.91 ± 1.36 <sup>**</sup>	11.89 ± 1.37 <sup>**</sup>
Acarbose (10 mg/kg/day)	15.85 ± 2.26 <sup>#</sup>	15.46 ± 4.05 <sup>#*</sup>	14.09 ± 2.03 <sup>**</sup>	12.49 ± 1.64 <sup>**</sup>

520 Postprandial blood glucose levels were measured at the indicated time points. Data represent as mean ± SD (n = 8).

<sup>#</sup>*p* < 0.05 vs. control group, \**p* < 0.05 vs. model group, \*\**p* < 0.01 vs. model group.

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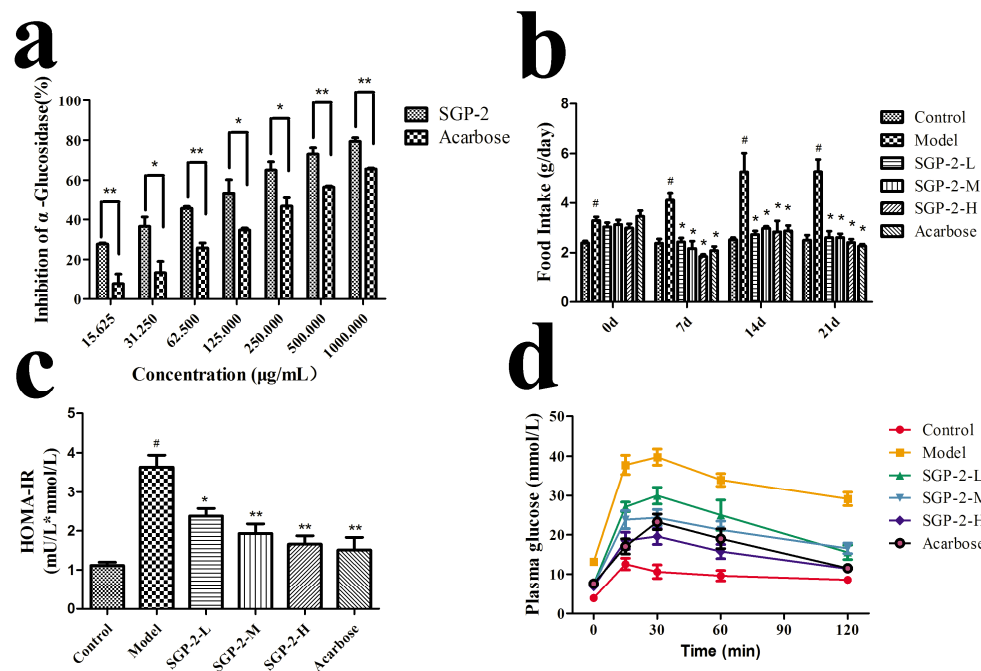


Fig. 1

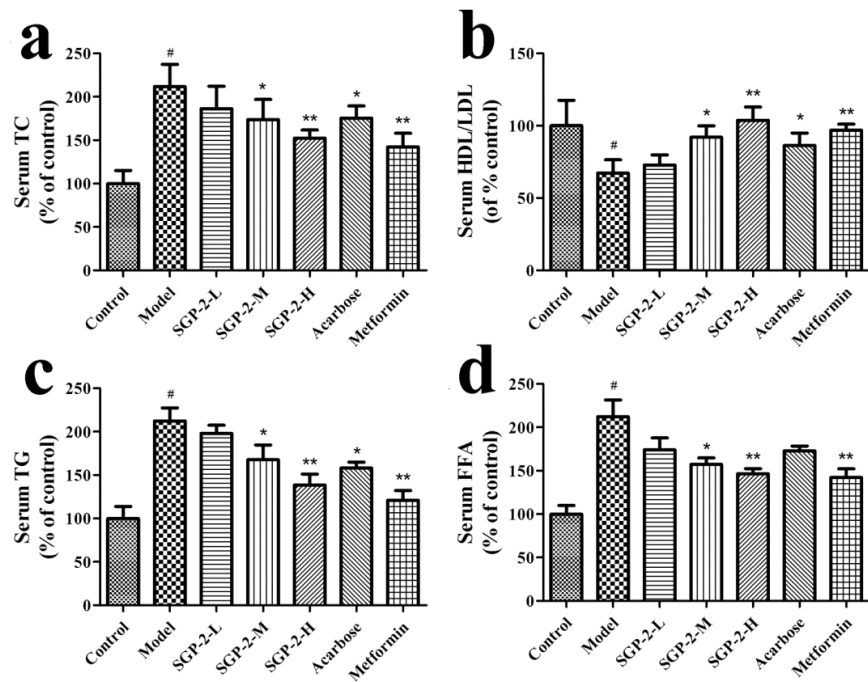


Fig. 2

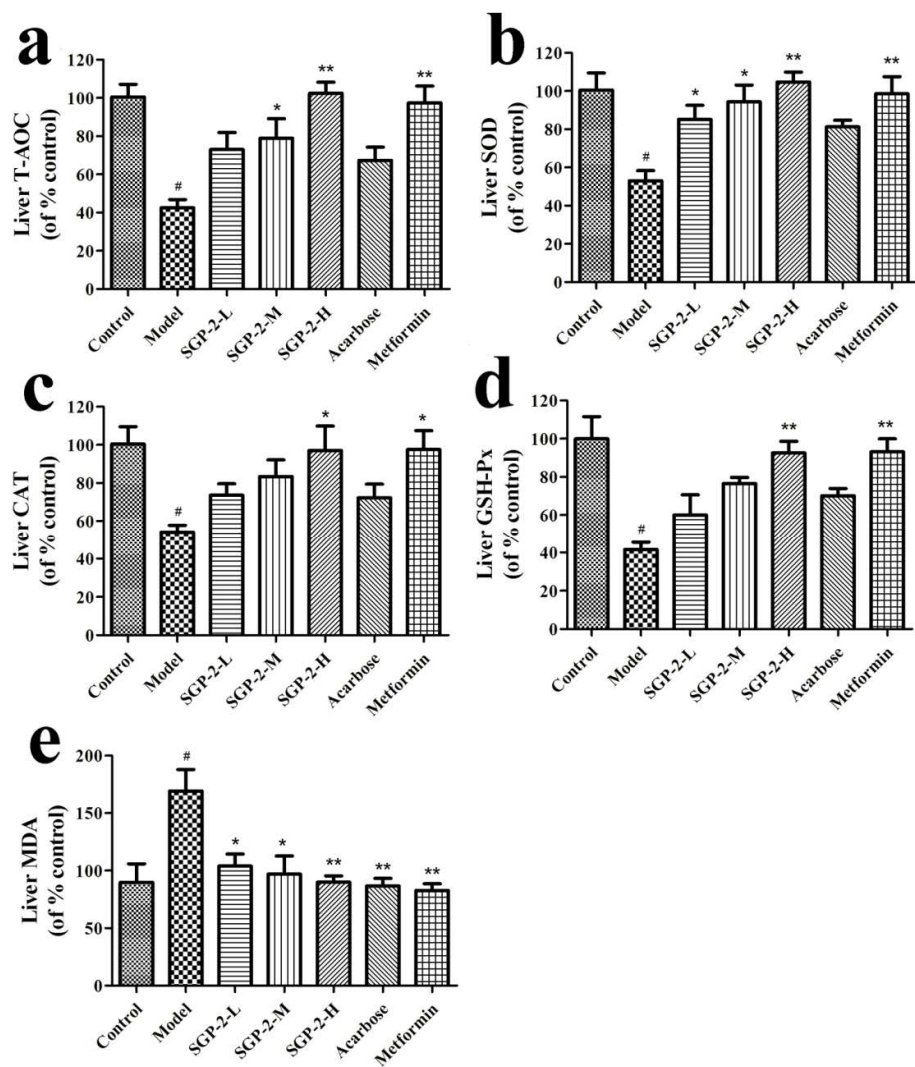


Fig. 3

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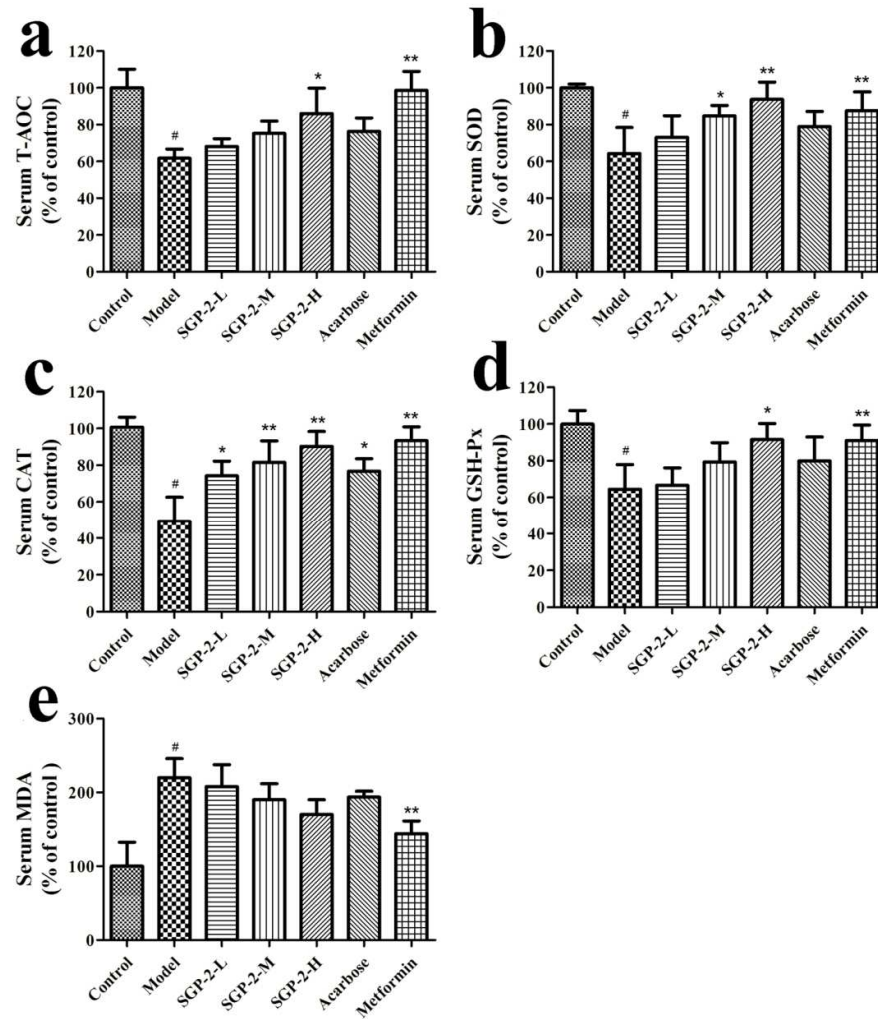


Fig. 4

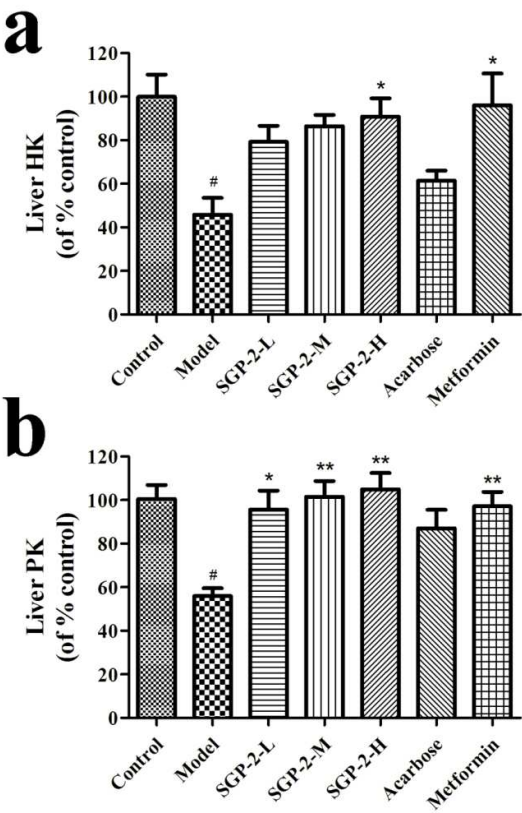


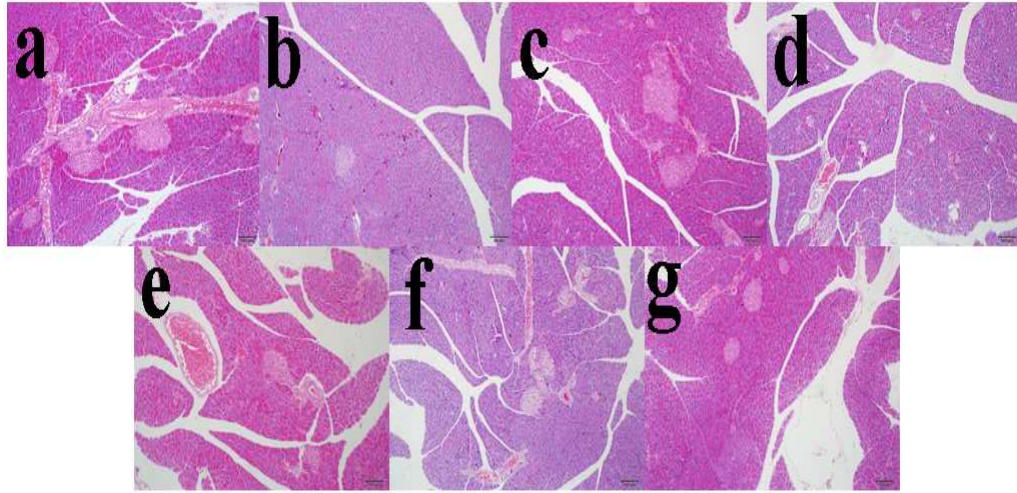
Fig. 5

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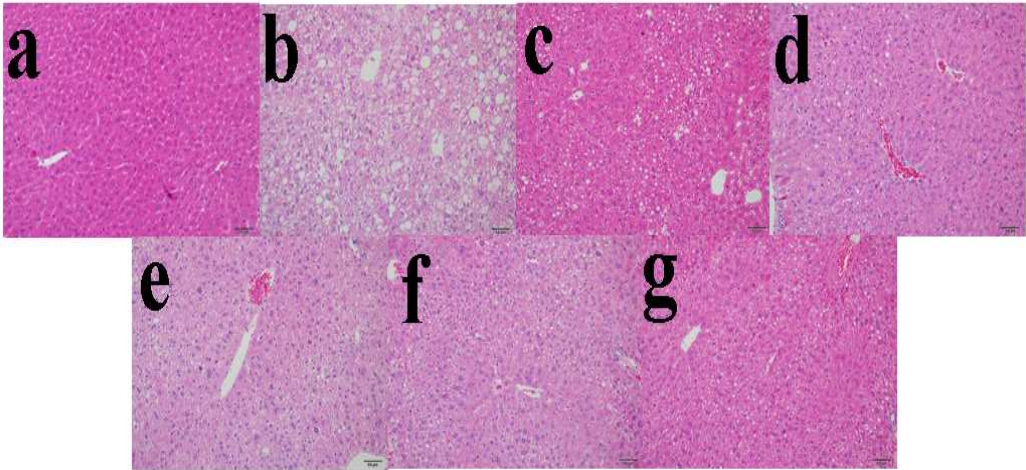
**Fig. 6**

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**Fig. 7**

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