



Original Article

Effects of total iridoid glycosides of *Picrorhiza scrophulariiflora* against non-alcoholic steatohepatitis rats induced by high-fat and high-sugar diet through regulation of lipid metabolism

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ABSTRACT

Objective: To investigate the therapeutic effect of total iridoid glycosides of *Picrorhiza scrophulariiflora* (TIGP) on non-alcoholic steatohepatitis (NASH).

Methods: SD rats were fed with high-fat and high-sugar diet for 8 weeks to establish NASH. TIGP were given orally at doses of 20, 40 and 80 mg/kg/d for 4 weeks. Triglycerides assay (TG), total cholesterol (TC), low density lipoprotein cholesterol (LDL-C), high-density lipoprotein cholesterol (HDL-C), aspartate aminotransferase (AST), alanine aminotransferase (ALT), fasting plasma glucose (FPG), fasting insulin (FINS), tumor necrosis factor- α (TNF- α), interleukin-6 (IL-6), chemokine-1 (MCP-1), leptin (LEP) in serum were tested. TG, TC, superoxide dismutase (SOD), malondialdehyde (MDA), and free fatty acid (FFA) in liver tissue were determined by colorimetric methods. Steatosis of hepatocytes and inflammation was performed by pathological examination.

Results: The results showed that TIGP significantly decreased TC, TG and FFA in liver tissue, increased SOD activity, decreased MDA content, decreased serum levels of TG, TC, HDL-C/LDL-C, ALT, AST, GLU, HOMA-IR, TNF- α and LEP, and in addition, improved steatosis of liver cells compared to NASH.

Conclusion: TIGP had anti-fatty liver effect against NASH rats induced by high-fat and high-sugar diet. Its mechanism was related to the regulation of lipid metabolism and reduction of insulin resistance, through inhibition of oxidative stress and inflammation.

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1. Introduction

Nonalcoholic fatty liver disease (NAFLD) is becoming the most common chronic liver disease, showing highly prevalent property worldwide, and its global prevalence is about 25% (Yu, Cai, She & Li, 2019). NAFLD is a spectrum of liver disease, comprising simple fatty liver and nonalcoholic steatohepatitis (NASH). NASH has been thought a progressive form of NAFLD, characterized

by inflammation, hepatocyte injury and fibrogenesis, and may progress to cirrhosis, liver failure and hepatocellular carcinoma (HCC) (Schuster, Cabrera, Arrese & Feldstein, 2018). Hepatomegaly and elevated serum aminotransferase are common in clinic. Histological manifestations include massive vesicular steatosis and lobular inflammation of hepatocytes, balloon-like deformation and/or fibrosis of hepatocytes. The pathogenesis of NASH is not yet clear. However, NASH is often accompanied by obesity, lipid metabolism disorder, insulin resistance or type II diabetes, which could induce metabolic stress, oxidative stress, and endoplasmic reticulum-related stress to develop in fatty hepatocytes (e.g. lipotoxicity) (Diehl & Day, 2017), having a closely relation on the

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increase of free fatty acids in the liver, the toxicity of cytokines and the damage of free radicals leading to lipid peroxidation.

Picrorhizae Rhizoma, the underground part (mostly rhizome) of *Picrorhiza scrophulariiflora* Pennell, distributed throughout the high altitude region (over 4400 m) in the southeast of Tibet and the northwest of Yunnan, China, has been traditionally used for diarrhea, jaundice and malaria (Li, Li, Tezuka, Namba & Kadota, 1998; Wang et al., 2013). It has been reported to contain cucurbitacins, sugars, phenolics, acetophenones and iridoid glycosides, etc. Total glycosides of *P. scrophulariiflora* (TIGP) are effective component obtained from the dried rhizome of *P. scrophulariiflora* by water extraction and macroporous adsorption resin separation. The main constituents are iridoid glycosides, which are composed of picoside-I, picoside-II, picoside-III, picoside-IV, 6-feruloylcatalpol, 6-isoferuloylcatalpol, and 6-cinnamyl- α/β -glucose, etc. Among which picoside-I and picoside-II are the main active constituents with relatively high content (Hou, Zhou, Shan & Liu, 2013). Studies have shown that *Picrorhiza* genus has the functions of protecting liver, lipid-lowering and cholagogic action. Among them, the main components against cytotoxic reaction are picoside-I and picoside-II. Earlier studies had showed that the plant had hepatoprotective, nephroprotective, and immunomodulatory properties (Baruah, Gupta, Nath, Patnaik, & Dhawan, 1998; Puri et al., 1992; Yadav & Khandelwal, 2009). However, there is no report of NASH which is closely related to the protecting liver and lowering lipid of treatment of *P. scrophulariiflora*. This study was to investigate the therapeutic effect of total iridoid glycosides of *P. scrophulariiflora* on NASH.

2. Materials and methods

2.1. Drugs and reagents

Silybin capsule was obtained from Tianjin Tasly Pharmaceutic Co., Ltd. Total cholesterol (TC) kit, triglycerides assay (TG) kit, low density lipoprotein cholesterol (LDL-C) kit, high-density lipoprotein cholesterol (LDL-C) kit, aspartate aminotransferase (AST) kit, alanine aminotransferase (ALT) kit, and glucose (Glu) kit were obtained from Biosino Bio-Technology and Science Incorporation. Free fatty acid (FFA) kit, superoxide dismutase (SOD) kit, and malondialdehyde (MDA) kit were obtained from Nanjing Jiancheng

Bioengineering Institute. Rat insulin kit was purchased from MERCODIA. Rat tumor necrosis factor (TNF- α) kit, rat leptin (LEP) kit, rat interleukin-6 (IL-6) kit, and rat chemokine-1 (MCP-1) kit were obtained from RapidBio Lab.

2.2. Preparation of total iridoid glycosides of *P. scrophulariiflora* (TIGP)

The medicinal material of *Picrorhizae Rhizoma* was crushed into the coarsest powder. Then, the coarsest powder was soaked in four times water for 1 h. Then it was put into the infiltration tube and immersed in water for 24 h. After adding water, it was percolated at a flow rate of 1.5 mL/min, and 16 times of the percolation fluid was collected. The percolation fluid was then evaporated and dried to brown and yellow powder. Previous study verified that the content of picoside-I and picoside-II was about 14% and 22% on average from three batches (Hou et al., 2013).

2.3. Animal treatment

Seventy male SD rats with specific pathogen-free condition were purchased from Beijing Vital River Laboratory Animal Technology Co., Ltd. The experimental animals were given one week to acclimatize to the environment and diet, which were housed in temperature and humidity-controlled barrier system with a 12/12 h light and dark cycle. Among these, 55 were given a high fat and high sugar diet to prepare the NASH model. Feed formula: lard 20%, cholesterol 1%, sucrose 10%, and sodium cholate 0.25%. The remaining 15 rats were given normal diet as normal control. After 8 weeks, five animals were respectively taken from normal group and model group, and histopathological method was then used to confirm the formation of NASH (Fig. 1). Animals in modeling group were randomly divided into five groups according to their body weight, with 10 animals in each group. The normal control group and model control group were orally given 0.5% CMC. The tested groups were orally given TIGP at doses of 20 mg/kg, 40 mg/kg and 80 mg/kg, respectively, the doses were referred from Zhuang, XU, Wang, and Xiang (2018). The positive drug group was given silybin by gavage at a dose of 160 mg/kg. Each group was administered once a day for 4 weeks; each dose was 10 mL/kg.

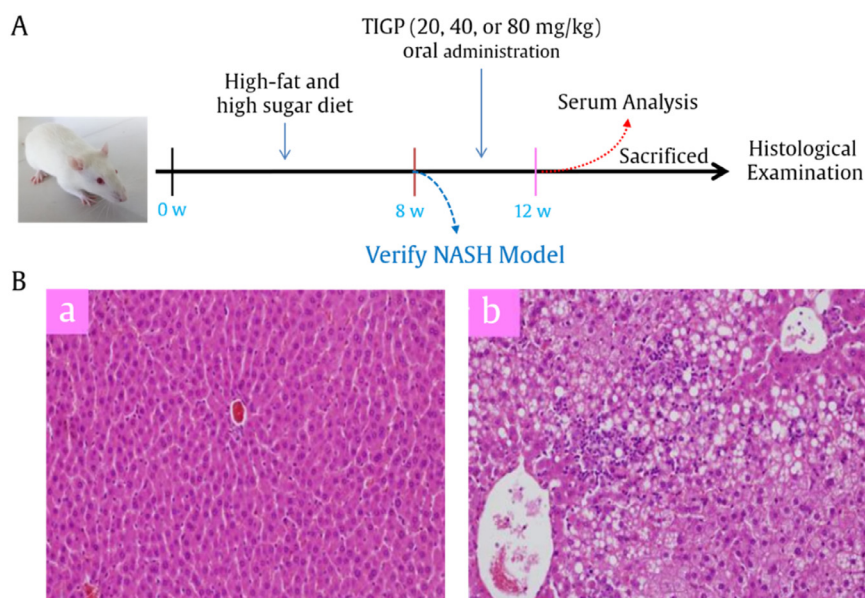


Fig. 1. Route of whole experiment (A) and verification of NASH model by H&E staining ($\times 200$) (B). (a) normal control rats; (b) NASH model rats.

2.4. Serum test

Animals were anesthetized after 1 h administration, fasting for 12 h before the last administration, and then abdominal aorta blood was taken to prepare serum, which was used to determination of TG, TC, HDL-C, LDL-C, AST, ALT, fasting plasma glucose (FPG), fasting insulin (FINS), TNF- α , IL-6, MCP-1, LEP in serum, and calculated as follows: Insulin resistance index (HOMA-IR) = fasting plasma glucose content (FPG, mmol/L) \times fasting insulin content (FINS, mIU/L) / 22.5.

2.5. Detection of liver homogenate

All rats were sacrificed and the liver tissues were removed and weighed 100 mg. The homogenate was prepared by grinding with 2 mL of acetone-ethanol mixture (1:1). The lipid was extracted, and after centrifugation, the supernatant was taken for colorimetric determination of TG and TC. Another 100 mg of liver tissue was ground with normal saline (NS) to prepare homogenate. After centrifugation, the supernatant was taken for colorimetric determination of SOD, MDA, and FFA.

2.6. H&E staining

The liver tissues were fixed with formalin solution, stained with HE, and the lipid changes of hepatocytes were observed under light microscope. The liver tissues were graded and scored according to the criteria improved from Knodell RG (Knodell et al., 1981) in attached Table 1, and the necrotic inflammations in 40 consecutive fields of vision were counted to evaluate the inflammations.

2.7. Statistical analysis

The data were presented as mean \pm SD. One-way ANOVA was used to evaluate differences between multiple groups with

Table 1
Semi-quantitative scoring criteria for hepatocyte liposis.

Conditions of hepatocyte liposis	Levels	Scores
Number of lipid-containing cells in a fat cell < 1/20	0	0
Number of lipid-containing cells in a fat cell < 1/4	I	1
Number of lipid-containing cells in a fat cell < 1/2	II	2
Number of lipid-containing cells in a fat cell < 3/4	III	3
Liver tissue was almost replaced by lipid droplets	IV	4

Table 2
Effect of high-fat and high-sugar diet on hepatocyte liposis (mean \pm SD, $n = 5$).

Groups	Severity of lipid degeneration					Total scores	Focal necrosis counts
	0	I	II	III	IV		
Normal	5	0	0	0	0	0	4.8 \pm 1.9
NASH	0	0	1	2	2	16***	29.8 \pm 7.9***

*** $P < 0.001$ significance vs normal control group.

Table 3
Effects of TGPS on TC, TG and FFA in liver of NASH rats (mean \pm SD, $n = 10$).

Groups	Doses/(mg·kg ⁻¹)	TC/(mmol·100 g ⁻¹ liver)	FFA/(μ mol·100 g ⁻¹ liver)	TG/(mmol·100 g ⁻¹ liver)
Normal	–	1.70 \pm 0.16	0.86 \pm 0.10	2.60 \pm 0.72
Model	–	3.28 \pm 0.86##	2.08 \pm 0.42##	4.38 \pm 0.50##
TIGP	20	2.85 \pm 0.79	1.83 \pm 0.35	3.72 \pm 0.64*
TIGP	40	2.35 \pm 0.75*	1.71 \pm 0.29*	3.50 \pm 0.66**
TIGP	80	2.19 \pm 0.72**	1.75 \pm 0.25*	2.98 \pm 0.74**
Silybin	160	2.26 \pm 0.77*	1.74 \pm 0.23*	3.00 \pm 0.47**

$P < 0.01$ vs corresponding normal control group.

* $P < 0.05$ and ** $P < 0.01$ vs corresponding model control group.

Duncan's test. Rank data were tested by Redit method. Significance was set at $P < 0.05$.

3. Results

3.1. Establishment of NASH rats by high-fat and high-sugar diet

In order to investigate the hepatoprotective effect of TIGP in this study, rats were fed on high-fat and high-sugar diet for 8 weeks to establish NASH model, commonly used on the research of hepatoprotection. H&E stained sections of the control group showed normal liver tissue structure with normal sized central vein, surrounded by rows and cords of normal hepatocytes and there were no inflammatory cells infiltration was observed (Fig. 1B, Table 2). However, the liver tissue sections of rats fed with high-fat and high-sugar diet showed diffusely micro vesicular hepatic steatosis, mostly developing grade II, III or IV, and presented moderate to severe hepatic lobular inflammation (Fig. 1B, Table 2), which was the evidence of the formation of NASH and most rats were at the degree of III or IV level.

3.2. Effects of TIGP on hepatic lipid metabolism

Excessive accumulation of TC and TG in hepatocytes are the hallmark of NASH. To investigate the role of TIGP on regulation of lipid metabolism, related parameters were detected. Successive perfusion of TIGP for 4 weeks in NASH rats could significantly reduce the elevated lipid in liver. TIGP at 20, 40 and 80 mg/kg inhibited the elevated TC by 27.0% ($P > 0.05$), 58.6% ($P < 0.05$) and 69.0% ($P < 0.01$), respectively. TG was inhibited by 37.0% ($P < 0.05$), 49.4% ($P < 0.01$) and 78.7% ($P < 0.01$) in rats, respectively. FFA was respectively inhibited by 20.5%, 30.3% ($P < 0.05$) and 27.0% ($P < 0.05$). The results were shown in Table 3.

3.3. Effect of TIGP on lipid metabolism

Rats were fed on high-fat and high-sugar diet for 12 weeks could markedly increase the level of TG and TC in serum ($P < 0.01$). Successive perfusion of TIGP for 4 weeks in NASH rats could significantly reduce blood lipid. TIGP at doses of 20, 40 and 80 mg/kg inhibited the elevated serum TG by 36.4% ($P < 0.05$), 78.4% ($P < 0.01$) and 99.4% ($P < 0.01$), respectively. And serum TC was inhibited by 14.1% ($P < 0.05$), 44.8% ($P < 0.01$) and 71.0% ($P < 0.01$), respectively. Additionally, the ratio of serum HDL-C/LDL-C decreased by 47.6% ($P < 0.05$), 132.0% ($P < 0.01$) and 175.8% ($P < 0.01$), respectively. High dose of TIGP showed stronger effect than positive group. The results were shown in Table 4

3.4. Effects of TIGP on liver function

ALT and AST levels were markedly higher in the model group than those in normal control group ($P < 0.01$), suggesting liver

Table 4
Effects of TIGP on lipid metabolism (mean \pm SD, $n = 10$).

Groups	Doses/(mg·kg ⁻¹)	TC/(mmol·L ⁻¹)	TG/(mmol·L ⁻¹)	HDL-C/(mmol·L ⁻¹)	LDL-C/(mmol·L ⁻¹)	HDL-C/ LDL-C
Normal	–	0.35 \pm 0.09	1.78 \pm 0.09	0.50 \pm 0.09	0.33 \pm 0.07	1.60 \pm 0.51
Model	–	0.53 \pm 0.08##	2.40 \pm 0.20##	1.22 \pm 0.27##	1.09 \pm 0.12##	1.11 \pm 0.17##
TIGP	20	0.46 \pm 0.05*	2.31 \pm 0.30	1.18 \pm 0.19	0.89 \pm 0.10**	1.34 \pm 0.30*
TIGP	40	0.39 \pm 0.09**	2.12 \pm 0.22**	1.19 \pm 0.24	0.72 \pm 0.21**	1.75 \pm 0.55**
TIGP	80	0.35 \pm 0.07**	1.96 \pm 0.22**	1.18 \pm 0.21	0.65 \pm 0.21**	1.97 \pm 0.55**
Silybin	160	0.39 \pm 0.07**	2.03 \pm 0.24**	1.24 \pm 0.19	0.65 \pm 0.14**	1.99 \pm 0.47**

$P < 0.01$ vs corresponding normal control value.* $P < 0.05$ and ** $P < 0.01$ vs corresponding model control group.

function was damaged in NASH rats. Successive perfusion of TIGPS for 4 weeks in NASH rats significantly improved liver function, reflecting in ALT and AST levels, especially in 40 and 80 mg/kg TIGP groups, whose effects were more powerful than the positive group. The results were shown in Table 5

3.5. Effect of TIGP on lipid peroxidation

MDA is a lipid peroxide, whose content could reflect the level of lipid peroxidation. SOD could remove oxygen free radical, which is a vital factor on balancing oxidation and antioxidation. The changes of MDA and SOD levels revealed lipid peroxidation injury occurring on liver cell biomembrane in NASH rats ($P < 0.05$). However, successive perfusion of TIGP for 4 weeks in NASH rats showed obvious anti-lipid peroxidation effect. TIGP at 20, 40 and 80 mg/kg increased SOD activity by 33.2% ($P > 0.05$), 73.9% ($P < 0.05$) and 113.8% ($P < 0.01$), respectively. MDA was decreased by 31.4% ($P < 0.05$), 59.5% ($P < 0.01$) and 80.1% ($P < 0.01$), respectively. High dose of TIGP showed similar effect with positive group. The results were shown in Table 6

3.6. Effect of TIGP on insulin resistance index

Rats fed on high-fat and high-sugar diet for 12 weeks showed significant insulin resistance. Successive administration of TIGP for 4 weeks in NASH rats significantly reduced the level of glucose and improved insulin resistance. At doses of 20, 40, and 80 mg/kg of TIGP, the elevated GLU was decreased by 87.5% ($P < 0.01$), 109.4%

Table 5
Effects of TIGP on serum ALT and AST in NASH rats (mean \pm SD, $n = 10$).

Groups	Doses / (mg·kg ⁻¹)	ALT / (U·L ⁻¹)	AST / (U·L ⁻¹)
Normal	–	39.1 \pm 10.7	116.3 \pm 25.7
Model	–	60.2 \pm 10.6##	196.8 \pm 25.3##
TIGP	20	52.9 \pm 7.4	161.1 \pm 31.6*
TIGP	40	43.6 \pm 11.9**	132.3 \pm 26.6**
TIGP	80	45.5 \pm 7.7**	133.9 \pm 36.4**
Silybin	160	47.6 \pm 7.0**	155.6 \pm 33.3**

$P < 0.01$ vs corresponding normal control group.* $P < 0.05$ and ** $P < 0.01$ vs corresponding model control group.**Table 6**
Effects of TIGP on SOD and MDA in liver of NASH rats (mean \pm SD, $n = 10$).

Groups	Doses/ (mg·kg ⁻¹)	SOD/(U·mL ⁻¹ ·mg ⁻¹ prot)	MDA/(mmol·L ⁻¹ ·mg ⁻¹ prot)
Normal	–	34.6 \pm 5.8	141 \pm 31
Model	–	25.4 \pm 5.3##	224 \pm 29##
TIGP	20	28.4 \pm 5.7	198 \pm 22*
TIGP	40	32.3 \pm 5.3*	175 \pm 26**
TIGP	80	35.8 \pm 5.5**	158 \pm 29**
Silybin	160	34.9 \pm 5.1**	160 \pm 30**

$P < 0.01$ vs corresponding normal control group.* $P < 0.05$ and ** $P < 0.01$ vs corresponding model control group.

($P < 0.01$) and 101.1% ($P < 0.01$), respectively. HOMA-IR was decreased by 79.9% ($P < 0.05$), 95.8% ($P < 0.01$) and 98.1% ($P < 0.01$), respectively. The positive drug silybin (160 mg/kg) had no significant effect on insulin resistance. The results were shown in Table 7

3.7. Effects of TIGP on cytokines

The levels of TNF- α , IL-6, MCP-1 and LEP in NASH rats were increased in varying degrees compared with normal control group ($P < 0.01$ and $P < 0.05$). Successive administration of TIGP for 4 weeks in NASH rats reduced the levels of TNF- α and LEP to varying degrees. TIGP at doses of 20, 40, and 80 mg/kg decreased TNF- α by 21.6% ($P > 0.05$), 33.2% ($P < 0.01$), and 66.9% ($P < 0.01$), and also decreased LEP by 15.2% ($P > 0.05$), 67.0% ($P > 0.05$) and 73.3% ($P < 0.01$), respectively. The positive drug silybin (160 mg/kg) mainly reduced TNF- α , while, had no obvious effect on other cytokines. The results were shown in Table 8

3.8. Effects of TIGP on steatosis of hepatocytes and inflammation

Pathological examination showed that the contours of the hepatic lobules in the normal group were clear, the structure of the portal area was normal, no hepatic cells were degenerated and necrotic, and there was no obvious inflammatory cell infiltration (Fig. 2A). Rats fed with high fat and high sugar diet for 12 weeks, hepatocyte swelling, cytoplasm loosening, hepatic sinusoidal dilatation, steatosis of hepatocytes, infiltration of inflammatory cells and focal necrosis were observed (Fig. 2B). Successive perfusion of TIGP (20, 40 and 80 mg/kg) for 4 weeks, fatty degeneration and focal necrosis of hepatocytes were reduced in rats (Fig. 2D–F). The results were shown in Table 8.

4. Discussion

Nonalcoholic fatty liver disease (NAFLD) is the most common cause of abnormal aminotransferase levels and has overtaken chronic hepatitis C infection to become the most common etiology of chronic liver disease and cirrhosis, and besides that, its prevalence has been steadily increasing, affecting 30% of the general population and 60%–80% of the type 2 diabetic population (Noureddin, Zhang & Loomba, 2016). NASH, the progressive sub-

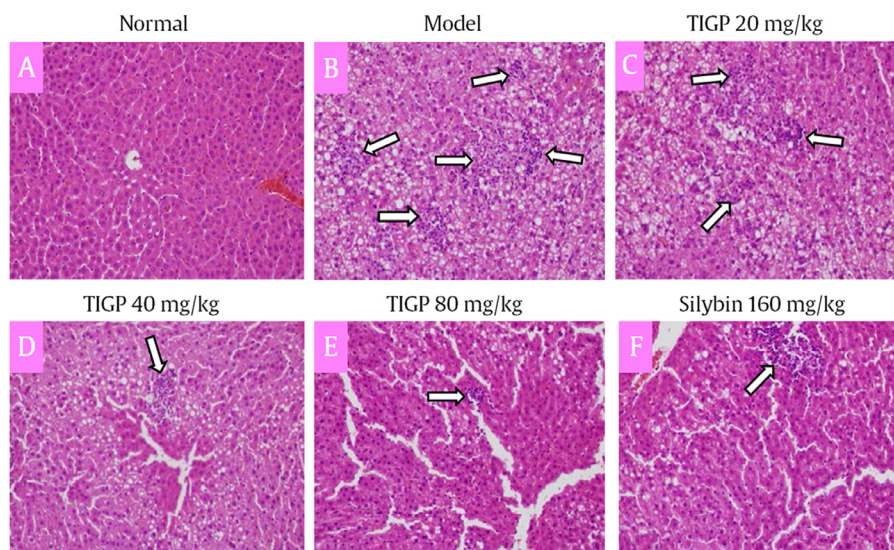
Table 7
Effect of TIGP on GLU, INS and HOMA-IR in NASH rats (mean \pm SD, $n = 10$).

Groups	Doses/(mg·kg ⁻¹)	GLU/(mmol·L ⁻¹)	INS/(ng·L ⁻¹)	HOMA-IR
Normal	–	4.73 \pm 0.81	356 \pm 39	74 \pm 7
Model	–	6.43 \pm 0.85##	388 \pm 29#	111 \pm 19##
TIGP	20	4.94 \pm 0.67**	371 \pm 33	81 \pm 12**
TIGP	40	4.57 \pm 0.88**	368 \pm 33	75 \pm 19**
TIGP	80	4.71 \pm 0.93**	357 \pm 47	71 \pm 15**
Silybin	160	5.96 \pm 0.69	372 \pm 31	98 \pm 13

$P < 0.05$ and ## $P < 0.01$ vs corresponding normal control group.* $P < 0.05$ and ** $P < 0.01$ vs corresponding model control group.

Table 8
Effect of TIGP on cytokines in NASH rats (mean \pm SD, $n = 10$).

Groups	Doses/(mg·kg ⁻¹)	TNF- α /(pg·mL ⁻¹)	IL-6/(pg·mL ⁻¹)	MCP-1/(pg·mL ⁻¹)	LEP/(pg·mL ⁻¹)
Normal	–	354 \pm 86	135 \pm 24	190 \pm 24	936 \pm 297
Model	–	571 \pm 120##	161 \pm 31#	231 \pm 44#	1336 \pm 302##
TIGP	20	524 \pm 108	148 \pm 21	226 \pm 53	1275 \pm 299
TIGP	40	499 \pm 100	139 \pm 34	198 \pm 39	1068 \pm 361
TIGP	80	426 \pm 67**	139 \pm 27	205 \pm 37	1043 \pm 229*
Silybin	160	444 \pm 119*	153 \pm 27	205 \pm 31	1231 \pm 240

$P < 0.05$ and ## $P < 0.01$ vs corresponding normal control group.* $P < 0.05$ and ** $P < 0.01$ vs corresponding model control group.**Fig. 2.** Effect of TIGP on steatosis and inflammation of hepatocytes (H&E, $\times 200$).

type of NAFLD, is recognized as a common liver injury, related with histopathologic abnormalities including steatosis with inflammation and fibrosis (Angulo, 2002). The two-hit hypothesis is a vital theoretical framework for understanding the pathophysiology of NAFLD and NASH. The 'first hit', an excessive accumulation of fat in the liver, increases susceptibility of the liver injury mediated by 'second hits', such as oxidative stress, lipid peroxidation, and pro-inflammatory cytokines, which in turn lead to steatohepatitis and/or fibrosis. Free radical damage is vital for in the pathogenesis of NASH, therefore, the improvement of the oxidant status may be a benefit on NASH pathology (Tung, Huang, Lin & Yen, 2018). Searching for anti-fatty liver components from natural plants is one of the current research hotspots. For example, Liu et al. have found that chlorogenic acid extracted from coffee beans or honeysuckle increased the cellular lipid droplets and the expression of SREBP-1c and PNPLA3 in the tested cells. Additionally, 2-NBDG uptake was significantly increased, whereas the cellular ROS and protein of CYP2E1 and CYP4A were markedly decreased (Liu, Zhai, Qing, Zhu & Chen, 2018).

Different animal models of NASH have been developed and could be classified into three basic categories: those caused by either spontaneous or induced genetic mutation; those produced by either dietary and pharmacological manipulations; and those involving genetic mutation and dietary or chemical challenges (Semiane et al., 2017). In this study, we adopted the high-fat and high-sugar diet-induced NASH model, inducing the damage of liver morphology (Fig. 1 and Table 2), especially resulting insulin (IR) in phenotype, the generation of oxidative stress and the increase of TC, FFA and TG (Table 3), which could resemble the symptoms of human NASH.

Total iridoid glycosides of *P. scrophulariiflora* (TIGP) was the main bioactive components in *Picrorhizae Rhizome*, which the content of picroside-I, picroside-II, picroside-III, and picroside-IV was most abundant. Recent studies showed that TIGP had hepatoprotective activity *in vivo* and *in vitro*, respectively, and their ability might be associated with the antioxidant and anti-inflammatory properties (Sun, Sun & Zhu, 2009; Yan et al., 2002). Moreover, picroside-I, picroside-II, picroside-III, and picroside-IV could decrease the red granular lipid droplets, reduce the generation of oxidative stress and inflammation in different degree at FFA-induced NASH *in vitro* (Chen et al., 2019). Another evidence *in vivo* proved 10 mg/kg picroside-II could up-regulate the expression of bcl-2 genes, leading to increase the bcl-2/bax ratio to protect hepatocytes apoptosis against D-galactosamine and LPS induced acute-liver injured mice (Gao & Zhou, 2005). The present study demonstrated that TIGP showed hepatoprotection through decreasing TC, TG and FFA in liver tissue, as well as the inflammation and improving the balance of oxidative and anti-oxidative enzymes.

The excess energy substance induced by overload or disruption of FFA and carbohydrates in the liver could be toxic, causing endoplasmic reticulum (ER)/oxidative stress and cell injury (Yu et al., 2019). FFA, partly from TAG, could switch to TAG and VLDL or other phospholipids after linked with lipoproteins. Once FFA homeostasis is impaired, the overload of FFA leads to steatosis and lipotoxicity. In this study, after fed on high-fat and high-sugar diet for 12 weeks, high-fat and high-sugar diet significantly increased TC, FFA, and TG in the liver, as well as the levels on TG and TC in serum, while decreased the ratio of HDL-C/LDL-C in serum (Tables 3 and 4). However, the successive administration of TGPS for 4 weeks could reverse the factors, showing hepatoprotective ability.

Apoptosis, one of the best-defined types of programmed cell death, is considered a bridge between lipotoxicity and fibrogenesis, which acts as the main features in the NASH. In accordance with previous study, TIGP could decrease the level of MDA, while increase the level of SOD at high-fat and high-sugar diet induced NASH rats (Table 6), which could explain the role of hepatoprotection through anti-oxidative stress.

Adipocytokines such as adiponectin, resistin and leptin, which are secreted by adipocytes, play key roles in regulating insulin sensitivity. In obesity, insulin mediated lipolysis in adipocytes is impaired, leading to an increase in non-esterified fatty acid (NEFA), which interferes with insulin to affect glucose intake. Furthermore, insulin (IR) is always linked to chronic mild inflammation, and lots of regulators released by adipocytes or immune cells can in turn promote IR, including TNF- α , IL-6, IL-1 and monocyte chemoattractant protein-1 (MCP-1) as well as the IKK/NF- κ B pathway (Yu et al., 2019). On the other hand, the increase of FFA and overexpression of CYP2E1 in liver were closely related to lipid peroxidation, which directly or indirectly aggravated IR. In this study, TIGP could improve the HOMA-IR (Table 7), as well as the adipocytokines, such as TNF- α , IL-6, MCP-1 and LEP (Table 8) in NASH rats.

The decrease of FFA, TC and TG in liver and serum showed TGPS could combat the ‘first hit’ inducing the liver susceptibility. Furthermore, the reduction of TNF- α , IL-6 and MDA, the increase of SOD and LEP means TIGP could eliminate lipid peroxidation, scavenge oxidative stress, and improve IR, the ‘second hit’ factors leading the progress of NASH. Therefore, this study demonstrated the beneficial protective effects of TIGP on liver histological and biochemical features of NASH in a rodent model, suggesting TIGP may decrease the formation of NASH.

5. Conclusion

TIGP may treat nonalcoholic steatohepatitis by reducing transaminase, reducing blood lipid, clearing liver lipid, resisting lipid peroxidation, lowering blood glucose, improving insulin resistance, clearing inflammatory factors, reducing lipid changes in hepatocytes, and reducing leptin.

Declaration of Competing Interest

The authors declare that they have no competing interests.

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