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Original Research Article

Anti-diabetic activity of aqueous extract of *Fructus Ligustri Lucidi* in a rat model of type 2 diabetes

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Abstract

Purpose: *To determine the anti-diabetic activity of an aqueous extract of Fructus Ligustri Lucidi (FLL) in a rat model of type 2 diabetes (T2D).*
Method: *Body weight (BW), food intake (FI), fasting blood glucose (FBG), glucose tolerance testing and insulin tolerance testing were used to determine the anti-diabetic activity of an aqueous extract of Fructus Ligustri Lucidi (FLL) in a rat model of streptozotocin-induced T2D. Anti-oxidant activity and oxidative stress were assessed by superoxide dismutase (SOD) and malondialdehyde (MDA) analyses, respectively.*
Results: *Following FLL extract treatment, diabetic rat BW increased, while FI and FBG levels decreased. FLL extract increased glucose tolerance and decreased insulin tolerance. Following treatment with 300 and 600 mg/kg FLL extract, MDA levels reached 13.5 ± 0.9 nmol/ml and 13.8 ± 1.1 nmol/ml, respectively. Compared to MDA levels of 19.5 ± 1.1 nmol/ml in diabetic control group, MDA levels was decreased by 30.8 % and 29.0 % after the treatment with 300 and 600 mg/kg FLL extract, respectively, indicating alleviated oxidative stress.*
Conclusion: *The results show that aqueous FLL extract has the potential to alleviate T2D, resulting in reduced FI and FBG, and increased BW. The anti-diabetic activity of FLL extract on T2D may be relevant to reduced oxidative stress burden.*

Keywords: *Fructus Ligustri Lucidi, Type 2 diabetes, Anti-oxidant activity, Glucose tolerance, Insulin tolerance, Anxiety-like behaviour*

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INTRODUCTION

Fructus Ligustri Lucidi (FLL) is widely used as traditional Chinese medicine for strengthening bones and treating kidney and liver diseases [1]. In recent pharmacological studies, FLL also showed anti-oxidant, anti-tumour, and anti-osteoporosis effects [2,3]. Another study reported positive effects of extracts in mice suffering from non-alcoholic fatty liver disease [1].

Type 2 diabetes (T2D), characterized by chronic hyperglycaemia with high glucose levels, can lead to dysfunctions in glycometabolism and

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lipometabolism. T2D accounts for 90 % of diabetes cases worldwide. This disease has been associated with increased risks of Alzheimer's disease and depression [4]. Insulin tolerance and high oxidative stress are regarded as primary pathogenetic factors underlying the development of T2D and its complications. T2D is a complex disorder caused by degenerating insulin function. High levels of free radicals [5] and attenuated anti-oxidant capacity [6]are concomitant with T2D. One study reported that the oxidant hydrogen peroxide decreased glucose transportation and the sensitivity of insulin signalling elements. Recently, several plant extracts were reported to have anti-diabetic effects, and many such compounds are beneficial in the setting of T2D [7]. The biguanide metformin is an oral hypoglycaemic agent originally synthesized by modify a certain compound extracted from plant *Galega officinalis*, which had been used to treat diabetes for centuries [8]. The extract of this plant is useful for designing the chemical structure of new anti-diabetes drug. In this study, FLL was used for extracting with an aqueous method. Its anti-diabetic activity was evaluated in a rat model of T2D.

EXPERIMENTAL

Plant material and extraction

FLL was purchased from the Second Affiliated Hospital of Shanxi University of Traditional Chinese Medicine and dried at 50 °C for 48 h. It was ground into powder and extracted in water for 2 days using a Soxhlet extractor. The liquid was dried with a lyophilizer and stored at 4 °C.

Experimental animals

Healthy male Sprague-Dawley (SD) rats weighing 240 g to 280 g and housed in specific pathogen-free conditions were used. All animals were reared in the same environment with a constant humidity of 50 % at 24 °C under a 12/12-h light/dark cycle. The study was approved by Animal Ethic Committee of the Affiliated Hospital of Shanxi University of Traditional Chinese Medicine (No. EA_20160107), and the experiments with rats were in full compliance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) [9]and with the Guidelines laid down by the NIH in the US [10].

Acute toxicity test

The acute toxicity of FLL extracts was analysed according to OECD Guidelines for Testing of Chemicals No. 423. Before testing, rats were fasted for 4 h with water available. The rats were orally treated by FLL extract at dosages of 5, 50, 500, or 2000 mg/kg. The animals were observed individually every day for 14 days. The behaviours observed include writhing, gasping, palpitation, decreased respiratory rate, and mortality.

Establishment of T2D rat model and animal grouping

Rats were supplied with 10 % fructose solution for 14 days after a 7-day adaptation period. This was followed by a 7-day period with daily injections of streptozotocin (STZ, 30 mg/kg) in citrate buffer to cause pancreatic β-cell dysfunction, while animals in normal control groups were provided with water and injected with citrate buffer [11]. After 7-day T2D induction, fasting blood glucose (FBG) was determined using glucose meter. Animals with FBG > 250 mg/dL were considered to be diabetic [12]. Animals were divided into four groups: normal control (NC), diabetic control (DC), diabetic + low dose of FLL extract (DLDFE, 300 mg/kg), and diabetic + high dose of FLL extract (DHDFE, 600 mg/kg). FLL extracts were orally administered to DLDFE and DHDFE groups via a force-feeding needle once a day for 35 days after model establishment. Only saline was administered to the NC and DC groups. Body weight (BW), food intake (FI), and FBG were recorded before/after 35-day treatment period.

Glucose tolerance testing (GTT)

GTT was carried out to analyse glucose tolerance in SD rats. Blood glucose was measured after a 12-h fast. The rats were treated with saline or FLL extracts 1 h prior to glucose administration. After sampling blood from the tail vein, glucose (1 g/kg) was intraperitoneally injected. FBG was recorded at 0, 0.5, 1, 1.5, and 2 h after glucose administration.

Insulin tolerance testing (ITT)

ITT was carried out on overnight-fasted rats 4 days after GTT. The rats received saline or FLL extracts 1 h prior to insulin administration. After sampling blood from the tail vein, a single dose of insulin solution (0.5 U/kg) was immediately subcutaneously injected. FBG was recorded at 0, 0.5, 1, and 1.5 h after insulin injection.

Anxiety-like behaviour test

An elevated plus maze was used to evaluate anxiety-like behaviour. Anxious rats are more

likely to explore safe and comfortable environment (closed arms) rather than uncomfortable, risky, or threatening environments (elevated open arms). The time each animal spent in different areas was recorded [13].

Oxidative stress analysis

After grounding liver tissues by liquid nitrogen, phosphate-buffered saline was used to extract superoxide dismutase (SOD) and malondialdehyde (MDA).Specific SOD activity (units/mg protein) was measured with SOD Activity Analysis Kits (Beyotime, China). MDA content (μmol/mg protein) was analysed by MDA assay kits (Beyotime). Total protein concentrations were measured with Bradford Protein Kits (Beyotime) [14].

Statistical analysis

Data analysis was conducted with SPSS 21 software (IBM, USA). Results are presented as mean ± standard deviation. *t*-Tests were used to compare differences between two groups, and $p < 0.05$ was considered statistical significant.

RESULTS

Acute toxicity

FLL extract of highest dosage did not cause any symptoms in acute toxicity analyses, and no rats died over the 14-day test, suggesting that FLL extract at 2000 mg/kg was nontoxic. Based on these results, experiments using 300 and 600 mg/kg were deemed safe and feasible.

Body weight, food intake and FBG

After drug / saline administration for 35 days, BW, FI and FBG were compared to values measured at baseline. As shown in Table 1, BW obviously decreased after T2D establishment. BW of diabetic rats continued to decrease during saline administration. In the DLDFE and DHDFE groups, BW started to increase after FLL extract treatment. Notably, the high dose (600 mg/kg) did not have a more significant effect on BW than the 300 mg/kg dose. T2D induction also increased FI. After treatment, FI decreased in an FLL dose-dependent manner. Compared with the NC group, animals in the DC group showed obviously higher FBG ($p < 0.05$). Continuous 35-day treatment with FLL extract resulted in an obvious decrease in diabetic rat FBG.

GTT and ITT analyses

FBG levels in the NC, DLDFE and DHDFE groups reached maximum values at 0.5 h in the GTT analysis. However, FBG in the DC group peaked at 1 h (Figure 1). After that, all four groups exhibited obvious downward trends. Animals treated with low and high doses of FLL extract had similar trends of FBG decrease after glucose administration. In NC and DC groups, FBG reached the lowest values 0.5 h and 1 h after insulin administration, respectively (Figure 2). In DLDFE and DHDFE groups, FBG values were lowest 1.5 h after insulin administration.

Anxiety-like behaviour

Elevated plus maze testing was performed to evaluate the effect of FLL extract on diabetes-induced changes in anxiety-like behaviour (Figure 3). Rats in the DC group passed open arms and central areas of elevated plus maze faster than those in NC group ($p < 0.05$) (Figure 3).

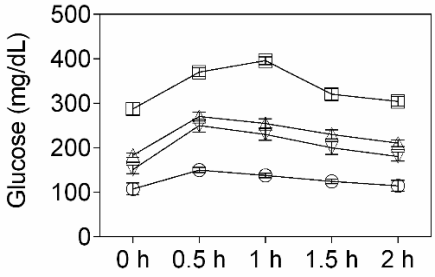

Figure 1: Effect of FLL extracts on glucose tolerance. NC, normal rats treated with vehicle; DC, T2D rats treated with vehicle; DLDFE, T2D rats treated with a low dose of FLL extract (300 mg/kg); DHDFE, T2D rats treated with a high dose of FLL extract (600 mg/kg); □: DC group; ○: NC group; △: DLDFE group; ▽: DHDFE group

Table 1: Effect of FLL extract on BW, FI and FBG. $^{\#}p < 0.05$ compared with NC group; * $p < 0.05$ compared with those before drug/saline administration

Group	Body weight (g)		Food intake (g/rat/day)		Fasting blood glucose (mg/dL)	
	Before	***After***	***Before***	***After***	***Before***	***After***
NC	275.4±3.1	287.0±3.9	17.1±4.0	19.9±2.7	95.0±3.9	117.8±7.6*
DC	213.1±5.5$^{\#}$	197.2±6.8$^{\#}$	34.9±2.5$^{\#}$	39.2±3.0$^{\#}$	290.1±5.3$^{\#}$	300.7±8.8$^{\#}$
DLDFE	230.6±10.1	265.4±5.5*	31.2±5.7	27.3±4.5	281.5±10.2	230.4±10.1*
DHDFE	229.5±6.3	270.2±3.1*	33.5±7.4	24.3±5.4	285.7±9.3	190.1±6.4*

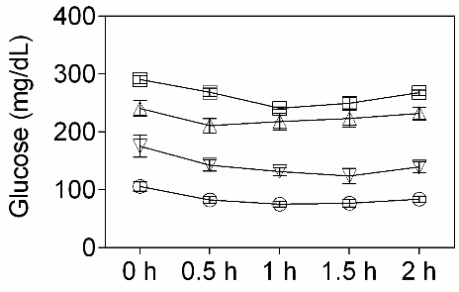

Figure 2: Effect of FLL extract on insulin tolerance. NC, normal rats treated with vehicle; DC, T2D rats treated with vehicle; DLDFE, T2D rats treated with a low dose of FLL extract (300 mg/kg); DHDFE, T2D rats treated with a high dose of FLL extract (600 mg/kg); □: DC group; ○: NC group; △: DLDFE group; ▽: DHDFE group

Low-dose FLL extract did not make rats pass open arms and central areas slower than DC group. However, high-dose FLL made rats pass open arms and central areas significantly slower compared to the DC group ($p < 0.05$).

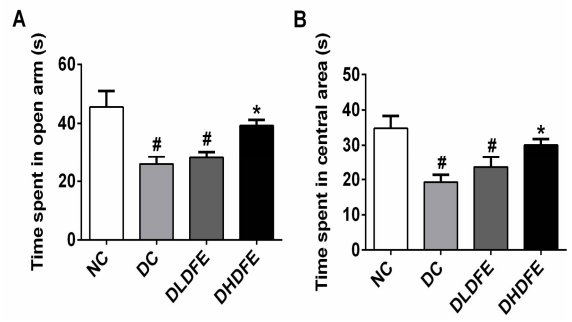

Figure 3: Time spent in open arm (A) and central area (B). $^{\#}p < 0.05$ compared with NC; $^{*}p < 0.05$ compared with DC. NC, normal rats treated with vehicle; DC, T2D rats treated with vehicle; DLDFE, T2D rats treated with a low dose of FLL extract (300 mg/kg); DHDFE, T2D rats treated with a high dose of FLL extract (600 mg/kg)

Anti-oxidant activity and oxidative stress

MDA levels and SOD activities were used to evaluate anti-oxidant activity and oxidative stress, respectively. Increased MDA level in DC group indicated significantly higher oxidative stress in rat liver following T2D establishment (Figure 4 A). After treatment with 300 and 600 mg/kg doses, MDA levels were decreased by 30.8 % and 29.0 %, respectively, indicating that FLL extract alleviated oxidative stress. The decrease in SOD activity supports the hypothesis that liver anti-oxidant activity was reduced after model establishment, and this was not significantly restored by FLL extract at either dose (Figure 4 B).

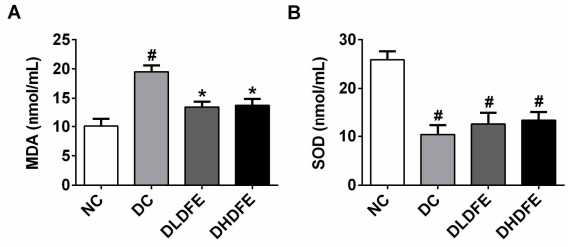

Figure 4: Effect of FLL extracts on MDA (A) and SOD (B). $^{\#}P < 0.05$ compared with NC, $^{*}p < 0.05$ compared with DC. NC, normal rats treated with vehicle; DC, T2D rats treated with vehicle; DLDFE, T2D rats treated with a low FLL extract dose (300 mg/kg); DHDFE, T2D rats treated with a high FLL extract dose (600 mg/kg)

DISCUSSION

In Asia, FLL is widely used to strengthen bones and treat kidney and liver diseases. There is evidence that FLL could also be used as an anti-oxidant, anti-neoplastic treatment [2,3]. In this study, two doses of FLL extracts were evaluated for their anti-diabetic and anti-oxidant effects. T2D is energy metabolism disorder that is characterized by chronic hyperglycaemia with high FBG levels. The STZ rat model of T2D is extensively used to study relevant disease mechanisms. We examined the acute toxicity of FLL extracts and their effects on FBG, BW, FI, GTT, ITT, anxiety-like behaviour, and oxidative stress were analysed in this study.

BW stability is an important indication of good health. Decreased BW and elevated FBG and FI are representative symptoms in T2D rats [15]. We found that diabetic rats had lower BW compared with NC animals. Diabetic rats gained weight after FLL extract administration. Importantly, this treatment also decreased FI and FBG. Previous studies reported that natural plant medicines alleviate T2D by maintaining glucose homeostasis, normalising gastrointestinal glucose absorption, exerting insulinotropic action, and promoting pancreatic β-cell regeneration [16]. Furthermore, GTT and ITT analyses showed that FLL extracts increased glucose tolerance and decreased insulin tolerance.

MDA analyses indicated that T2D establishment increased oxidative stress. FLL extracts significantly decreased MDA in diabetic rats and restored liver anti-oxidant activity. Persistent hyperglycaemia can lead reduce anti-oxidant levels and enhance the production of reactive oxygen species [17]. Importantly, SOD levels in diabetic rats were increased after FLL extract administration. Treatment with the high dose significantly increased the time spent in open arms and central areas of the elevated plus

maze compared to animals in DC group ($p < 0.05$). These results are in line with our other findings. These anti-diabetic effects of FLL extract could be related to its regulation of lipid metabolism since the total glycosides extracted from FLL could decrease serum and hepatic lipids, reduce lipid peroxidation, and regulate lipid metabolism [1].

CONCLUSION

The findings of this study demonstrate that FLL extract has the potential to alleviate T2D manifestations, resulting in decreased FI and FBG and increased BW. Glucose and insulin tolerance were also improved after FLL extract treatment. It is likely that these results are related to FLL extract-mediated decreases in oxidative stress and improved lipid metabolism regulation.

DECLARATIONS

Conflict of Interest

No conflict of interest associated with this work.

Contribution of Authors

We declare that this work was done by the authors named in this article and all liabilities pertaining to claims relating to the content of this article will be borne by the authors. Lanxiu Cao and Juan Lv designed all the experiments and revised the paper. Min Li, Rui Zhang, Fu Bai and Pengfei Wei performed the experiments, Lanxiu Cao and Juan Lv wrote the paper.

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