

Full Length Research Paper

Investigating Flavonoid-Induced Glycemic and Lipid Regulation from *Nelumbo Nuficera* Leaves in a Diabetic Mouse Model

Taoying Zhou^{1*}, Denghong Luo¹, Xingyuan Li¹ and Yunbo Luo²

¹Department of Biology, HuangGang Vocational and Technical College, HuangGang 438002, P. R. China.

²College of Food Science and Nutritional Engineering, China Agricultural University, Beijing 100083, P. R. China.

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This study evaluated flavonoids extracted from lotus (*Nelumbo nuficera* Gaertn) on reducing hyperglycemia and hyperlipidemia in alloxan-induced diabetic mice. flavonoids from lotus(FLL) was orally administered once a day after 3 days of alloxan -induction at 50 and 200 mg/kg for 28 day, and the results showed that fasting blood glucose ,serum TC and TG levels were significantly decreased, whereas serum HDL-c level were increased. The dosage of 200 mg/kg is more effective than that of 50 mg/kg. In addition, FLL did not exhibit any toxic symptoms in the limited toxicity evaluation in male mice. The above results suggested that FLL could control blood glucose and modulate the metabolism of glucose and blood lipid. So we conclude that the FLL should be evaluated as a candidate for future studies on diabetes mellitus.

Key words: Hypoglycemic, hypolipidemic, flavonoids from lotus leaf.

INTRODUCTION

Diabetes mellitus (DM) is among the most common disorder in developed and developing countries (Mukund et al., 2008). And the disease is increasing rapidly in most parts of the world (Kumar et al., 2008). In 1995, the World Health Organization reported that approximately 150 million persons worldwide had diabetes mellitus, and this number may well be double by 2025 (Bnouham et al., 2006). Hyperlipidemia is a metabolic abnormality frequently associated with diabetes mellitus (Yoshino et al., 1996). Pharmacological treatment of DM is based on oral hypoglycemic agents and insulin, but these approaches currently used in clinical practice either do not succeed in restoring normoglycaemia in most patients or fail after a variable period of time. Moreover, continuous use of the synthetic anti-diabetic drugs causes side effects and toxicity (Luo, 2004; Alarcon-Aguilar et al., 2000). Therefore, there is a need for discovering more effective and safe oral hypoglycaemic gents. DM is known from ancient time onwards and

numerous medicinal plants are used to control diabetes in traditional medicine (Ajikumaran et al., 2006).

Lotus (*Nelumbo nucifera* Gaertn) is a perennial aquatic crop with stout creeping yellowish white colored rhizo-mes. It is both an ornamental plant and a dietary staple in Eastern Asia, particularly in China (Hu and Skibsted, 2002). All parts of Lotus are used for various medicinal purposes in oriental medicine (Kashiwada et al., 2005). The leaf of lotus is bitter, sweet and neutral. It is aromatic and blue-green in color. It considered best for 'over-coming body heat', and stopping bleeding (Bensky et al., 2004). It is used as a drug for hematemesis, epistaxis, hemoptysis, hematuria (diabetic) obesity and metro-rrhagia in traditional Chinese medicine (Ono et al., 2006). Flavonoids are considered to be one of main components of lotus leaf (Onishi et al., 1984; Cour et al., 1995). The health benefits of flavonoids are well known and are displayed as a remarkable range of biochemical and pharmacological properties (Middleton et al., 2000). But an absence of study on the effect of Flavonoids extracted from lotus leaf on diabetes mellitus. The present study was designed to evaluate hypoglycemic and hypoli-pidemic effects of flavonoids from lotus leaf (FLL) on alloxan induced diabetic mice.

*Corresponding author. E-mail: cnkiedu@qq.com or cnki0601@tom.com. Tel: +8613972736991. Fax: +8607138345265.

Table 1. Ingredience of basal diet for the tested mice.

Ingredience	Content (%)	Ingredience	Content (%)
Corn starch	20	bone meal	3
bran	19	yeast powder	2.3
rice	16	salt	0.5
soybean oil meal	20	Vitamin mix	0.1
Calcium flour	3	microelement	0.1
fish flour	16	—	—

MATERIALS AND METHODS

Preparation of FLL

The shade dried lotus leaf was crushed in an electrical grinder and then powdered. Out of this powder, 50 g was extracted with 50% ethanol solution (v/v) at 80°C for 8 h (Jin and Qin, 2007; Fan et al.). Then under ultrasonication for 30 min in ultrasonic cleaner. After filtration with Whatman No. 1 filter paper and centrifugation (10000 rpm, 5 min) at 20°C, the solvent was evaporated and the aqueous extract was condensed by using a rotary evaporator. The NKA-9 macroporous resins were chosen to separate and purify the FLL from the crude extract. The FLL solution was diluted to 1.5 mg ml⁻¹ and stored at 4°C before use. The 752 spectrophotometer was used to determine the content of flavonoids in the above extracted product at 510 nm (Jin et al., 2007; Zhu and Tao, 2007; Jiang, 2006). The flavonoid content was calculated using the following linear equation based on the calibration curve that was prepared by rutin:

$$Y = 0.0904 X + 0.037, r = 0.9992$$

Where X is the absorbance; Y is the flavonoid content in g / ml. When extraction yield of FLL was calculated, the following

formula can be used:

$$K = \frac{Y \times a \times V}{W} \times 100\%$$

Where K is the extraction yield of FLL in %; Y is the flavonoid content in g / ml; a is dilution multiple; V is volume of extracting solution in ml; W is weight of lotus leaf in mg.

Selection of animals

Mice of original Kun-ming strain, five to six weeks of age, weighing 18-20 g were housed in colony cages at an ambient temperature of 25 ± 2°C with 12 h light and 12 h dark cycle. The mice were fed basal diet. The ingredient and nutrient compositions of the basal diet fed to mice are given in Table 1.

The animals were allowed to acclimatize to the laboratory environment for 1 week and then randomly divided into four groups (n = 8 mice per group) as given below:

- Group I – normal control group (NC);
- Group II – diabetes control group (DC);
- Group III – diabetic mice treated with 50 mg/kg FLL (DLFLL).
- Group IV – diabetic mice treated with 200 mg/kg FLL (DHFLL).

All the experiments with animals were carried out according to the guidelines of the institutional animal ethical committee and had prior

approval.

Induction of diabetes mellitus, followed by FLL treatment

Overnight-starved experimental mice from groups II, III and IV (as described above) were injected with a intraperitoneally injection of 4% alloxan that prepared freshly dissolved in ice-cold sodium chloride at a dose of 200 mg/kg body weight respectively. Diabetes was confirmed by the determination of tail vein blood glucose levels on the third day after administration of alloxan. The mice having blood glucose levels greater than 11.1 mol/l were considered diabetic. The mice belonging to group III and IV were treated with an oral dose of FLL once every day for 28 consecutive days, while groups I and II mice received only 0.5 ml distilled water/100 g.

Testing of fasting blood glucose (FBG)

During the FLL supplement for 28 days, fasting blood glucose level was measured once every week. Blood was collected from tip of the tail vein and fasting blood glucose level was measured by using a glucose analyzer (Akhtar et al., 2007).

Estimation of lipid profile in blood samples

On 28th day of experiment, the mice were sacrificed by decapitation under light ether anesthesia and blood samples was collected from dorsal aorta and serum was separated by centrifugation for 5 min and was kept at -20°C for the biochemical assay of total cholesterol (TC), high-density lipoprotein cholesterol (HDL- c) and triglyceride (TG). TC, TG, and HDL-c were determined by enzyme methods (Akhtar et al., 2007).

Acute toxicity evaluation in mice

FLL was tested for its acute toxicity (if any) in male mice. The test was carried out by single oral administration of FLL at doses of 200, 400, 600 and 1200 mg/kg to different groups of mice (5 mice in each group). The mortality and general behavior was observed continuously for one hour, four hour, and intermittently for next six hour and again at 24 and 48 h. (Kar et al., 2003; Han et al., 2006). The parameters observed were gross behavioral changes, grooming, alertness, sedation, loss of righting reflex, tremors convulsions (Mukund et al., 2008).

Statistical analysis

All the data were expressed as mean ± S.D. Statistical significance was calculated using one-way analysis of variance (ANOVA). Significance was accepted at the p < 0.01.

Table 2. Effect of FLL on fasting blood glucose levels in diabetic mice.

Groups	Fasting blood glucose levels(mmol/l)				
	Day 0	Day 7	Day 14	Day 21	Day 28
NC	4.64±0.27	4.49±0.16	4.71±0.25	4.53±0.49	4.59±0.31
DC	15.38±0.34	15.43±0.72	15.01±0.44	15.69±0.84	15.86±0.13
DLFLL	15.16±0.36	12.37±0.29	11.05±0.43*	10.06±0.28*	8.24±0.74*
DHFLl	15.25±0.31	12.01±0.45	10.67±0.74*	9.42±0.63*	7.15±0.29*

Each value represents mean ± S.E. of six mice per group; * Represents statistical significance vs. diabetes control (p < 0.01).

Table 3. Effect of FLL on blood lipids (mmol/l) in diabetic mice.

Groups	TC(mmol/l)	TG(mmol/l)	HDL-c(mmol/l)
NC	2.57±0.24	1.61±0.28	1.67±0.27
DC	3.39±0.46*	3.31±0.19*	0.74±0.09*
DLFLL	3.16±0.32**	2.43±0.47**	0.93±0.24**
DHFLl	2.81±0.27*	1.75±0.24*	1.26±0.24**

Each value represents mean ± S.E. of six mice per group. *Represents statistical significance vs. normal control group (p < 0.01). **Represents statistical significance vs. diabetes control group (p < 0.01)

RESULTS

Acute toxicity studies

In the present study, toxicity test was carried up to high concentration of 2400 mg/kg (12 times more than chosen dose). Even at this dose extract did not exhibit any sign of toxicity. Since the main purpose of this test is to get some idea on conspicuous behavioral changes and death, if any, and the FLL did not exhibit any toxic symptoms in the limited toxicity evaluation in male mice.

Effect of FLL on fasting blood glucose levels in mice

Blood glucose levels, estimated in 16 h fasting diabetic mice and was measured for once every week. The results were summarized in Table 2. The blood glucose levels in the FLL treated groups showed no significant differences at the end of the first week of drug administration (p>0.05), but those groups were all lower than that in the DC group (p<0.01) after 14 d of treatment. On 28th day of experiment, blood glucose levels in the DLFLL and DHFLl groups decreased by 45.65 and 53.11%, respectively. The NC and DC groups did not show any significant variation on the blood glucose level throughout the experimental period (p>0.05). These results indicated FLL decreases hyperglycemia in diabetic mice.

Effect of FLL on blood lipids levels in mice

Various parameters of blood lipid profiles were tested in the normal mice and diabetic mice. The results were su-

mmarized in Table 3. The levels of TC and TG were significantly elevated and the levels of serum HDL-C was decreased in the DC group as compared to the NC group (P<0.01). After supplementation with the FLL, the alteration in lipid metabolism was partially attenuated as evidenced by decreased serum TG and TC levels and increased HDL-C concentration in diabetic mice. The response was better in the DHFLl group compared with the DLFLL group.

DISCUSSION

Diabetes mellitus is a serious chronic disease. Effective control of the blood glucose level is a key step in preventing or reversing diabetic complications and improving the quality of life in both types 1 and 2 diabetic patients (Abraira et al., 1995; Ohkubo et al., 1995; DeFronzo., 1999). Antihyperglycemic potency of the FLL in diabetic mice has been indicated here by the study of fasting blood glucose levels as the important basal parameter for monitoring of diabetes and the dosage of 200 mg/kg is more effective than that of 50 mg/kg.

Diabetes is also associated with hyperlipidemia (De Sereday et al., 2004). The levels of TC and TG have been decreased significantly in diabetic mice after the FLL supplementation. These effects may be due to low activity of cholesterol biosynthesis enzymes and or low level of lipolysis which are under the control of insulin (Sharma et al., 2003). The FLL supplementation also results to the significant attenuation in the level of serum HDL-c toward the control level which again strengthens the hypolipidemic effect of this extract. There are reports

that other medicinal plants have hypoglycemic and hypolipidemic effects that could prevent or be helpful in reducing the complications of lipid profile seen in some cases of diabetes in which hyperglycemia and hypercholesterolemia coexist (Sharma et al., 2003).

In conclusion, the data obtained from the present study indicates that the FLL may have beneficial effects as both hypoglycemic and antihyperglycemic agents. Toxicity data have already proved that the FLL did not show any toxic reactions. Other experiments are necessary to determine the other mechanisms of antihyperglycemic action of FLL and the active fractions involved in this effect. Considering all these facts, it is reasonable to undertake further studies on possible usefulness of FLL in the treatment of diabetes mellitus.

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