Hypoglycemic and lipid-lowering effects of extracted from the aerial part of Urtica cannabina L. on alloxan-induced hyperglycemic mice

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Abstract
To evaluate the potential effect of Urtica cannabina L. (UC) in type 1 diabetes, we herein investigated the hypoglycemic and hypolipidemic effects and the underlying mechanism of ethanol extracted from the aerial part (AUC) and underground part of Urtica cannabina L (UUC) using alloxan-induced hyperglycemic mice model. The aerial part and underground part of Urtica cannabina L was extracted with 95% ethanol was administered orally (AUC and UUC at 500, 1600 mg/kg body weight) every day for 1 weeks to investigate the anti-diabetic effects in alloxan-induced mice. Blood glucose and body weight of the mice were recorded daily, and serum levels of total cholesterol, triglycerides and glycosylated hemoglobin were measured. Compared with the diabetic mice, treatment group affected the weight loss of diabetic mice to different degrees and reduced the blood glucose level. The crude extract of Urtica cannabina L also normalizes lipid metabolism parameters. Compared with the diabetic control group, the administration of the high-dose urtica high-dose group significantly (P <0.05) reduced the levels of serum triglycerides (P <0.05) and cholesterol (P <0.05). This study suggests that in the mouse model of hyperglycemia caused by diabetic alloxan, Urtica cannabina L has significant hypoglycemic and hypolipidemic activity, these provided scientific basis for the high-value utilization of Urtica cannabina L and healthy consumption.

1. Introduction
Diabetes (DM) is a comprehensive disease in which the secretion of insulin is absolutely or relatively insufficient, or the metabolism of sugar, lipids and proteins in the body is caused by insulin deficiency [1]. According to World Health Organization projections, around 300 million or more people will be affected by diabetes by the year 2025[2]. So far, its pathogenesis is not clear. Diabetes is the third most common high-risk disease after cardiovascular and cerebrovascular diseases and cancer [3,4]. Many experts point out the three principles of diabetes treatment, diet, exercise and medication [1]. Among them, diet therapy is the basic measure in the comprehensive treatment of diabetes. So far, the treatment of diabetes can meet the needs of some but not all patients. Therefore, it is of great significance to study natural hypoglycemic active ingredients for the development of hypoglycemic drugs and even functional foods. The natural hypoglycemic active ingredient currently found are mainly lignans [5,6], flavonoids [7-9], terpenoids [10,11], miscellaneous [12].

At present, antioxidants are found in plants including vitamins, flavonoids, fatty acids and tea polyphenols. Therefore, it has been suggested that one of the important activities of plant medicines may be antioxidant effect [13]. Urtica dioica L. (Urticaceae) is commonly known as nettle in folk and it has a variety of pharmacological activities. Urtica dioica has been successfully used to treat diseases such as diabetes mellitus (DM) [14], rheumatism, benign prostatic hyperplasia and hypertension and...
cardiovascular disease\cite{15-18}.

\textit{Urtica cannabina} L is an annual or perennial plant belonging to the nettle family, belonging to the genus \textit{Urtica}. Although \textit{Urtica cannabina} L belongs to the same genus as \textit{Urtica dioica} L, its physiological functions are very different. It contains polysaccharides, phenols, lignans and other nutrients\cite{19}. Studies have shown that ramie has pharmacological effects of lowering blood sugar, but the existing literature does not report on its hypoglycemic mechanism. In this study, we investigated the mechanism of the hypoglycemic effect of castor extract from the aspects of mouse body weight and fasting blood glucose level.

2. Materials and methods

2.1 Chemicals and reagents

Alloxan monohydrate was purchased from Sigma, Germany. Metformin hydrochloride was got from Tianfang Pharmaceutical Co., Ltd. GA-3 Nano glucometer and strips were obtained from Sinocare Inc Co., Ltd (Mannheim, Germany). The ELISA kit of mouse total cholesterol (TC), triglyceride (TG) and glycated hemoglobin (GHb) was from Nanjing Jiancheng Bioengineering Institute (Nanjing, China). The chemical reagents involved in the experiment are all analytically pure standards, and the test water is distilled water.

2.2 Experimental Animals

The Kunming strain mice used in the experiment were purchased from the Experimental Animal Center of Dalian University and weighed 20-22g. They were Kunming mice. They were fed adaptively for 12 weeks at room temperature and 12 hours each light and dark.

2.3 Plant materials and preparation of Crude Extract

\textit{Urtica cannabina} L(UC). was collected from the suburb of Ji’an City, Jilin Province in August 2016, and was identified as the family of Urticaceae, genus \textit{Urtica} by Professor Chen Chen of Liaoning Normal University. The specimen (No. XYQM-005) was deposited at the Institute of Materia Medica, School of Life Science and Technology, Dalian University.

The aerial part and underground part of dried \textit{Urtica cannabina} L. was weighed (13.7, 14.0 kg), powdered, placed in an extraction tank, added with 95% ethanol to completely immersed in the medicinal material, soaked overnight, then heated and refluxed for 3 hours, 3 times each time. The extract was recovered, filtered, and concentrated under reduced pressure at low temperature to obtain 1.5 and 1.3 kg of extract.

2.4 Acute oral toxicity study

Before the experiment, we conducted an acute toxicity test according to Economic Cooperation and Development guidelines. Five 6-8 weeks old female Kunming strain mice were used (nucleated, non-pregnant). A female mouse was fasted for two hours, but was free to drink water on first day. 2000 mg / kg crude extract was gavaged. The behavior or physical changes of the mice were strictly observed within 24 hours. Observe whether mice have anorexia, diarrhea, vomiting and tremor toxic reactions within 7 days.

2.5 Induction of Experimental Diabetes

After fasting for 12 hours, each mouse was injected intraperitoneally with freshly prepared alloxan monohydrate (200 mg / kg body weight) to model diabetic mice. Control group was given saline. Injecting once every three days for a total of three injections. Injecting alloxan cause the mice to release a lot of insulin. In order to prevent the mice from being killed due to hypoglycemia, each mouse is given an appropriate amount of 5% aqueous glucose solution after the administration. Three days after modeling, the tail vein blood of mice was collected to measure the fasting blood glucose level. The mice with fasting blood glucose levels higher than the 11.1 mmol/L were considered hyperglycemic and...
selected for the further experiment.

2.6 Grouping and Dosing of Animal Hypoglycemic Test on Experimental Mice

The experiment shared 70 mice, 60 diabetic mice and 10 non-diabetic mice. The experiment was divided into 7 groups (n = 10). The grouping is as follows:

- **Group I (control, non-diabetic mice, NC)**, normal mice were given saline;
- **Group II (diabetic mice, MC)**, mice were given saline only;
- **Group III (positive control, PC)**, mice were used as the positive control and administered by gavage every day with 500mg/kg metformin hydrochloride;
- **Group IV (diabetic mice, AUC-500)**, mice were administered by gavage with 500mg/kg the aerial part Urtica;
- **Group V (diabetic mice, AUC-1600)**, mice were administered by gavage with 1600 mg/kg the aerial part Urtica;
- **Group VI (diabetic mice UUC-500)**, mice were administered by gavage with 500 mg/kg the underground part of Urtica;
- **Group VII (diabetic mice UUC-1600)**, mice were administered by gavage with 1600mg/kg the underground part of Urtica every day. The experiment lasted a total of 7 days.

2.7 Biochemical assay

Diabetic mice (BGL > 11.1 mmol / L) were randomly divided into 6 groups (n = 10). There is also a normal control group (non-diabetic control group). The first group (non-diabetic control group, NC) and the second group (diabetic control group, MC) were treated with physiological saline, respectively, at a therapeutic dose of 10 mL / kg. The third group (positive control group, PC) was treated with the standard drug metformin hydrochloride (500 mg / kg). Groups IV, V, VI and VII were administered with 500, 1600 of aerial part of *Urtica cannabina* L(AUC-500,AUC-1600) and 500,1600mg/kg underground part of *Urtica cannabina* L(UUC-500,UUC-1600). All groups were treated by gavage daily for 7 days.

Blood samples were collected from the tail vein of animals everyday and measured by a glucometer. After 7 days of experiment, all mice were sacrificed after an overnight fast, the weight of the mice was weighed, the blood was cut off at the tail, and blood glucose was measured. All the mice were fast overnight after being sacrificed. Blood samples were collected and allowed to stand at room temperature for 30 minutes, and then centrifuged at 3,000 rpm for 10 minutes at 4°C to obtain serum. Serum samples were stored at -20°C until measurement. The separated plasma was placed on ice. After complete thawing, the contents of total cholesterol (TC), triglyceride (TG) and glycated hemoglobin in plasma were measured by an ultraviolet spectrophotometer according to the relevant kit operating instructions.

2.8 Statistical analysis

All the data were presented as mean ± standard deviation (SD), and statistical data were processed by SPSS 21.0. Student's t test was used for comparison between the two groups. Duncan analysis was performed by one-way ANOVA between groups. P <0.05, there are statistical differences.

3. Results

3.1 Acute Oral Toxicity Study

2000 mg / kg crude extract of the aerial part (AUC)and underground part of *Urtica cannabina* L by oral administration to Kunming strain mice did not produce any signs of toxicity such as Loss of appetite, vomiting, coma, and death during 24 h. These reactions did not appear in the next 7 days.

3.2 Effects of UC extract on body weight in diabetic mice

Contrasted with the MC group, the body weights of mice in UC extract intervention group and the body weights of mice in PC group increased gradually 7 days later. On the contrary, the body weights of mice in the MC and the UUC-500 group did not increase significantly.
Table 1. Effect of UC extract on body weights of the hyperglycemic mice induced by alloxan

| Groups   | Body weight (g)       |
|----------|-----------------------|
|          | Day0                  | Day7                  |
| NC       | 26.06±2.09            | 30.43±1.91            |
| PC       | 26.76±2.91            | 28.88±1.98            |
| MC       | 27.39±3.33            | 25.64±1.68            |
| AUC-500  | 26.80±1.94            | 32.28±2.13            |
| AUC-1600 | 25.56±1.75            | 27.35±1.91            |
| UUC-500  | 27.36±2.23            | 28.43±2.29            |
| UUC-1600 | 25.96±2.32            | 27.51±2.13            |

3.3 Effect of UC extract on blood glucose level and oral glucose tolerance
Table 2 describes the effect of crude extract of Urtica cannabina L on the blood glucose levels of alloxan-induced hyperglycemia mice and normal mice after 7 days of treatment. Throughout the experiment, the blood glucose levels of the NC group remained at normal levels. The blood glucose level of mice in MC group has been maintained at a relatively high level. The experimental data showed that after the drug was administered, the PC, high and low doses of the Urtica cannabina L. were reduced blood glucose when compared with that of MC group (P<0.05). The AUC-500, AUC-1600, and UUC-500 groups have better hypoglycemic effect than the UUC-1600 group (Table.2).

Table 2. Effect of UC extract on blood glucose of the hyperglycemic mice induced by alloxan

| Groups   | Fasting Blood Glucose Level (mmol/L) | Day0                  | Day7                  |
|----------|--------------------------------------|-----------------------|-----------------------|
| NC       | 5.60±0.77                            | 6.70±0.84             |
| PC       | 17.98±2.93                           | 8.87±1.28             |
| MC       | 15.86±2.70                           | 12.06±2.38            |
| AUC-500  | 10.30±1.08                           | 8.54±1.46             |
| AUC-1600 | 18.21±1.85                           | 6.05±1.32             |
| UUC-500  | 16.13±1.40                           | 8.46±2.58             |
| UUC-1600 | 18.22±2.22                           | 10.53±1.80            |

3.4 Effects of UC extract on total cholesterol in diabetic mice
The serum TC level of diabetic mice was detected 7 days after the animal experiment. Compared with the NC group, the serum TC level of each group was significantly increased (P<0.05), indicating that the blood lipid level of diabetic mice was significantly higher than that of the normal group; Compared with the NC group, the TG content was significantly decreased in the MC group and the therapy group (P<0.05). In this study, the decrease of TG level in the serum suggested that administration with all group of UC significantly reduced the TC level in alloxan-induced hyperglycemic mice.(Fig.1).

Fig.1. Effects of UC extract on total cholesterol of normal mice.
Notes: Diabetic mice were orally administered for 7 days. *P <0.05 compared with the normal control group (group NC); **P <0.05 compared with the before administration; #P <0.05 compared with the positive control group (group PC); ※P <0.05 compared with the model control group (group MC). Data represent mean ± SD (n = 10 per group).
3.5 Effect of Triglyceride on serum in diabetic mice
Before administration, the TG levels of the mice in the other groups except the NC group were higher than the normal values. After administration, the TG levels in the PC group, the AUC-500, and the AUC-1600 group decreased, and the difference was statistically significant compared with the MC group (P<0.05). The TG level in the UUC group did not decrease. It shows that the underground extract of *Urtica cannabina* L. has no effect on reducing TG, and the aerial part has a significant effect on reducing TG (Fig.2).

![Fig.2 Effects of UC extract on triglyceride of normal mice](image)

Notes: Diabetic mice were orally administered for 7 days. *P < 0.05 compared with the normal control group (group NC); **P < 0.05 compared with the before administration; #P < 0.05 compared with the positive control group (group PC); ※P < 0.05 compared with the model control group (group MC). Data represent mean ± SD (n = 10 per group).

3.6 Effect of Glycated hemoglobin on serum in diabetic mice
As shown Fig.3, compared with NC group, the level of Glycated hemoglobin(GHb) of the diabetic mice were significantly increased (P<0.05). However, AUC treatment for 7 days significantly reduced the GHb levels in serum of diabetic mice, and the difference was significant compared with the MC group (P<0.05). Among them, the AUC-500 group had the most significant effect, and the difference was significant compared with the PC group (P<0.05). It indicated that the extracts from the aerial part of *Urtica cannabina* L had the effect of reducing GHb, and the high dose group on the *Urtica cannabina* L was the best (Fig.3).

![Fig.3 Effects of UC extract on Glycated hemoglobin of normal mice](image)

Notes: Diabetic mice were orally administered for 7 days. #P < 0.05 compared with the positive control group (group PC); ※P < 0.05 compared with the model control group (group MC). Data represent mean ± SD (n = 10 per group).

4. Discussion
Traditional Chinese medicine has great development prospects in the field of diabetes treatment. Compared with western medicine, it has different characteristics in the treatment of diabetic complications, and its effects are diversified and the effect is more significant [20]. In conclusion, the current study provides evidence that the aerial part of *Urtica cannabina* L. low dose group by reducing
blood glucose and improve lipid metabolism. In this study, the hypoglycemic effect of ramie extract was analyzed from the overall level of mice. The hypoglycemic effect and possible mechanism of action of different parts of castor extract were investigated from the aspects of blood sugar level and blood lipids, which laid a pharmacological basis for the isolation of monomer with hypoglycemic effect from *Urtica cannabina* L.

In this experiment, the blood glucose values of diabetic mice compared with normal mice were higher than 11.1 mmol/L, and the difference was significant ($P<0.05$), indicating that the diabetic mice were successfully modeled. Alloxan-induced diabetic mouse model has been widely used to evaluate hypoglycemic effect of natural compounds including polysaccharides [21,22]. Alloxan can selectively destroy islet $\beta$ cells, increase blood sugar levels in animals, and is not easy to relieve within a certain period of time. The data show that most diabetic patients have complications such as lipid metabolism disorders and the patient's posture is mostly obese. Abnormal disorder of blood lipid levels can lead to abnormal accumulation of lipids in the body, which induces hyperlipidemia in the body. These changes in the lipid profile can represent a risk factor of cardiovascular disease [23]. At present, blood biochemical analysis results for diabetics show that serum cholesterol (TC), triglyceride (TG), and glycated hemoglobin (GHb) are abnormally increased. Serum cholesterol and triglycerides are generally considered to be risk factors for abnormal lipid metabolism in the body [23]. Glycosylated hemoglobin reflects the average blood glucose level over a period of time, and also reflects the effect of the drug on non-enzymatic glycosylation in vivo. Compared with the fasting blood glucose index, the external interference is small and more clinically meaningful.

Our findings are the first to demonstrate that short-term treatment with ethanolic extract of *Urtica cannabina* L antidiabetic and antioxidant effects in alloxan-induced diabetic mice. Compared with the diabetic mouse model group, the blood glucose, TC, TG and GHb levels of the diabetic mice were compared. There are different degrees of reduction. The data showed that the low dose of castor had the most obvious effect on glucose and lipid metabolism in diabetic mice, and the underground part was slightly worse. It shows that ramie has a better effect on the disorder of glycolipid metabolism. Therefore, it is concluded that the ramie extract has a hypoglycemic effect, and its hypoglycemic mechanism may be to improve the lipid metabolism disorder caused by diabetes, thereby preventing and treating diabetic complications. The extract of the aerial part of the castor is better than the underground part, and the active ingredient of the hypoglycemic agent may exist in the aerial part. The lipid-lowering effect of UC may be related to insulin-like activity, thereby alleviating diabetic hyperlipidemia. Taken together, our results support that UC improved blood glucose in the diabetic state, and also contributed to lipid-lowering effects.

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