Arch. Pharm. Res. 13(3), 269-273

Hypoglycemic and Hypolipemic Effects of *Ixeris dentata* in Diabetic Rats

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(Received August 8, 1990)

Abstract□Blood glucose and total lipid levels in rats with streptozotocin-induced diabetes were determined after intraperitoneal administration of methanolic extract of *Ixeris dentata* whole plants and its main component, cynaroside (= luteolin 7-O-β-D-glucoside). From the data obtained, it was concluded that intraperitoneal administration of the methanolic extract produced a significant hypoglycemic effect. Total blood lipids were also decreased. Cynaroside in the streptozotocin-diabetic rats failed to exhibit hypoglycemic effect but a significant hypolipemic activity was observed. Thus, it is suggested that this methanolic extract may contain one or more hypoglycemic and hypolipemic principles including the main flavone glucoside, cynaroside, which can significantly reduce the levels of triglyceride and total cholesterol in streptozotocin-diabetic rats.

Keywords□*Ixeris dentata,* compositae, hypoglycemic effect, hypolipemic effect, cynaroside, streptozotocin-induced diabetic rats.

Species of the genus *Ixeris* (Compositae) are used as strengthening of stomach, sedative and diuretic agents[1]. Among them, the whole plants of *Ixeris dentata* have been used for treatment of diabetes in Korean folk medicines. It was previously reported that the whole plants contain aliphatics[2], triterpenoids[2] and sesquiterpene lactones[3]. However, no extensive chemical and pharmacological studies about this plant have been done yet.

In the course of screening for hypoglycemic and hypolipemic drugs among Korean folk medicines, we found that intraperitoneal administration of a methanolic extract of this plant resulted in a significant decrease of blood glucose in alloxan diabetic mice[4].

The present study was undertaken to provide an evaluation of its possible hypoglycemic effect in streptozotocin-diabetic rats and chemical constituents of the methanolic extract of *I. dentata* whole plants. This paper also reports the effects of the main flavone glycoside, cynaroside (= luteolin 7-O-glucoside) on serum constituents in streptozotocin diabetic rats.

MATERIALS AND METHODS

Animals

Male rats of JCL: Wistar strain, each weighing about 150g, were employed in this experiment. Diabetes was induced in the rats by intraperitoneal administration of streptozotocin (65 mg/kg body weight) dissolved in 10 mM citrate buffer (pH 4.5). Several days after the injection, the blood glucose level was determined and rats with a level of 350 mg/dl or more were used as diabetic rats. All rats were maintained in an air-conditioned room with lighting from 0600 to 1800 h. The room temperature (about 23°C) and humidity (about 60%) were controlled automatically. A laboratory pellet chow (CLEA Japan Inc., Tokyo, Japan; protein 24.0%, lipid 3.5%, carbohydrate 60.5%) and water were given freely.

Preparation of methanolic extract

Dried whole plants (1.0 kg) of commercially available *I. dentata* were extracted with methanol under reflux. The extracts were concentrated to dyness *in vacuo* at 40°C to give the methanolic extract (87g, yield; 8.7%).

Isolation of cynaroside

The above methanolic extract (85g) was partitioned with chloroform, ethyl acetate, *n*-butanol and water successively. The ethyl acetate-soluble fraction (3.9g) was chromatographed over silica gel using an EtOAc-MeOH-H_2O (600:99:81) mixture to give the main flavone glucoside, cynaroside (870 mg, yield;

8.7×10^{-2}%) as yellowish powder. Mp, 250-252°C; IR ν_{max}^{KBr} (cm^{-1}): 3400-3200 (br. −OH), 1650 (C=O), 1595, 1490 (C=C), 1100-1000 (glycoside); UV λ_{max}^{MeOH} nm (log ε): 259 (4.74), 270 (sh. 4.71), 353 (4.77); UV $\lambda_{max}^{MeOH + NaOMe}$ nm: 265 (4.75), 400 (4.83); UV $\lambda_{max}^{MeOH+NaOAc}$ nm: 260 (4.76), 370 (sh. 4.64), 400 (4.64); UV $\lambda_{max}^{MeOH + NaOAc + H_3BO_3}$ nm: 260 (4.84), 375 (4.80); $\lambda_{max}^{MeOH+AlCl_3}$ nm: 274 (4.72), 300 (sh. 4.46), 330 (sh. 4.25), 430 (4.91); UV $\lambda_{max}^{MeOH+AlCl_3+HCl}$ nm: 274 (4.72), 298 (sh. 4.53), 360 (4.69), 392 (4.71); ^{1}H-NMR (DMSO-d_6, 200 MHz) δ ; 13.1 (1H, brs., C_5-OH), 7.54 (1H, d, J=7.7 Hz, H-6′), 7.52 (1H, s, H-2'), 6.99 (1H, d, J=7.7 Hz, H-5′), 6.89 (1H, d, J=1.8 Hz, H-8), 6.84 (1H, s, H-3), 6.54 (1H, d, J=1.8 Hz, H-6), 5.16 (1H, d, J=6.8 Hz, anomeric); ^{13}C-NMR (DMSO-d_6, 50 MHz) δ ; 181.85 (C-4), 164.48 (C-2), 162.94 (C-7), 161.12 (C-5), 156.93 (C-9), 149.94 (C-4′), 145.77 (C-3′), 121.38 (C-1), 119.15 (C-6′), 116.00 (C-5′), 113.56 (C-2′), 105.35 (C-10), 103.16 (C-3), 99.96 (C-1″), 99.56 (C-6), 94.76 (C-8), 77.16 (C-5″), 76.41 (C-3″), 73.13 (C-2″), 69.61 (C-4″), 60.67 (C-6″).

Experimental procedure

The methanolic extract and the cynaroside component, suspended in 5% ethanol-saline were each administered intraperitoneally to test rats, while control rats were treated with an equal volume of 5% ethanol-saline. At predetermined times after intraperitoneal administration of the above samples, the rats were killed by a sharp blow on the head and exsanguinated. Blood was collected into a conical centrifuge tube and the serum was separated by centrifugation immediately.

Chemicals

Nicotinamide adenine dinucleotide (NAD) was purchased from P.-L Biochemical Inc. (Milwaukee, WI, USA). Streptozotocin was purchased from Sigma Chemical Co. (St. Louis, MO, USA) and lactic acid dehydrogenase was from Oriental Yeast Co. (Tokyo, Japan). All other reagents were of the highest grade commercially available.

Statistics

The significance of differences between the control and methanolic extract-treated or cynaroside-treated groups was tested using Student's *t*-test.

Determination of glucose, triglyceride, total cholesterol, pyruvate and lactate in serum

Glucose, triglyceride and total cholesterol were determined using commercial reagents ("Glucose B-test Wako", Wako Pure Chemical Industries, Ltd., Osaka, Japan; "TG-Five Kainos", Kainos Laboratories, Inc., Tokyo, Japan; "Cholesterol E-Test Wako", Wako Pure Chemical Industries, Ltd.). Pyruvate was determined by the 2,4-dinitrophenylhydrazone method[5]. Lactate was determined by a spectrometric method, based on measurement of the increase in optical density at 340 nm[6].

RESULTS AND DISCUSSION

Previously we reported that the methanolic extract of *I. dentata* affected total cholesterol and blood glucose levels in animal experimental models with hypercholesterolemia or hyperglycemia[4]. In the present study, we investigated effects of the extracts of *I. dentata* on blood glucose and lipid levels after a single or repeated intraperitoneal administration in rats with streptozotocin-induced hyperglycemia.

Fig. 1 shows the time course changes in hyperglycemic activity after a single administration of doses of 40 mg/kg or 80 mg/kg. Blood glucose was significantly lowered only in the groups given a 40 mg dose. In the groups given dose of 40 mg was decreased the glucose level significantly by 7% at 4 h after the treatment. However, there was no significant

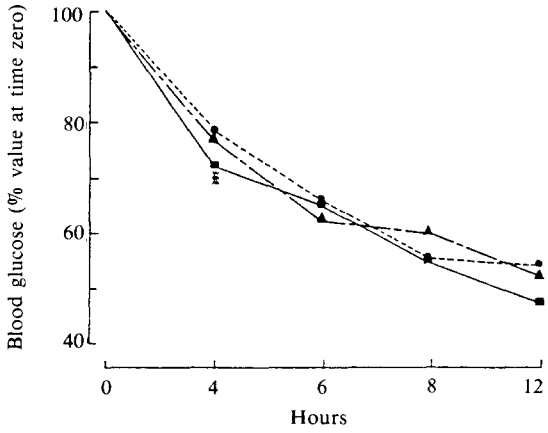

Fig. 1. Time-course changes in hypoglycemic activity after single administration of methanolic extract of *I. dentata*.

Blood samples were obtained after decapitation at the times indicated on the graph. Each point indicates the percent value calculated from mean values of six rats at time zero.

Control (●----●), 40 mg/kg (■——■) and 80 mg/kg (▲—·—▲)

*$p<0.05$

Table I. Effect of single administration of methanolic extract of *I. dentata* on levels of serum components in rats treated with streptozotocin

Treatment[a]	Dose (mg/kg BW)	Triglyceride (mg/dl)	Total cholesterol (mg/dl)	Pyruvate (mg/dl)	Lactate (μmol/ml)
Control	–	178.2±27.4 (100)	104.5±6.5 (100)	1.89±0.17 (100)	2.10±0.09 (100)
MeOH ext.	40	81.7±16.1 (46)*	80.1±3.7 (77)*	1.82±0.07 (96)	2.01±0.04 (96)

Values are mean±S.E. in six rats. Figures in parenthesis are percentages of the control value. [a] Rats were injected intraperitoneally once with the test dose. Four hours after single administration, blood samples were obtained after decapitation of the animals. [b]Singificantly different from the control value: *$p<0.05$

difference in the level of glucose during 12 h with the maximal effects at 4 h. The finding that the methanolic extract produced a very rapid reduction of serum glucose may imply that this extract is absorbed rapidly through the intestinal tract.

The effects on other serum constituents after a single intraperitoneal administration of the methanolic extract at the dose of 40 mg/kg are shown in Table I. Triglyceride was significantly lowered by 54%. Total cholesterol showed similar behavior to that of the triglyceride, being significantly reduced by 23%. The rats in the extract-treated group showed a less marked effect on the levels of serum pyruvate and lactate, indicating that the decreased glucose was not accumulated in the form of these metabolites.

The most common lipid abnormality in diabetes is hypertriglyceridemia. The extent to which these abnomalities occur and their mechanism in different types of diabetes remain to be defined fully. Diabetes also often have an elevated cholesterol level. The present results shows that administration of the methanolic extract to diabetic rats decreased the level of triglycerides, indicating an improvement of hypertriglyceridemia. In addition, treatment of rats with this extract caused a decrease of total cholesterol, although this was less marked than the significant decrease of serum triglyceride. It was thus shown clearly that the methanolic extract had an improving effect on the hyperlipemia induced by streptozotocin.

This is perhaps the first report on the hypoglycemic, hypotriglyceridemic and hypocholesterolemic activities of extracts from *Ixeris* species in rats. These actions are similar to those of insulin. We are planning a further study on whether methanolic extract acts as a kind of modifier which enhances the action of insulin at the insulin receptor or whether methanolic extract reduces the secretion of adrenalin, glucagon or adrenocorticotropic hormone which acts as an antagonist against insulin.

To confirm the hypoglycemic effect of the methanolic extract, we observed the hypoglycemic effect after repeated administration. As shown in Fig. 2, there are no significant differences in the groups given an 80 mg-dose. However, in rats given a 40 mg-dose, the blood glucose level fell to 434.2 mg/dl (a significant decrease of 7% from the control value). The present study demonstrated that administration of a rather low dose of the extract had a more pronounced hypoglycemic action. The present study was also carried out to investigate whether cynaroside would be a useful agent for the treatment of diabetes.

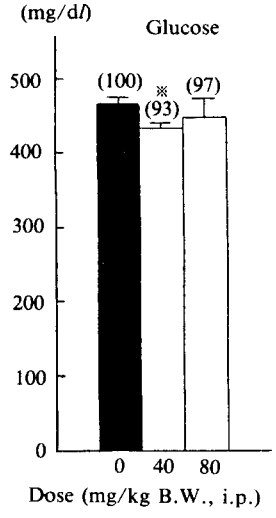

Fig. 2. Effect of methanolic extract of *I. dentata* on blood glucose after repeated administration.
Rats were injected intraperitoneally daily for three days with test dose. Four hours after the last dose, blood samples were obtained after decapitation of the animals.
Values are mean±S.E. in six rats. Figures in parenthesis are percentages of the control value.
*$p<0.05$

The effects of cynaroside on serum constituents after a single intraperitoneal administration are shown in Table II. In rats given cynaroside (10 mg/kg B.W),

Table II. Effect of single administration of cynaroside on levels of serum components in rats treated with streptozotocin

Treatment[a]	Dose (mg/kg BW)	Glucose (mg/dl)	Triglyceride (mg/dl)	Total cholesterol (mg/dl)
Control	–	394.9± 8.3 (100)	178.2±27.4 (100)	104.5±6.5 (100)
Cynaroside	10	308.7±46.0 (88)	105.5±29.9 (59)	87.1±5.2 (83)*

Values are mean±S.E in six rats. Firugre in parenthesis are percentages of the control value.
[a] Rats were injected intraperitoneally once with the test dose. Four hours after single administration, blood samples were obtained after decapitation of the animals.
[b] Significantly different from the control value: *$p<0.05$

Table III. Effect of repeated administration of cynaroside on levels of serum components in rats treated with streptozotocin

Treatment[a]	Dose (mg/kg BW)	Glucose (mg/dl)	Triglyceride (mg/dl)	Total cholesterol (mg/dl)
Control	–	478.47± 6.25 (100)	140.70±5.94 (100)	107.82±4.90 (100)
Cynaroside	10	524.92±23.02 (109)	114.90±10.08 (82)	87.53±5.14 (81)*

Values are mean±S.E in six rats. Firugre in parenthesis are percentages of the control value.
[a] Rats were injected intraperitoneally daily for three days with test dose. Four hours after the last dose, blood samples were obtained after decapitation of the animals.
[b] Significantly different from the control value: *$p<0.05$

the blood glucose level showed a reduced tendency although it was but statistically insignificant. The data in Table II further indicates that the cynaroside-treated group showed a striking decrease (41% as compared with that of the control) of triglyceride. The rats treated with cynaroside also showed a significant decrease of total cholesterol from 104.5 to 87.1 mg/dl.

Because the blood glucose level remained almost unchanged after a single intraperitoneal administration of isolated major flavone glucose, cynaroside, we examined the effects of cynaroside on serum constituents in rats given repeated administration.

As shown in Table III, the repeated administration of 10 mg/kg cynaroside to diabetic rats decreased the levels of triglyceride and total cholesterol even though it failed to demonstrate hypoglycemic effect. From the above results, thus, cynaroside was found to be one of the active principles improving the hyperlipemic state in diabetic rats. Supplementary testing seems required before concluding that this compound is inactive as a hypoglycemic agent on the basis of these experimental conditions.

Biochemical and pharmacological studies on the action of cynaroside including inhibition of a variety of enzymes e.g., lens aldose reductase[7], xanthine oxidase[8], alkaline phosphatase[9] and cyclic AMP phosphodiesterase[10,11] and bacteriostatic[12], hypolipidemic[13,14], anti-inflammatory[15,16], anti-herpetic and anti-poliomyeletic[17] activities have been reported. However, no report on the hypotriglyceridemic and hypocholesterolemic activities in rats with streptozotocin-induced diabetes has appeared. The findings of the present study indicate that the methanolic extract of *I. dentata* whole plants may be useful for treatment of diabetes, specifically that associated with a hyperlipemic state.

Further comprehensive chemical and pharmacological investigations are needed to elucidate exact mechanisms of these effects and to isolate the active principles responsible.

ACKNOWLEDGEMENT

One of the authors (J.S. Choi) express as sincere thanks to KOSEF for financial support to carry out this research at the Institute of Wakan-Yaku, Toyama Medical and Pharmaceutical University, Japan.

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