HYPOGLYCEMIC AND ANTIHYPERGLYCEMIC EFFECTS OF AQUEOUS EXTRACT OF *IXERIS GRACILIS* DC. ON NORMAL AND ALLOXAN-INDUCED DIABETIC MICE

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**Key words:** *Ixeris gracilis* DC., *alloxan*, hypoglycemic, antihyperglycemic, antidiabetic

**SUMMARY**

The potential blood glucose lowering effects of *Ixeris* (I.) gracilis (Asteraceae), an edible wild plant found in parts of Meghalaya, India, is reported. Varying doses (250, 450, 650, 850 and 1000 mg/kg body weight) of aqueous extract of the leaves of *I. gracilis* were administered intraperitoneally to female Swiss albino mice. The optimum effect was observed at a dose of 450 mg/kg body weight, which lowered blood glucose level in normal mice by 24% 2 h after extract administration. Toxic effects were not evident even at a dose of 1000 mg/kg body weight. At a dose of 450 mg/kg b.w., significant antihyperglycemic effect was observed in alloxan-induced diabetic mice with 55% inhibition of blood glucose level 2 h following administration of the extract. Glucose tolerance was also improved in both normal and diabetic mice. The results were compared with those of the standard drugs glibenclamide, metformin and insulin.

**INTRODUCTION**

Diabetes mellitus is a group of syndromes characterized by hyperglycemia; altered metabolism of lipids, carbohydrates, and proteins; and an increased risk of complications from vascular disease (1). Patients have been classified clinically as having either type 1 diabetes mellitus (insulin-dependent diabetes mellitus, IDDM), or type 2 diabetes mellitus (non-insulin dependent diabetes mellitus, NIDDM) (1). Type 2 diabetes mellitus, more commonly referred to as diabetes, is more prevalent and considered to be a world-wide epidemic, which is projected to affect 366 million people by 2030 (2). According to estimates, the number of persons with diabetes in India will rise from 31.7 million to 79.4 million by 2030 (2).

Oral agents are clearly a popular method of treatment in the battle for glycemic control (3). These drugs are, however, associated with a number of side effects. Sulfonylureas (e.g., glibenclamide) reportedly cause severe hypoglycemia, biguanides like metformin are considered unsafe for patients with renal impairments, while α-glucosidase inhibitors cause dose-related malabsorption, flatulence, diarrhea, and abdominal bloating (3). These associated problems necessitate the search for better drugs with fewer side effects. Plant materials that have been used as traditional medicine...
for the treatment of diabetes are considered one of the good sources for a new drug or a lead to make a new drug (4-7).

Many plants are seen to possess hypoglycemic and antihyperglycemic properties (8-11). Some of the notable and recent ones are *Andrographis paniculata* (12), *Gymnema sylvestre* (13), *Allium sativum*, (14,15), *Berberis lyceum* (16), *Tinospora cordifolia* (17), *Madhuca longifolia* (18) and *Momordica charantia* (19,20). Plants with antidiabetic properties act via different mechanisms. There are those which are similar to sulfonylureas (21,22), some similar to biguanides (23,24), while still others act by mimicking the action of insulin (25). While many plants which lower blood glucose levels have been used traditionally by ethnic communities of the northeastern part of India, only a few of them have been scientifically investigated and reported. The few plants studied are *Flemingia macrophylla*, *Potentilla fulgens* L., *Osbeckia chinensis* L., *Albizia lebbek*, *Curcuma amada* and *Gymnopetalum cochinchinensis* (26-31).

*Ixeris (I.) gracilis* DC. is a plant found in certain areas of Khari Hills in Meghalaya, India. It is bitter in taste and is consumed raw as a salad together with rice by the Khasi community. It belongs to the family *Asteraceae* and flowers during the months of April-May.

**MATERIALS AND METHODS**

**Chemicals**

Alloxan monohydrate was procured from Sigma Co., USA, glibenclamide from Aventis Pharma Ltd., insulin from Torrent Pharmaceuticals Ltd., metformin from USV Ltd., while other chemicals used were of analytical grade.

**Test animals**

Healthy, adult female Swiss albino mice weighing 20-30 grams were used for the study. Mice were housed in a room kept under controlled conditions with temperature maintained at 22 °C on a 12-hour light/dark cycle and were fed with balanced mice feed obtained from Pranav Agro Ltd. New Delhi.

Institutional ethical guidelines were followed in all experiments.

**Plant material**

Leaves of *Ixeris gracilis* DC. were collected from Smit village of East Khasi Hills district of Meghalaya (Voucher No: NEHU_11875). The specimen was submitted and identified by Dr. P.B. Gurung Curator Herbarium, Department of Botany, NEHU, Shillong, Meghalaya.

**Extraction**

The leaves were separated, weighed, washed, shredded and dried in the shade. Then were they powdered and repeatedly extracted with 10-times the volume of distilled water. The mixture was filtered and the filtrate evaporated to dryness in a Heto lyolab 3000 lyophilizer. The dried mass obtained was used for the investigation. The yield of the aqueous extract (w/w from dried starting material) was 2.7%. Prior to use, weighed powder was dissolved in distilled water and used for the study.

**Administration of extract to normal mice**

Mice were divided into five test groups and one control group, each group comprising a minimum of six mice (n=6). The mice were starved overnight prior to performing the experiment. The crude extract in varying doses ranging from 250 to 1000 mg/kg b.w. was administered to test groups by intraperitoneal injection and glucose level was monitored at different time intervals up to 24 h following the extract administration. Control groups received only distilled water being the solvent used for preparation. Food but not water was withheld during the 6-hour experiment period. After determination of blood glucose level at 6 h, food was given to the mice and blood glucose level was monitored again at 24 h.
Preparation of diabetic mice

Diabetes was induced by the administration of alloxan monohydrate to mice according to Syiem et al. (27). Mice with two- to three-fold increase in blood sugar levels were considered diabetic and used for further tests.

Administration of extract to alloxan-induced diabetic mice

Alloxan-induced diabetic mice were administered (i.p.) a single dose of 450 mg/kg b. w. of the extract. It was the optimum dose observed in normoglycemic studies. Blood glucose level was measured at varying time intervals for 24 h.

Oral glucose tolerance test and collection of blood for determination of blood glucose level

The oral glucose tolerance test and collection of blood for determination of blood glucose level were performed according to the protocol described by Syiem et al. (27). Mice were fed after measuring blood glucose level at 120 minutes and blood glucose level was monitored again at 1440 minutes (24 h). The standard drugs used in the present study were insulin, glibenclamide and metformin.

Toxicity studies

Normoglycemic mice administered a single injection (i.p.) of varying doses of 250, 450, 650, 850 and 1000 mg/kg b.w. of the extract were kept under observation for a period of 4 weeks for any signs of distress, convulsion, coma or death (32).

RESULTS

Normal mice

The effect on blood glucose level in normoglycemic mice after intraperitoneal administration of the aqueous extract of *I. gracilis* at varying doses showed a reduction in blood glucose levels in a dose- and time-dependent manner (Fig. 1). The lowest dose taken, i.e. 250 mg/kg b.w., showed very mild hypoglycemic activity suppressing glucose level by 17% at 2 h of extract administration. The dose of 450 mg/kg b.w. was observed to exert a greater glucose lowering effect at 2 h of extract administration, with 24% reduction as compared to the dose of 250 mg/kg b.w. The higher doses of 850 and 1000 mg/kg b.w. exerted a more prolonged effect on blood glucose with a reduction of 38% and 41%, respectively, at 24 h after extract administration as compared to lower doses. There were no adverse effects observed with different doses studied.

Diabetic mice

Administration of a selected dose of 450 mg/kg b.w. to diabetic mice resulted in 55% suppression of blood glucose level at 2 h following extract administration (Fig. 2). Lesser reduction was observed at other time intervals (41% at 4 h and 43% at 6 h) as compared to the control group. As in the normoglycemic studies, mice were fed after blood glucose reading at 6 h. There was marked suppression of glucose level even at 24 h, with 55% reduction observed. No toxic effects were apparent during the study period.

Glucose tolerance

Administration of the aqueous extract of *I. gracilis* improved glucose tolerance in both normal and alloxan-induced diabetic mice (Figs. 3 and 4). There
was noticeable suppression (51%) of the glucose peak in extract treated normal mice at 30 minutes (Fig. 3). Blood glucose lowering effect was seen to continue in extract treated mice even at 24 h of extract administration. Glucose tolerance was more improved in extract treated mice than in those treated with metformin. The overall pattern of glucose tolerance resembled that of glibenclamide with respect to the magnitude and pattern of glucose suppression.

Glucose tolerance in diabetic mice was also observed to improve considerably (Fig. 4). There was a 30% suppression of glucose at 30 minutes in comparison with 28% produced by metformin. Glibenclamide and insulin, however, produced higher levels of suppression of the glucose peak at 30 minutes with 43% and 94% inhibition, respectively. Marked glucose suppression comparable to glibenclamide was seen at 120 minute with 74% reduction of blood glucose level as compared to 34% by metformin and 70% by insulin. Following blood glucose determination at 120 minutes, mice were again fed and blood glucose level measured at 24 h. It was observed that extract treated diabetic mice exhibited lower blood glucose level, similar to those treated with insulin. No fatality was observed in extract treated normal and diabetic mice.

DISCUSSION

The results obtained in this study showed that the aqueous extract of *I. gracilis* exerted hypoglycemic as well as antihyperglycemic activity. The normoglycemic studies indicate that the aqueous extract of *I. gracilis* has mild hypoglycemic effects and is nontoxic even at doses of 850 and 1000 mg/kg body weight. Other plants previously reported (*F. macrophylla, P. fulgens* and *O. chinensis*) were toxic at the higher doses, death being observed in either normal or diabetic mice, or both (26-28). The optimum response was observed at a dose of 450 mg/kg b.w. *I. gracilis* was seen to possess significant antihyperglycemic activity at the selected dose of 450 mg/kg b.w., lowering blood glucose levels by half at 2 h following extract administration.
The oral glucose tolerance test is seen as the “gold standard” in diagnosing diabetes mellitus. The suppression of glucose levels in both normal and diabetic mice suggest that *I. gracilis* possesses blood glucose lowering properties that are comparable to the sulfonylurea glibenclamide in terms of the degree and pattern of glucose reduction. Sulfonylureas act by increasing insulin secretion, while biguanides, on the other hand, act by decreasing hepatic glucose production and intestinal glucose absorption, increasing peripheral glucose uptake, and by increasing insulin sensitivity (3). *I. gracilis*, therefore, displays an insulin-secretagogue type of action as reported for *S. nigra* (33) and *F. bengalensis* (34).

Other mechanisms cannot be ruled out at this stage and more comprehensive studies are, however, required in order to ascertain the actual mechanism of this plant.

Based on the results obtained from both the hypoglycemic as well as antihyperglycemic studies, one can say that *I. gracilis* shows promise as an antidiabetic plant and merits further investigation.

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## REFERENCES


