

Anti-Diabetic Activity of Plant Extract of *Urtica Parviflora* in Alloxan Induced Type 2 Diabetes in Rats

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ABSTRACT

Diabetes mellitus is the most common and significant chronic endocrine disorder affecting approximately 200 million individuals worldwide. The Research objective was phytochemical investigation and anti-diabetic activity of polar/non-polar solvent extracts of *Urtica parviflora* leaves. The extracts were subjected to qualitative chemical tests and steroids, triterpenoids, carbohydrates, glycosides and tannins and phenolic compounds were found to be present. The presence of steroids was confirmed by TLC & HPTLC. The effect of different extracts such as ethanolic, petroleum-ether, aqueous and chloroform of *Urtica parviflora* leaves were evaluated for their antidiabetic activity by using alloxan induced diabetes model (albino rats) and Glibenclamide (10 mg/kg p.o.) used as standard drug. LD₅₀ cut-off dose of *Urtica Parviflora* leaves extracts of petroleum ether, ethanolic, chloroform and aqueous extracts were found to be 2000 mg/kg. 1/10th of LD₅₀ cut-off dose of *Urtica parviflora* leaves extracts were taken for screening of antidiabetic activity. The present work confirms that ethanolic, aqueous and chloroform extracts showed significant antidiabetic activity.

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Introduction

Urtica is a genus of annual or perennial herbs, commonly known as Nettle distributed in the temperate and subtropical zones, armed with stinging hairs on the leaves and stems which on contact with the skin, cause irritation and symptoms of urticaria and nettle rash. Acetylcholine, histamine and 5-hydroxytryptamine have been implicated in urticaria and itching from the stinging hairs. Four species occur in India of which *Urtica parviflora* is found abundantly in Kumaun and Garhwal between 1,500ft to 7000ft elevation. Young branches and leaves of *Urtica parviflora* are used as delicious pot herbs. Seed oil is edible as well as medicinal in sciatica, rheumatism and several skin ailments; hair wash from leaf extract is believed to avoid baldness.

The leaves are used in dysentery, joint pain and liver disorders.^[1] The roots are employed for the treatment of fractures of bone and dislocations of joints.^[2] Despite several experimental studies on other *Urtica* species,^[3-8] there is currently only one literature report depicting protective effect of *Urtica parviflora* in CCl₄ induced hepatotoxicity.^[9] Studies on antidiabetic effects have been carried out on other species of urtica. With this background present study was designed to evaluate anti-diabetic activity of *Urtica parviflora* in alloxan induced diabetes models (albino rats).^[10-12]

Materials and Methods

Plant identification

The leaves of *Urtica parviflora* were collected in the month of June from Nainital and were authenticated from Department of Botany, Forest Research Institute, Dehradun, India.

Macroscopy

The following macroscopic characters for the fresh leaves were noted: size and shape, colour, surfaces, venation, presence or absence of petiole, the apex, margin, base, lamina, texture, odour and taste.^[13,14]

Microscopy

The outer epidermal membranous layer (in fragments) was cleared in chloral hydrate, mounted with glycerin and observed under a compound microscope. The presence / absence of the following were observed: epidermal cells, stomata (type and distribution) and epidermal hairs (types of trichomes and distribution). The transverse sections of the fresh leaves through the lamina and the midrib as well as a small quantity of the powdered leaves were also cleared, mounted and observed.^[15]

Quantitative investigation

Quantitative leaf microscopy to determine palisade ratio, stomata number, stomata index, vein-islet number and vein let termination number were carried out on epidermal strips. Other parameters determined for the powdered leaves were moisture content, total ash, acid-insoluble ash, water-soluble ash, alcohol (90% ethanol) and water soluble extractive values.^[16]

Preparation of extracts

The obtained leaves of *Urtica parviflora* were dried in shade and powdered. The powdered leaves were subjected to successive hot-solvent extraction process with the solvents in order of increasing polarity, viz. petroleum ether (40°-60°C), chloroform, ethanolic and aqueous. Aqueous extract was performed by cold maceration process. The extracts were subjected to qualitative chemical tests. Steroids were isolated

from ethanolic extract of *Urtica parviflora*. The isolated compounds were subjected and partly confirmed to spectral studies Viz. UV and FT-IR.

Experimental animals

Male Westar rats weighing 250–350g of both sex were kept in a room maintained at temperature of $23\pm 2^\circ\text{C}$ and under 12 h light/dark cycle.

Experimental design

The extracts of *Urtica parviflora* were evaluated for the antidiabetic activity using following experimental model. Animals were divided into 7 groups each containing 6 animals. Grouping was done as:

Group I- Control group: 2ml Saline p.o.

Group II- Diabetic Control: 2ml Saline p.o.

Group III- Petroleum Ether Extract: 220 mg/kg p.o.

Group IV- Chloroform Extract: 240 mg/kg p.o.

Group V- Ethanol Extract: 200 mg/kg p.o.

Group VI- Aqueous Extract: 180 mg/kg p.o.

Group VII- Glibenclamide 10 mg/kg p.o.

All the animals were fasted for 24 hrs and then blood glucose level was determined immediately before treatment and then 7 hrs after treatment.

Oral glucose tolerance test (OGTT)

Animals were fasted for 16 h before the OGTT. Glucose (1g/kg) was administered by gavage 30min after oral administration of 250mg/kg of *Urtica parviflora* extracts. Glibenclamide at dose of 10mg/kg was used as a standard drug. Blood glucose level was measured each hour after glucose loading in rats under light ether anesthesia. Blood was obtained from tail vein using heparin zed capillary tubes and immediately centrifuged for 5min. Plasma was analyzed for glucose content using a glucose oxygenase method (Sigma diagnostics).

Blood sampling

Approximately, 1 ml blood sample obtained from each animal was placed into an Eppendorf tube, centrifuged at 3000 rpm. The sera were collected and the blood glucose was estimated by glucose oxidase method.^[17]

Phytochemical screening

Preliminary Phytochemical screening of extracts of *Urtica parviflora* leaves was done for their chemical constituents.^[18] The presence of different chemical constituents was confirmed by treatment of the extract with different chemical reagents. For instances, Alkaloids with Dragendorff's reagent, flavonoids with metallic magnesium plus HCl, saponins with the ability to produce foam, reducing sugars with Fehling's reagent, glycosides with Lieberman's test, tannins with ferric chloride and polysaccharides with iodine solution.

Statistical Analysis

Results of Anti-diabetic activity were reported as Mean \pm SEM. Significant intergroup difference of each parameter was analyzed separately and one-way analysis of variance (ANOVA) was carried out. The calculated mean tabulated along with the SEM, Dunnet's 't' test was used for individual comparison (Pulsatum Health Care Pvt. Ltd., Bangalore).

Results and Discussion

Macroscopically, the leaf is simple in composition, most of the leaves and stalks are arranged across opposite sides of the stem. The leaf blades are elliptic, lanceolate, ovate or circular. The leaf blades usually have three to five, rarely up to seven veins. The leaf margin is usually serrate to more or less coarsely tooth. The often-lasting bracts are free or fused to each other. Fresh leaves are green in colour, odourless with a slightly acid taste.

Microscopic and morphological features revealed that leaf constants are fixed for all plant species, but they may vary from species to species. Determination of leaf constants is also one of the methods of standardization. It is helpful in identification of correct plant variety and also useful in predicting adulteration. The results of leaf constants & numerical data are provided in Table-1 and Table-2 respectively.

In the present study, the leaves of *Urtica parviflora* (Urticaceae) were subjected to Pharmacognostic investigations to identify the leaf constants. The Phytochemical investigation of various extracts is as follows; petroleum ether extract showed the presence of steroids, fats and oil, chloroform extract showed the presence of carbohydrates, steroids, triterpenoids, ethanolic extract showed the presence of steroids, triterpenoids, carbohydrates, tannin and phenolic substances and aqueous extract showed the presence of glycosides, steroids, triterpenoids and tannins and phenolic compounds

Table 1. Morphological screening of leave of *Urtica parviflora*

S. No.	Parameter	Constants	Mean
1	Stomatal number	4-6	04.8 \pm 0.312
2	Stomatal Index	1.3-2.7-6.3	1.2 \pm 0.142/2.7 \pm 0.089/ 6.2 \pm 0.125
3	Vein-Islet number	21-28	22.71 \pm 0.624
4	Vein-Termination No.	09-17	09.91 \pm 0.414
5	Palisade cells Ratio	1 - 3	01.21 \pm 0.718

Table 2. Phytochemical screening of leave extract of *Urtica parviflora*

S. No.	Parameter	Mean (% w/w)
1	Moisture Content	13.65 \pm 0.152
2	Total Ash	14.50 \pm 0.225
3	Acid Insoluble Ash	05.36 \pm 0.175
4	Water Soluble Ash	04.10 \pm 0.005
5	Alcohol Soluble Extractive	08.88 \pm 0.015
6	Water Soluble Extractive	33.44 \pm 0.009
7	Loss on Drying	02.00 \pm 0.075

Acute toxicity study was carried out according to OECD guidelines. The LD₅₀ values were obtained for various extracts (Table-3) and 1/10th (200 mg/kg b.w.) of this lethal dose was taken as effective dose (therapeutic dose) for subsequent anti-diabetic activity.

Table 3. Oral acute toxicity study of leave extract of *Urtica parviflora*

Sr. No.	Extracts	LD ₅₀ Cut-off mg/kg b.w.
1	Petroleum Ether	2200 mg/kg b.w.
2	Chloroform	2400 mg/kg b.w.
3	Ethanol	2000 mg/kg b.w.
4	Aqueous	1800 mg/kg b.w.

The petroleum ether (40°-60°C), chloroform, ethanol and aqueous extracts were given orally at a dose of 200mg/kg b.w. with the help of gastric tube to alloxan induced diabetic rats. Further the blood glucose levels were analyzed initially (0 hr), 1st hr, 3rd hr, 5th hr and 7th hr. after single dose and 7th day after prolonged treatment of extracts.

Normal control and diabetic control animals received equal volume of normal saline and Glibenclamide tablet (10 mg/kg b.w.) served as standard. Blood glucose level was measured in all groups by using glucometer, (Pulsatum, Pulsatum Health Care Pvt. Ltd., Bangalore).

Results obtained from alloxan induced diabetes indicated that ethanol, chloroform, and water showed more significant ($p < 0.01$) anti-diabetic activity (200.3 ± 3.84 , 202.0 ± 4.239 , 203.5 ± 3.201 respectively) in acute as well as prolonged treatment (178.3 ± 2.916 , 192.7 ± 4.009 , 195.0 ± 2.897 respectively) compared to diabetic control (222.10 ± 4.058). The results were comparable with standard Glibenclamide (186.18 ± 3.906 , 192.18 ± 4.35). Petroleum ether extract did not show significant anti-diabetic activity on prolonged treatment but showed significant ($p < 0.05$) activity at 7th hour in acute study (207.71 ± 4.427) compared to diabetic control.

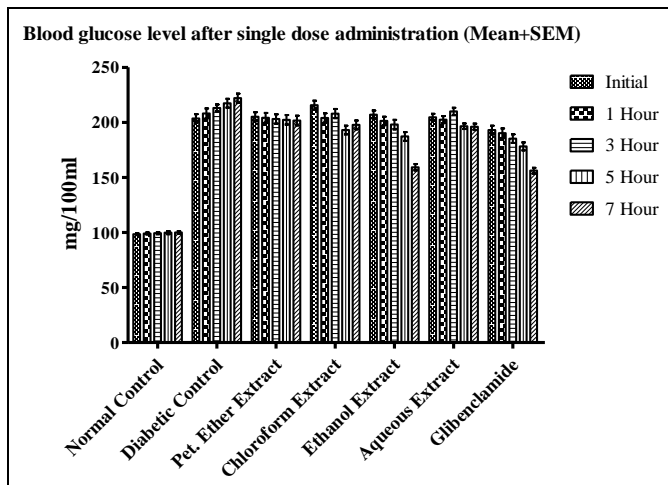


Fig 1. Blood glucose level of Alloxan induced diabetic rats after single dose administration

The single dose of alcoholic extract has more significantly reduced the blood glucose level at 3rd hr (202.0 ± 3.796) and at 0 hr (192.5 ± 4.311). Glibenclamide (10 mg/kg b.w.) has also significantly reduced blood glucose level at 3rd hr (196.13 ± 3.924) and 170.18 ± 2.583 at 0 hr and significant hypoglycemia was maintained for another four hours (Figure-1).

On prolonged treatment, the effect of Ethanolic extracts were (178.3 ± 7.916) nearly equal to that of the standard drug Glibenclamide (170.18 ± 6.583). These findings clearly established that alcoholic extract exhibited better anti-diabetic activity than other extracts (Figure-2).

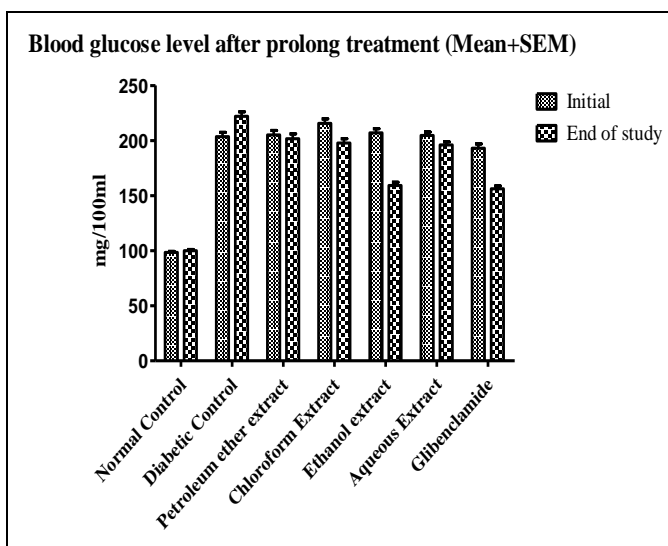


Fig 2. Blood glucose level of Alloxan induced diabetic rats after prolonged treatment

Conclusion

The overall results of the antidiabetic activity have led to the conclusion that ethanolic extract has exhibited more significant antidiabetic activity which compared with other extracts as it contains major chemical constituents viz. steroids and tannins. Aqueous and chloroform extracts also showed significant activity as compared to diabetic control which may be due to the combination of various active constituents. However, this claim demands for further study to pinpoint the mechanism of the extracts and formulation of *Urtica parviflora* leaves in combination or single for the development of herbal formulation.

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