EVALUATION OF ANTI DIABETIC ACTIVITY OF URTICA URENS LINN IN STZ INDUCED DIABETIC RATS

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Abstract: This study has been undertaken to investigate the anti diabetic activity of Urtica urens linn in streptozotacin induced diabetic rats. The present study shows that the ethanolic extract of Urtica urens has potential antidiabetic action in STZ induced diabetic rats and the effect was found to be more similar to the reference drug Metformin.

Index Terms: Diabetes, Streptozotacin, Metformin, *Urtica urens*

1.INTRODUCTION

Diabetes mellitus is a combination of heterogeneous disorders commonly presenting with episodes of hyperglycaemia and glucose intolerance, as a result of lack of insulin, defective insulin action, or both. Such complications arise due to derangements in the regulatory systems for storage and mobilization of metabolic fuels, including the catabolism and anabolism of carbohydrates, lipids and proteins emanating from defective insulin secretion, insulin action, or both. The incidence of type 1 diabetes is increasing in both rich and poor countries. Furthermore, a shift towards type 1 diabetes occurring in children at earlier ages is imminent. In 2010, about 285 million people in the age group 20-79 were envisaged to have diabetes worldwide, about 70% of whom live in developing nations. This estimate is expected to increase to about 438 million, by 2030. Further, by 2030, the number of people with IGT is projected to increase to 472 million, or 8.4% of the adult population. In the last few decades eco-friendly, bio-friendly, cost effective and relatively safe, plant-based medicines have moved from the fringe to the main stream with the increased research in the field of traditional medicine**.**

Urtica urens L. is a member of Family Urticaceae which includes about 48 genera and 1050 tropical and warm temperate species. It's an annual herbaceous shrubs, native of Europe and has become naturalized throughout North America, Africa, Asia, Australia and South America. *U. urens* L. is one species of 3 *genera* grows in Egypt as wild weeds in the cultivated lands, around the River Nile, Mediterranean regions and Isthemic desert. This plant is a rich source of phenolic compounds. It is widely used as folk medicine and exhibitedanti-nociceptive, antioxidant and hepatoprotective effects. Plant material of *Urtica urens* is quite evident from the literatures surveyed that these plants number of pharmacological activity and therefore the extracts of these herbs alone or in combination may have the potential to treat many diseases in an effective manner without exhibiting side effect or toxicity as indicated by synthetic molecules. The aim of our study was to provide scientific evidence concerned to the medicinal values of this herb.

2. MATERIAL AND METHODS

2.1 Plant material collection

Aerial parts of *Urtica urens* were purchased from Puspita nursery from Kolkata.

2.2 Extraction of plant material

Dried powdered plant material aerial parts of *Urtica urens* has been extracted with ethanol using maceration process for 48 hrs, filtered and dried using vaccum evaporator at 40^0C.

2.3 Determination of percentage yield

The percentage yield of each extract was calculated by using following formula:

$$\text{Percentage yield} = \frac{\text{Weight of Extract}}{\text{Weight of powder drug Taken}} \times 100$$

2.4 Phytochemical Screening

Phytochemical examinations were carried out for all the extracts as per the standard methods.

2.4 In vivo* Antidiabetic activity of *Urtica urens

2.4.1 Source of data

The standard information was collected from various journals, standard text books available in the library and digital library and from various standard websites.

2.4.2 Screening of Diabetes

I Animals

Wistar rats (150–200 g) were group housed (n= 6) under a standard 12 h light/dark cycle and controlled conditions of temperature and humidity (25±2 °C, 55–65%). Rats received standard rodent chow and water *ad libitum*. Rats were acclimatized to laboratory conditions for 7 days before carrying out the experiments. All the experiments were carried in a noise-free room between 08.00 to 15.00 h. Separate group (n=6) of rats was used for each set of experiments. The animal studies were approved by the Institutional Animal Ethics Committee (IAEC), constituted for the purpose of control and supervision of experimental animals by Ministry of Environment and Forests, Government of India, New Delhi, India.

II Toxicity Study

For the acute oral toxicity determination the organization for economic co-operation and development (OECD) guideline 423 was followed. As per OECD guidelines a stepwise procedure with the use of 3 animals of a single sex per step was followed. Absence or presence of compound related mortality of the animal doses at one step will determine the next step i.e.

- No further testing needed
- Dosing of three additional animals, with the same dose
- Dosing of three additional animals at the next higher or the next lower dose levels.

III Experimental model:

Induction of Experimental Diabetes in Rats

Streptozotocin was dissolved in 100 mM citrate buffer (pH 4.5) and calculated amount of the dose (60 mg/kg) of the fresh solution was injected intraperitoneally to overnight fasted rats, 15 min after the intraperitoneal administration of 110 mg/kg of nicotinamide. Blood glucose was checked 48 h later and animals showing blood glucose value more than 250 mg/dl were included in the experiments and termed as diabetic.

Group I- Normal

Group II- Diabetic rats received only distilled water (negative control)

Group III- Diabetic rats was treated with Metformin (500mg/kg p.o.)

Group IV- Diabetic rats received *Urtica urens* (100 mg/kg/day p.o.)

Group V- Diabetic rats received *Urtica urens* (200 mg/kg/day p.o.)

IV Antidiabetic screening

Blood sampling and glucose estimation

For blood glucose determination, blood was withdrawn by tail snipping technique. For various lipid profile and biochemical parameters estimation, blood was collected from ophthalmic venous plexus by retro-orbital bleeding technique.

Estimation of Total Cholesterol (TC)

Total cholesterol in serum was estimated by using CHOD/PAP methods.

Estimation of Triglycerides (TG)

Triglycerides are the main constituent of vegetable oil, animal fat, LDL and VLDL, and play an important role as transporters of fatty acids as well as serving as an energy source.

Estimation of High Density Lipoproteins-Cholesterol (HDL-C)

HDL-cholesterol in serum was estimated by using PEG method.

Classification	Cholesterol	HDL	Triglyceride	LDL
Desirable	<200	>60	<150	<130
Borderline	200-239	35-59	200-399	130-159
High	>240	-	>399	>160
Low	-	<35	-	-

Estimation of Total Protein (TP)

Proteins are present in all body fluids but show very high concentration (> 3 g/dl) in plasma, lymphatic fluids, and some exudates.

Estimation of SGPT

The enzyme alanine aminotransferase is widely reported in a variety of tissue sources.

V Statistical analysis

Variables of interest were entered and all data analyzed using GraphPad Instant 3.06 software version 14 for windows XP (Microsoft Corporation). All statistical analysis is expressed as mean ± standard error of the mean (SEM). Data were analyzed by one way ANOVA, where applicable $p<0.05$ was considered statistically significant, compared with vehicle.

3. RESULTS AND DISCUSSION

I Determination of Percentage Yield

II Yield of Extraction: The crude extracts so obtained after the maceration process, each extracts were further concentrated on water bath evaporation the solvents completely to obtain the actual yield of extraction. To obtain the percentage yield of extraction is very important phenomenon in phytochemical extraction to evaluate the standard extraction efficiency for a particular plant, different parts of same plant or different solvents used. The yield of extracts using ethanol as solvents is depicted in the table.

Table 1: % Yield of ethanolic extract

S. No.	Plant	Yield (w/w)
1.	Aerial parts of *Urtica urens*	6.98%

III Phytochemical screening of extract

A small portion of the dried extracts were subjected to the phytochemical test using Kokate (1994) methods to test for alkaloids, glycosides, saponins, flavonoids and phenol separately for extracts of all samples. Small amount of each extract is suitably resuspended into the sterile distilled water to make the concentration of 1 mg per ml. The outcomes of the results are discussed separately in the table

Table No. 2: Result of phytochemical screening of ethanolic extract of *Urtica urens*

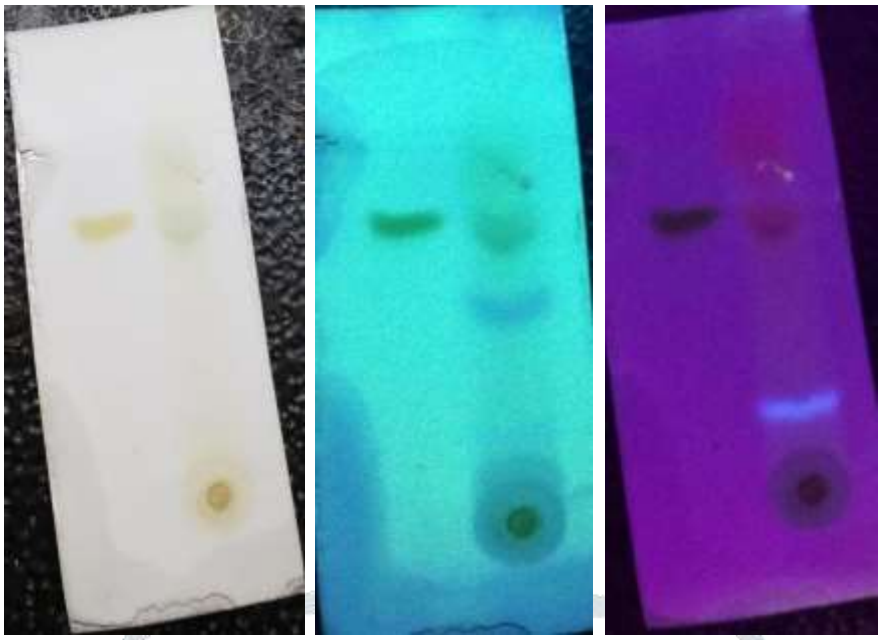
S. No.	Constituents	Ethanolic extract
1.	**Alkaloids**	
	Dragendroff's test	-ve
	Wagner's test	-ve
	Mayer's test	-ve
	Hager's test	-ve
2.	**Glycosides**	
	General glycosides test	-ve
3.	**Flavonoids**	
	Lead acetate test	+ve
	Alkaline test	-ve
5.	**Phenolics**	
	$Fecl_3$ test	-ve
6.	**Amino acids**	
	Ninhydrin test	+ve
7.	**Cabohydrates**	
	Molichs test	-ve
8.	**Diterpines**	-ve
	Copper acetate test	
9.	**Saponins**	-ve

IV Results of comparative thin layer chromatography of ethanolic extract

From the Rf value it was confirmed the presence of Quercetin as Flavanoids in the extract.

Table No. 3: TLC of extracts

S. No.	Toluene: Ethyl acetate: Formic acid (5:4:1) Quercetin (R_f value)
1.	0.82



Normal Light Short U.V. Long U.V.

Figure 1: Photograph of T.L.C (Quercetin)

V Results of estimation of total flavonoid contents

Total flavonoid content estimation (TFC)

The content of total flavonoid compounds (TFC) content was expressed as mg/100mg of quercetin equivalent of dry extract sample using the equation obtained from the calibration curve: **$Y = 0.06X+0.019$, $R^2= 0.999$,** where X is the quercetin equivalent (QE) and Y is the absorbance.

VI Calibration Curve of Quercetin

Table No.4: Preparation of calibration curve of Quercetin

S. No.	Concentration	Absorbance
0	0	0
1	5	0.352
2	10	0.61
3	15	0.917
4	20	1.215
5	25	1.521

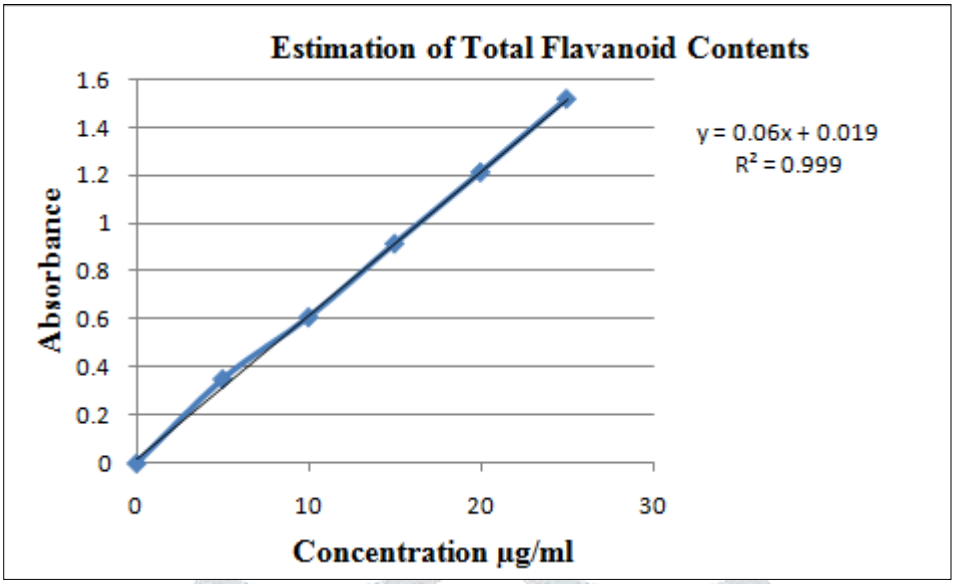


Figure 2: Graph of Estimation of Total flavonoid content

Table No.5: Total flavonoid content of *Urtica urens* extract

S. No.	Extract	Total flavonoid (mg/100mg)
1.	Ethanolic	0.876

VI Results of *in vivo* anti-diabetic activity

The various results obtained from different experiments carried out were compiled here under.

Table No.6: Mean Body Weight Change

Group	Initial weight	Final weight
Normal	200.00±8.00	230.18±8.00
Control	210.20±15.62	190.00±15.60
Metformin	230.00±10.00	190.00±10.45
***Urtica urens*-100**	230.00±7.00	199.20±7.00
***Urtica urens*-200**	235.00±8.00	197.00±8.00

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at $p<0.05$ vs. control group respectively (One-way ANOVA followed by Dunnett's test).

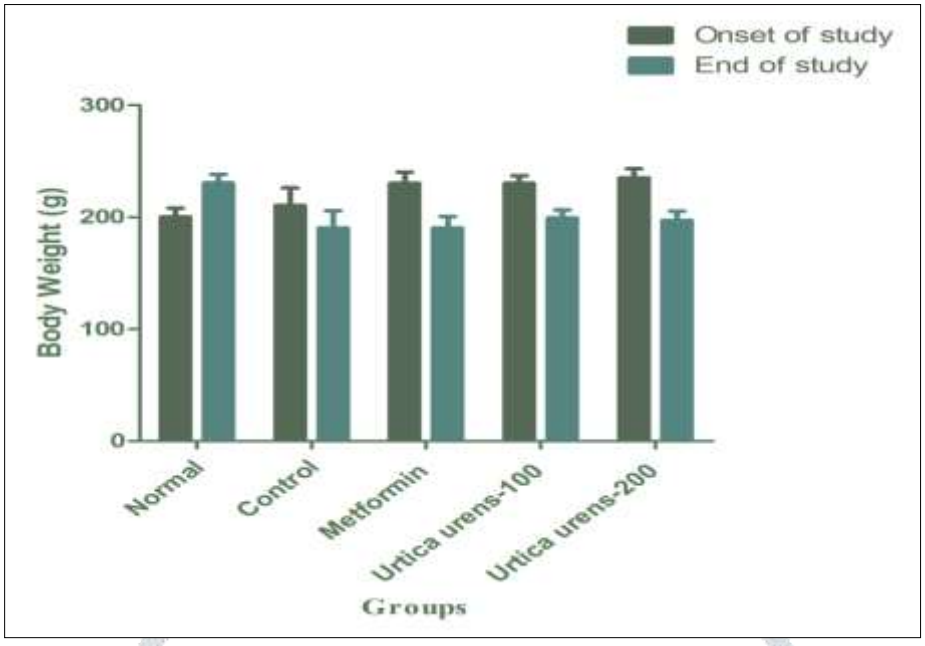


Figure 3: Mean Body Weight Change

Table No.7: Antidiabetic activity of *Urtica urens* on blood glucose level in STZ induced diabetic rats

Group	0 day	8 day	21 day
Normal	90.00±4.00	92.00±4.00	100.00±5.00
Control	295.00±5.00	380.00±5.00	391.00±5.00
Metformin	245.00±7.00	130.00±7.00	120.00±7.00
***Urtica urens*-100**	255.00±5.50	157.00±5.50	140.00±5.50
***Urtica urens*-200**	250.00±6.00	142.00±6.00	125.00±6.00

Values are expressed as mean ± S.E.M. (n = 6).Values are statistically significant at $p<0.05$ vs. negative control group respectively (One-way ANOVA followed by Dunnett's test)

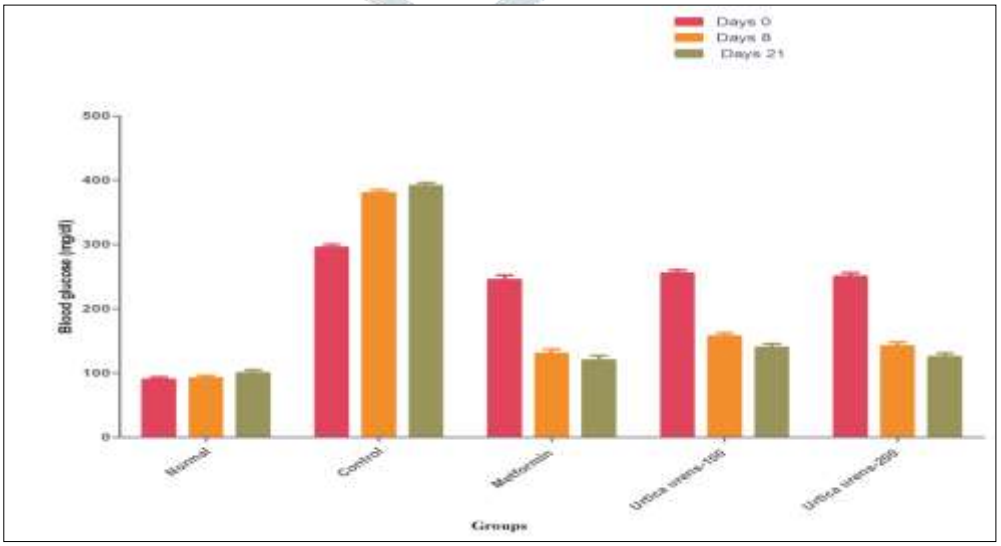


Figure 4: Antidiabetic activity of *Urtica urens* on blood glucose level in STZ induced diabetic rats

Table No.7.8: Effect of *Urtica urens* on total cholesterol level in STZ-induced diabetic rats

Group	TC (Total Cholesterol)
Normal	80.50±5.50
Control	200.00±5.00
Metformin	115.00±7.00
Urtica urens-100	125.00±5.00
Urtica urens-200	121.00±5.00

Values are expressed as mean ± S.E.M. (*n* = 6).Values are statistically significant at p<0.05 (One-way ANOVA followed by Dunnett's test).

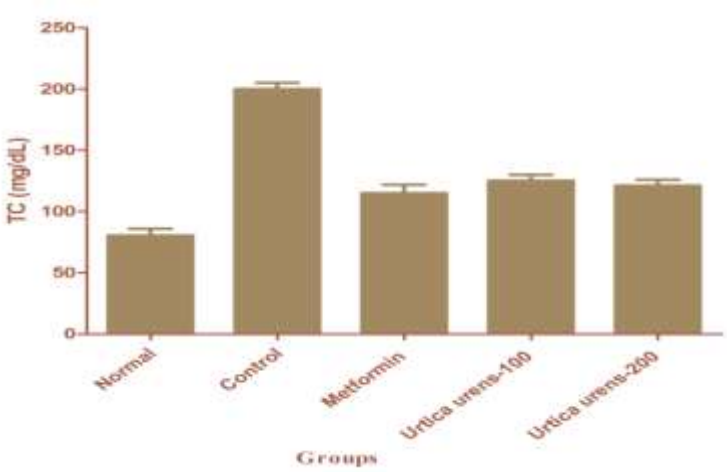


Figure 7.5: Effect of *Urtica urens* on total cholesterol level in STZ induced diabetic rats

Table No.9: Effect of *Urtica urens* on triglyceride level in STZ -induced diabetic rats

Group	TG (triglyceride)
Normal	80.00±5.00
Control	138.00±6.50
Metformin	89.00±9.00
Urtica urens-100	99.00±7.00
Urtica urens-200	87.00±6.00

Values are expressed as mean ± S.E.M. (*n* = 6).Values are statistically significant at p<0.05 (One-way ANOVA followed by Dunnett's test).

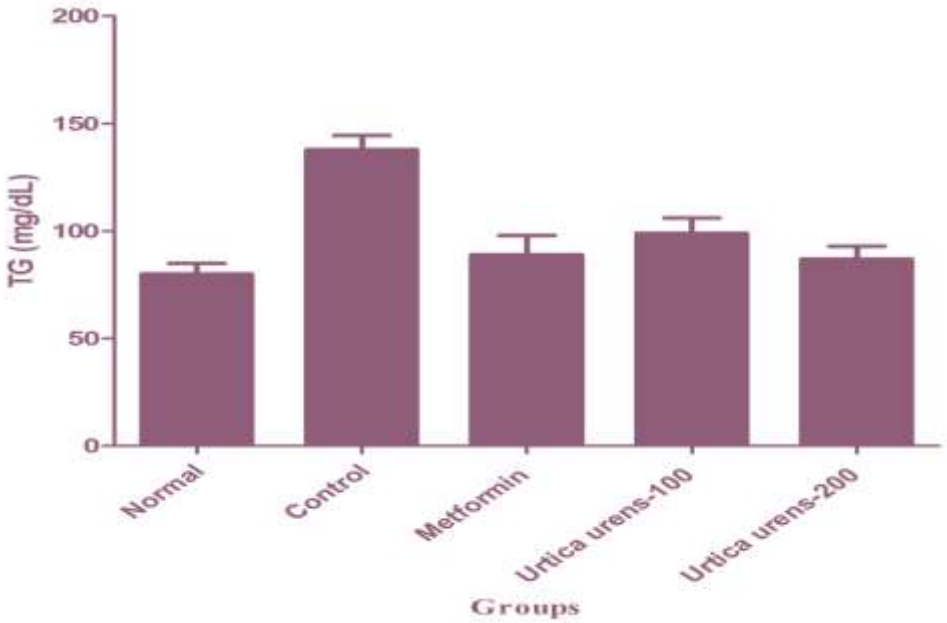


Figure 7.6: Effect of *Urtica urens* on triglyceride level in STZ induced diabetic rats

Table No.10: Effect of *Urtica urens* on HDL in STZ induced diabetic rats

Group	**HDL**
Normal	53.00±5.00
Control	29.00±7.00
Metformin	50.00±6.00
Urtica urens-100	38.00±5.00
Urtica urens-200	45.00±6.00

Values are expressed as mean ± S.E.M. (*n* = 6).Values are statistically significant at p<0.05 (One-way ANOVA followed by Dunnett's test).

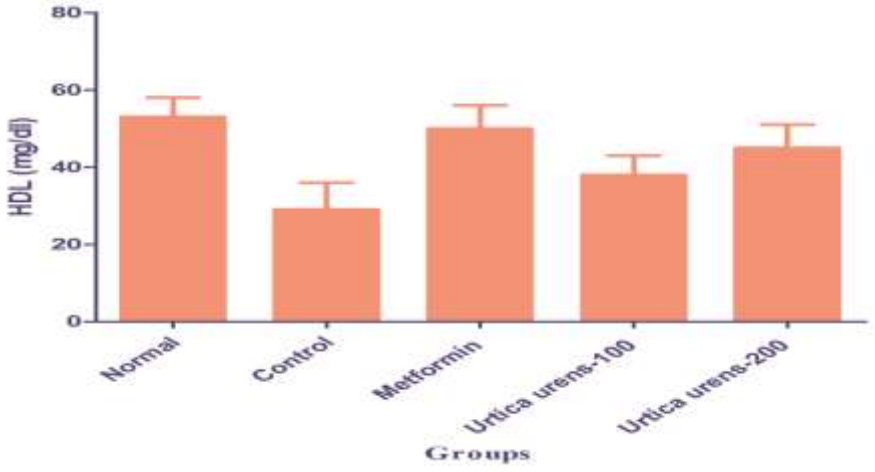


Figure 7: Effect of *Urtica urens* on HDL in STZ -induced diabetic rats

Table No.11: Antidiabetic effect of *Urtica urens* on serum lipid profile i.e. total protein (TP) level in STZ -induced diabetic rats

Group	Total protein (TP)
Normal	80.00±5.00
Control	130.00±6.00
Metformin	75.00±7.00
Urtica urens-100	94.00±5.00
Urtica urens-200	83.00±5.00

Values are expressed as mean ± S.E.M. (*n* = 6).Values are statistically significant at p<0.05 (One-way ANOVA followed by Dunnett's test).

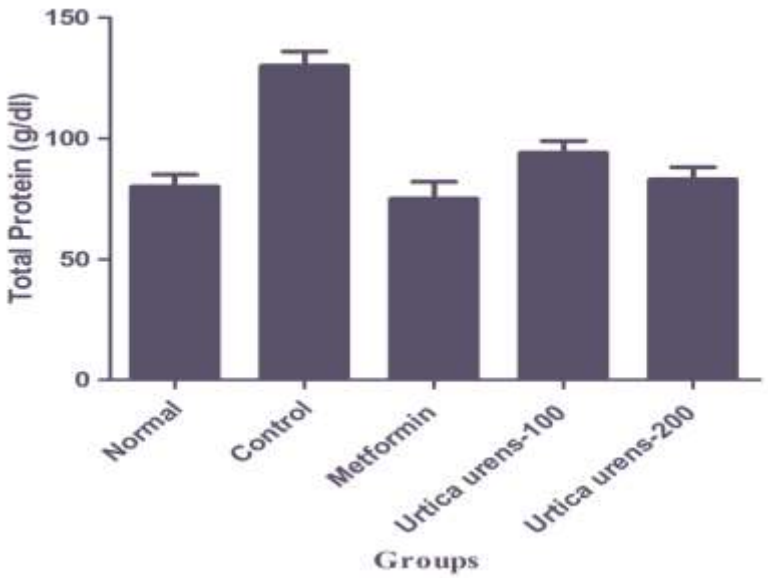


Figure 8: Effect of *Urtica urens* on TP in STZ induced diabetic rats

Table No.12: Effect of *Urtica urens* on SGOT in STZ induced diabetic rats

Group	SGOT
Normal	60.00±5.00
Control	125.00±7.00
Metformin	70.00±6.00
Urtica urens-100	80.00±5.00
Urtica urens-200	75.00±5.00

Values are expressed as mean ± S.E.M. (*n* = 6).Values are statistically significant at p<0.05 (One-way ANOVA followed by Dunnett's test).

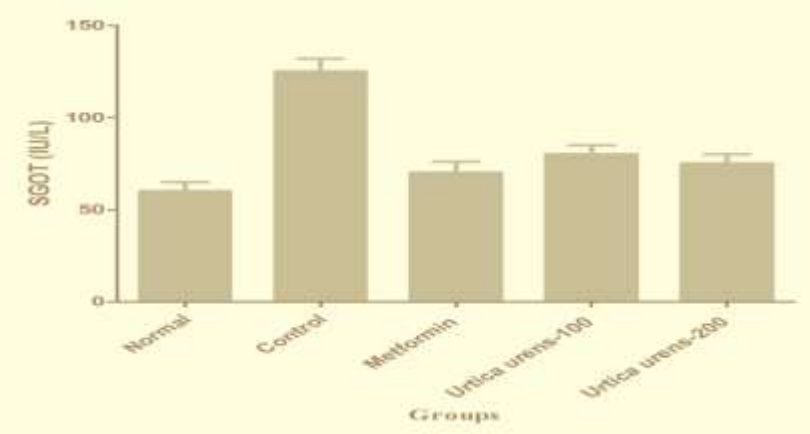


Figure 9: Effect of *Urtica urens* on SGOT in STZ -induced diabetic rats

Table No.13: Effect of *Urtica urens* on SGPT in STZ -induced diabetic rats

Group	**SGPT**
Normal	50.00±5.00
Control	118.00±7.00
Metformin	60.00±5.00
Urtica urens-100	70.00±5.00
Urtica urens-200	65.00±6.00

Values are expressed as mean ± S.E.M. (*n* = 6).Values are statistically significant at p<0.05 (One-way ANOVA followed by Dunnett's test).

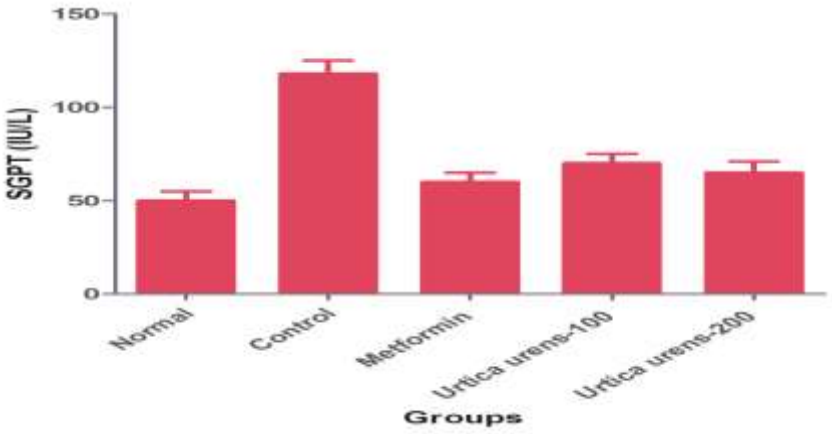


Figure 10: Effect of *Urtica urens* on SGOT in STZ -induced diabetic rat

4. Conclusion

In conclusion, the present study shows that the ethanolic extract of *Urtica urens* has potential antidiabetic action in STZ induced diabetic rats and the effect was found to be more similar to the reference drug Metformin.

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