ANTI-INFLAMMATORY ACTIVITY OF HYDROALCOHOLIC EXTRACT OF AERIAL PARTS OF URTICA URENS L.

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Abstract *:* With an aim to study anti-inflammatory activity of hydroalcoholic extract of aerial parts of *Urtica urens* L, anti-inflammatory activity was measured using formalin (0.2 ml of 2% v/v) induced rat paw edema assay in albino wistar rats by intraperitoneal route. Rats were treated with hydroalcoholic extract of aerial parts of *Urtica urens* L and anti-inflammatory activity of aerial parts of *Urtica urens* L (100mg/kg and 200mg/kg, p.o.) was observed. Antioxidants have been reported to play a significant role in diminishing the formalin induced paw edema. Hydroalcoholic extract of aerial parts of *Urtica urens* L prevented formalin induced paw edema in a dose dependent manner shows significant anti-inflammatory effect, percentage inhibition shown in group 3 (given extract of 100mg/kg p.o.) and 4 (given extract of 200 mg/kg p.o.) was 69.17% and 72.27%. On the basis of the results obtained in the present study, it is possible to conclude that hydroalcoholic extract of aerial parts of *Urtica urens* L has significant anti-inflammatory activity in rats induced with formalin.

IndexTerms **– Herbal, Hydroalcoholic extract, Urtica urens, Anti-inflammatory, Safe and Effective.**

I. INTRODUCTION

Inflammation is a part of the complicated biological reaction of vascular tissues to harmful stimuli, including pathogens, damaged cells or irritants. It is characterized via redness, swollen joints, joint pain, its stiffness and lack of joint characteristic. Inflammation is presently treated via NSAIDs. Unfortunately these capsules motive elevated danger of blood clot ensuing in heart assaults and strokes. Inflammation is a normal, protective reaction to tissue damage caused by physical trauma, noxious chemical compounds or microbiological marketers.

Inflammation is a stereotyped reaction, inherent to vascularized tissues, which has the goal of reestablishing tissues homeostasis. The inflammatory process has cell and humoral additives, such as leucocytes (neutrophils, macrophages, eosinophils, mast cells and lymphocytes) and the humoral proteolytic structures (complement, kinins and coagulation), respectively. These components paintings synergistically and concurrently, inflicting vascular changes and leukocyte recruitment to the lesion.

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 exhibited anti-nociceptive and antioxidant.

2. MATERIAL AND METHODS

2.1. Plant collection

The aerial parts of Urtica urens L. were collected from local area of Bhopal in the month of October, 2018.

2.2. EXTRACTION OF PLANT

Aerial parts of Urtica urens L was shade dried at room temperature. The shade dried plant material was coarsely powdered and subjected to extraction with petroleum ether by maceration. The extraction was continued till the defatting of the material had taken place. 45gm of dried powdered of aerial parts of Urtica urens L. has been extracted with hydroalcoholic solvents (70:30: ethanol: water) using maceration

process for 48 hrs, filtered and dried using vacuum evaporator at 40ºC. The percentage yield of each extract was calculated by using following formula:

% yield = weight of the extract/weight of plant material × 100

2.3. Phytochemical investigation

Qualitative phytochemical investigation was carried out for by alkaloids, glycosides, flavonoids, phenolics, amino acids, carbohydrates, Diterpene diterpenes and saponins.

2.4. Anti-inflammatory activity

2.4.1. 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 bef 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= group (n=6) of rats was used for each set of experiments.

2.4.2. DRUGS AND CHEMICALS

Diclofenac injections (Voveran), formalin (Sigma Chemical) were used in present study.

2.4.3. Toxicity study

Prelimina Preliminary experiments were carried out on rats (n=6). Hydroalcoholic extract aerial parts of Urtica urens L were administered orally in differen different doses to find out the range of doses which cause zero and 100 % mortality of animals. Acute oral toxicity was conducted according to the method of Organisation for Economic Cooperation and Development (OECD) (OECD, 2001). Animals were kept fasting providing only wat only water, extract were given p.o. in doses of 500, 1000 and 2000 mg/kg/p.o. administered orally for 4 days of different groups of rats (n=6) and the animals were kept under observation for mortality as well as any behavioral changes for evaluation of a possible anti-inflammatory effect.

2.4.4. Grouping of animals

The animals were randomly divided into following four groups; each group consists of six animals. Animal grouping and their treatment is as follows

Group –1: Control (formalin -0.2 ml of 2% v/v)

Group –2: Diclofenac sodium (10 mg/kg, bw, Standard) + Formalin (0.2 ml of 2% v/v)

Group –3: Hydroalcoholic extract aerial parts of Urtica urens L (100mg/kg, p.o.) + Formalin (0.2 ml of 2% v/v)

Group –4: Hydroalcoholic extract aerial parts of Urtica urens L (200mg/kg, p.o.) + Formalin (0.2 ml of 2% v/v)

2.4.5 . Formalin induced hind paw edema model

Antiinflammatory activity was measured using formalin induced rat paw oedema assay. The rats were divided into 4 groups of 6 animals eac each (plant extract was dissolved and administered per oral at different dose levels). Group 1 was treated as formalin (0.2 ml of 2% v/v

freshly prepared formalin solution prepared in distilled water) was used as edematogenic agent, Group 2 was administered Diclofenac sodium sodium (10 mg/kg, bw) and considered as standard a(100mg/kg, p.o.) and + Formalin (0.2 ml of 2% v/v). Group 4 were treated with H Hydroalcoholic extract aerial parts of Urtica urens L (200mg/kg, p.o.) and Formalin (0.2 ml of 2% v/v). The thickness was measured before

injecting the formalin and after injecting the formalin everyday at a fixed time. The volumes of oedema of the injected were measured after the induction of inflammation using a plethysomgraph to calculate the percentage of paw oedema inhibition.

Percentage Inhibition = Vc-Vt/Vc X 100

Where, Vc- Edema volume of control group

Vt- Edema volume of test group

2.4.6. Statistical Analysis

All analysis was performed using graph pad prism for Windows. 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 followed by Dunnett's test.

3.RESULT

3.1. Percentage Yield

Yield of Extraction: The yield of extracts obtained from different samples using Pet. ether, hydroalcoholic solvents are depicted in the table n o . 1 .

Table no. 1

S. No.	Solvents	% Yield
1	Pet ether	4.45
2.	Hydroalcoholic	8.55

3.2. Phytochemical analysis

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 no. 2

Table no. 2

S.NO.	EXPERIMENT	OBSERVATION
1	**Alkaloids**	Absent
	Mayer's reagent test	Fail
	Wagner's reagent test	Fail
	Hager's reagent test	Fail
2	**Glycosides**	Absent
	General glycosides test	Fail
3	**Flavonoids**	Present
	Lead acetate test	Pass
	Alkaline test	Pass
4	**Phenolics**	Absent
	FeCl3 test	Fail
5	**Amino acids**	Present

	Ninhydrin test	Pass
6	**Carbohydrates**	Absent
	Molisch test	Fail
7	**Diterpenes**	Absent
	Copper acetate test	Fail
8	**Saponins**	Absent
	Froth test	Fail

3.3 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, R2= 0.999, where X is the quercetin equivalent (QE) and Y is the absorbance.

3.4 Calibration Curve of Quercetin

Table No. 3: Preparation of calibration curve of Quercetin

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

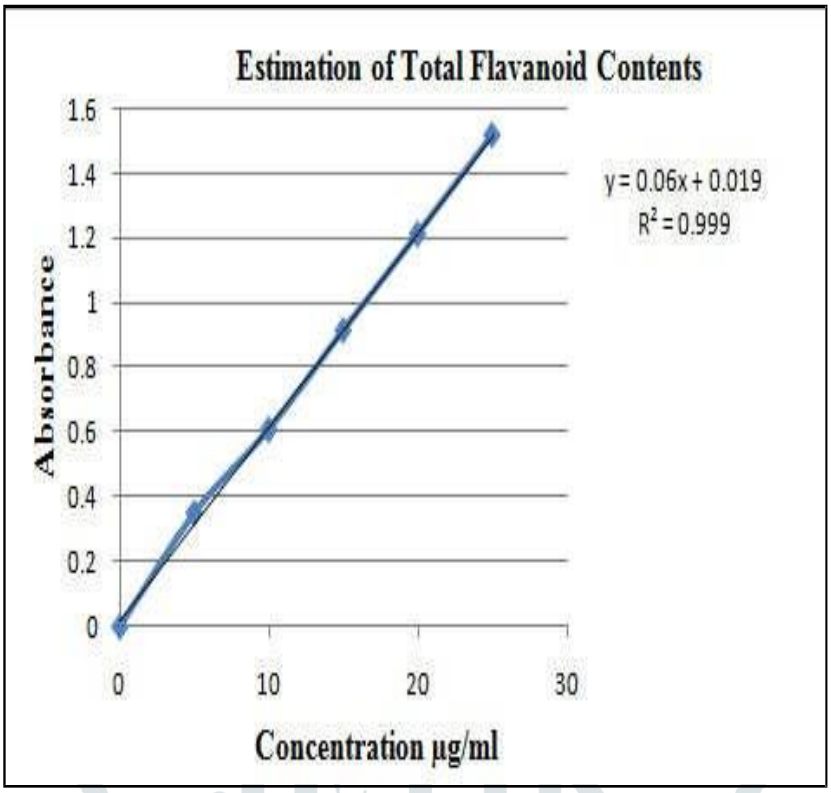

Figure No.1: Graph of Estimation of Total flavonoid content

Table No. 4: Total flavonoid content of Hydroalcoholic extract of *Urtica urens*

S. No.	Extract	Total flavonoid (mg/100mg)
1.	Hydroalcoholic extract	0.897

3.5 Results of anti-inflammatory activity

Table No. 5 : Effect of Hydroalcoholic extract aerial parts of Urtica urens L on paw edema induced by formalin in rats

Treatment	Dose (mg/kg)	Mean differences in Paw Volume (ml)	Percentage of Inhibition (%)
Control (formalin)	0.2 ml of 2% v/v	3.00 ±0.50	--
Diclofenac sodium + Formalin (0.2 ml of 2% v/v)	10	0.90±0.50 ***	70
Hydroalcoholic extract aerial parts of *Urtica urens L* + Formalin (0.2 ml of 2% v/v)	100	1.20±0.50 **	60
Hydroalcoholic extract aerial parts of *Urtica urens L*+ Formalin (0.2 ml of 2% v/v)	200	0.99±0.50 ***	67

Values are expressed as mean ± SD.

*P < 0.05-significant compared to formalin treated group.

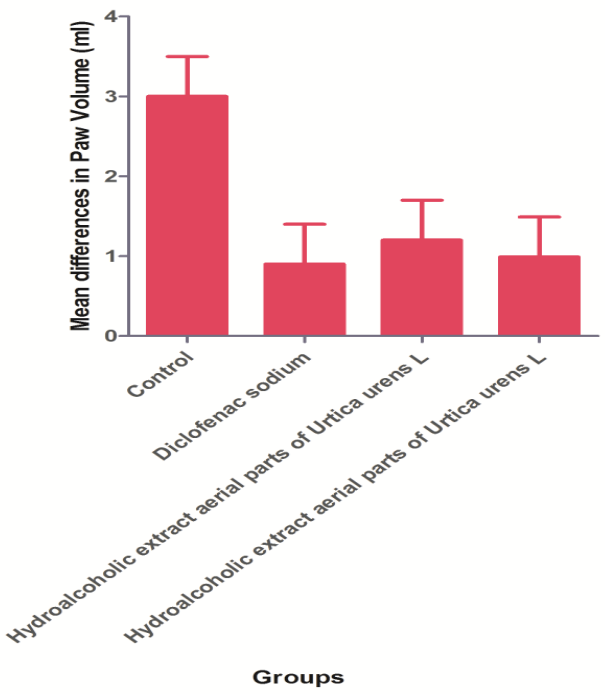

Figure No.2 : Effect of Hydroalcoholic extract aerial parts of *Urtica urens L* on paw oedema induced by formalin in rats

4. DISCUSSION AND CONCLUSION

Tissue damage and injury are always associated with pain and inflammation. Formalin test is a biphasic response where first phase is the direct effect of formalin, which involves neurogenic pain. Formalin-induced paw edema is one of the most suitable test procedures to evaluate chronic anti-inflammation, as it closely resembles human arthritis (Greenwald, 1991; Arzi et al., 2015). Inflammation is currently treated by NSAIDs. Unfortunately these drugs cause increased risk of blood clot resulting in heart attacks and strokes. Therefore, the developments of potent anti-inflammatory drugs from the natural products are now under considerations. Formalin induced acute inflammation is one of the most suitable test procedure to screen anti-inflammatory drugs. As shown in Table no. 5 and fig no.2, administration of Hydroalcoholic extract aerial parts of Urtica urens L prevented formalin-induced paw edema in a dose-dependent manner showing significant antiinflammatory effect, percentage inhibition shown was found to be 69.17% and 72.27% at dose of 100 and 200 mg/kg, respectively. Hence, it is suggested that Hydroalcoholic extract aerial parts of Urtica urens L may provide benefits in the management of inflammation. As a result, Hydroalcoholic extract aerial parts of Urtica urens L was found to diminish the formalin-induced paw edema in a dose-dependent manner. Although it has been considered that it might exert the benefits through its antioxidant features, the exact mechanism of effect is yet unknown. In conclusion, it could be suggested that Hydroalcoholic extract aerial parts of Urtica urens L provides considerable anti-inflammatory effects and the efforts to focus on the underlying mechanism/s of Hydroalcoholic extract aerial parts of Urtica urens L may provide new opportunities for the development of new drugs.

Inflammation is a body defence reaction to prevent the spread of injurious agent and to remove the necrosed cells and tissues. Inflammatory abnormalities are a large group of disorders which underlie a vast variety of human diseases. During treatment of inflammatory diseases, many conventional therapies (non-steroidal antiinflammatory drugs) used to relief pain and inflammation. Continuous use of the intended drugs is frequently associated with serious side effects, whereas plants still hold their unique place, by way of having no side effects. Therefore, a systematic approach should be made to find out the efficacy of plants against inflammation to exploit them as herbal anti-inflammatory agents with a better safety profile.

In conclusion, it could be suggested that Hydroalcoholic extract aerial parts of Urtica urens L provides considerable anti-inflammatory effects and the efforts to focus on the underlying mechanism/s of Hydroalcoholic extract aerial parts of Urtica urens L may provide new opportunities for the development of new drugs.

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