IJPSR (2011), Vol. 2, Issue 11 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 07 May, 2011; received in revised form 16 August, 2011; accepted 30 October, 2011

ANTI-INFLAMMATORY, ANTI-ARTHRITIC AND ANALGESIC ACTIVITY OF THE ALCOHOLIC EXTRACT OF THE PLANT *URGINEA INDICA* KUNTH.

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Keywords:
Urginea indica Kunth, anti-inflammatory activity, anti-arthritic activity, analgesic activity

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ABSTRACT

This study was designed to present the observation of the pharmacological properties to the bulb of the plant. The extract of the bulb of *Urginea indica* Kunth were collected by using of alcoholic extraction. The anti-inflammatory action of the alcoholic Extract of the bulb of the plant *Urginea indica* was evaluated in rats (female) against carrageenan induced edema i.e., using plethysmographic method. Besides this method, this extract was also assessed for Cotton pellet test and Hot plate test for anti-inflammatory and analgesic effects respectively. The effects of the extract were compared with the classical anti-inflammatory drug - Ibuprofen. The crude extract and the standard drug were orally administered. A significant anti-inflammatory effect was produced with the Alcoholic Extract of the plant part. This effect was then compared with the effect from the classical anti-inflammatory drug.

INTRODUCTION: *Urginea indica* Kunth, Fam: *Liliaceae* (bengali: banpiaaj, janglipiaaj), is a perennial plant with fibrous roots proceeding from the base of a large, tunicated, nearly globular bulb, 4 to 6 inches long, the outer scales of which are thin and papery, red or orange-brown in color. It is also known as Maritime Squill, *Scilla maritima* (Linn.), *Urginea maritime*, White-squill, Red-squill [1].

The flowers are in bloom in April and May and are followed by oblong capsules. It is a very variable plant, the bulb differing greatly in size and color, and the leaves of the flower presenting similar varieties, which has led to the formation of several species, about twenty-five species having been described. Two varieties of Squill, termed respectively white and red, are distinguished by druggists. In the first named, the bulb scales are whitish or yellowish in color, whereas the red species has deep, reddish brown outer scales and yellowish white inner scales, covered with a pinkish epidermis, intermediate forms also occurring. No essential difference exists in the medicinal properties of the two kinds. Merck, in 1879, separated the three bitter glucosidal substances Scillitoxin, Scillipicrin and Scillin.

Other constituents are mucilaginous and saccharine matter, including a peculiar mucilaginous carbohydrate named Sinistrin, an Inulin-like substance. The bulb has antifungal [2,] 3, antiangiogenic and proapoptotic activity[4], asthma [5], emmenagogue, antihelmintic, purgative, alexiteric, useful in paralysis, bronchitis, dropsy, rheumatism, renal calculi, leprosy, skin diseases, headache, disease of nose, internal pains, scabies [6]. Three novel flavonoid glycosides, 5, 6-dimethyoxy- 3', 4''- dioxymethylene- 7- O- (6''- beta- D-

glucopyranosyl-beta-D-glucopyranosyl) flavanone, 5, 4'-dihydroxy-3-O-alpha-L-rhamnopyranosyl- 6- C- gluco pyranosyl-7-O-(6''- para- coumaroyl- beta- D- gluco pyranosyl) flavone and 5, 4'-dihydroxy-3-O-(2'''''-beta-glucopyranosyl- alpha- L- rhamnopyranosyl) - 6- C-glucopyranosyl-7-O-(6''-para-coumaroyl-beta-D-gluco pyranosyl) flavone were isolated from the 1-butanol soluble fraction of the bulbs of the plant *Urginea indica* [7].

MATERIALS AND METHOD: The whole plant of *Urgenia indica* was weighed 39 kg. The leave and roots were cut out from the bulb of plant and then the weight was 24 kg. The bulb of the plant was sliced transversely and was subjected to air dry for four days. The air dried sliced pieces were then transferred into oven for drying. After final drying the weight was found 9 kg. Oven dried crispy pieces were finally crushed to form powder. After crushing the weight was found 4.5 kg. The plant was collected from Joydevpur in August 2009 and was taxonomically identified by a Scientific Officer, Bangladesh National Herbarium (BNH) and one voucher specimen has been deposited there.

The experiments were carried out on albino rates (Swiss Strain) were obtained from Animal House of BCSIR, Chittagong. 150-200g rats were used. The rats were housed in plastic cages. They were maintained at room temperature under conditions of natural light and dark schedule. The rats were fed with Vat chow - prepared according to the formula developed by the Bangladesh Council for Scientific and Industrial Research (BCSIR), Chittagong. They were allowed to drink normal water; the drug and extract were administered with the help of feeding needle.

In our study, extraction of dried and powdered plant (4.5 kg) of *U. indica* was done by cold extraction process by using methanol as a solvent [8]. The air dried and pulverized plant material (141.86gm) was cold extracted with methanol. And after that the fractions were evaporated by roto-dryer at low temperature (40−50°C) to dryness. Crude alcoholic extract was subjected to anti-inflammatory activity, anti-arthritic activity and analgesic activity study.

Ibuprofen was bought from retail pharmacy whose strength was checked within the laboratory and used as standard. Solutions of the drug were prepared according to various dosages regimens of distilled water. Doses of Ibuprofen were selected as reported in different literature and pilot experiments were carried out in the study. All drugs were administered orally.

Among the many methods used for screening and evaluation of anti-inflammatory activity, the most commonly employed techniques is based upon the ability of such agents to inhibit the acute inflammatory edema produced in the hind paw of the rat following injection of a phlogistic agent, like Carrageenan; a mucopolysaccharide derived from Iris sea moss, *Chondrus carrageenin* (Sigma, Japan) was prepared as 0.1% solution in water for injection.

- **Method for the study on inflammatory activity**: Acute inflammation was induced as described by Winter *et al.,* [9]. A volume of 0.05 ml 1% carrageenan was injected into the planter apponeurosis of the right hind paw of the rats. The rats were orally administered with solution of the extract or Ibuprofen and 1 hour prior to carraageenan injection to observed preventive effects. The volumes of the treated paw of the experimental rats were measured with Plethysmometer Cat.No.-7140.

  Measurements were recorded immediately after carageenan injection and then on hourly basis during the study period. The mean increase in volume of the injected paw of each group at 0, 1st, 2nd, 3rd and 4th hour after carrageenan injection was calculated to observe the activity of standard and the extract. For standard drug testing, increase in paw-volume 4 hours after carrageenan administration was considered as a measurement of effect. The percentage of edema reduction with test drug and extracts were calculated and recorded for comparison against the control group.

- Method for the study of anti-arthritic activity: The method was based on that of Meir *et al.* [10] and was carried out male rats weighing 150-160 gm with some minor modification. Surgical grade cotton was rolled in pellet (10±0.5mg) and sterilized at 120°C for two hours in an electric oven. These were soaked in 1% solution of crystalline penicillin just before use. The cotton pellets were implanted at the plantar surface under skin of abdomen. Treated animals received the test drugs one hour before

the implantation and thereafter daily for 7 days. Control animals received the vehicle only. On eight day rats were sacrificed and pellets with granulomatous tissues over them were recovered. These were cut into two part and dried to a constant weight at 60°C for 24 hours and carefully reweighed. Mean increase in weight of the pellets for control and animals were calculated.

- Method for the study of analgesic activity: The analgesic study was conducted on mice by the "Hot-Plate" (Scorel - DS37, UGO Basile, Italy) method described by Woolfe and Wood [11]. Hot plate was maintained at a constant temperature of 55.0±0.5°C. Each mouse was placed on the hot surface and the time of response of this thermal stimuli, indicated by the licking of hind and/or for paws or by licking of the legs or by trying to jump out, was recorded and the results were compared with that of control group.

All data from each treated and control group was analyzed by using *t*-test.

RESULT AND DISCUSSION

Anti-inflammatory activity study: Twenty rats were selected for total study and all the rats were classified into four groups as - Gr-A (Normal control: Rats with no drug and served as normal control), Gr-B (Anti-inflammatory control: 3.0 ml of water is given orally. After one hour this group was served with carrageenan solution (1%) at the rate of 0.5mg/kg body weight, injected into the planter aponeurosis), Gr-C (Sample treatment: 3.0 ml of alcoholic extract of *Urginea indica* (at dose of 1.5gm/kg, oral) was given to each rat.

After one hour this group was served with carrageenan solution (1%) at the rate of 0.5mg/kg body weight, injected into the planter aponeurosis) and Gr-D (Drug treatment: 3.0 ml Ibuprofen at dose of l.5gm/ kg was given to each rat orally. After one hour each rat of this group was served with carrageenan solution (1%) at the rate of 0.5mg/kg body weight, injected into the planter aponeurosis and served as positive control).

TABLE 1: ANTI-INFLAMMATORY EFFECT OF ALCOHOLIC EXTRACT OF *URGINEA INDICA*

Groups	Dose	Time interval (In Hour)	Paw volume (In C.C) mean + SEM	% of increase(t)/% of inhibition(4<)	
Normal	-----	-----	0.60 + 0.016	------	------
Control	2 ml /rat	01	0.91 ± 0.026	34.06 (↑)	------
		02	1.15 ± 0.018	47.82 (↑)	------
		03	1.31 ± 0.020	54.03 (↑)	------
		04	1.41 ± 0.025	57.44 (↑)	------
AEUI	5/kg	01	0.74 ± 0.027**	18.91 (↑)	18.68 (4)
		02	0.84 ± 0.030***	28.57 (↑)	26.95 (4)
		03	0.93 ± 0.036***	35.48 (↑)	29.00 (4)
		04	0.99 + 0.032***	39.39 (↑)	29.78 (4)
Ibuprofen	6mg/kg	01	0.70 ± 0.021***	14.28 (↑)	23.07 (4)
		02	0.76 ± 0.019***	21.05 (↑)	33.91 (4)
		03	0.83 ± 0.019***	27.71 (↑)	36.64 (4)
		04	0.82 ± 0.013***	26.82 (↑)	41.84 (4)

n = 05, AEUI means Alcoholic Extract of *U. Indica*; Result are expressed as Mean + SEM. Unpaired *t*-test were performed as the test of significance. **p<0.002, ***p<0.001.NB: As value of t is greater than p0.00l. Hence, difference is highly significance at level (p<0.001) 85 significance at level (p<0.005).

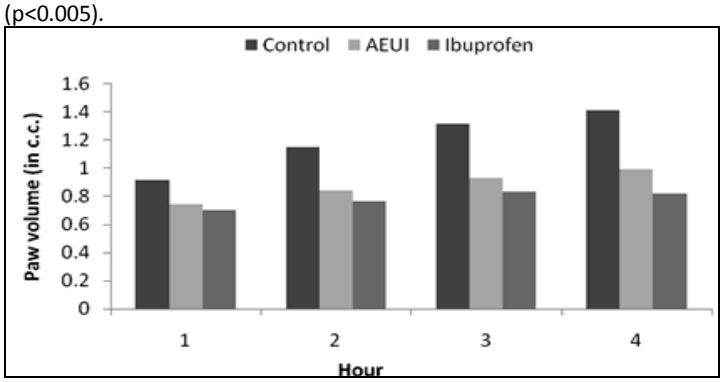

FIG. 1: DETERMINATION OF ANTI-INFLAMMATORY ACTIVITY BY CARRAGEENAN INDUCED ANTI-INFLAMINATION TEST

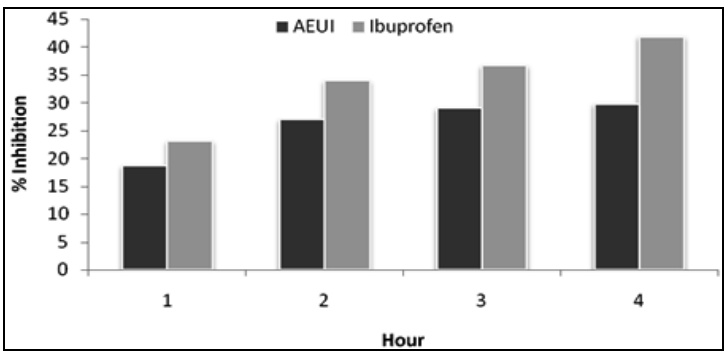

FIG. 2: ANTI-INFLAMMATION ACTIVITY TEST ON THE BASIS OF PERCENT OF INHIBITION

Anti-arthritis activity test by cotton pellet method: Eighteen rats were selected for the study and grouped into equal three classes as – Gr-A (Anti-inflammatory control: 3.0 ml of water is given orally. After one hour this group was served with carrageenan solution (1%) at the rate of 0.5mg/kg body weight, injected into the planter aponeurosis), Gr-B (Sample treatment: 3.0 ml of alcoholic extract of *Urginea indica* (at dose of 1.5gm/kg, oral) was given to each rat. After one hour this group was served with carrageenan solution (1%) at the rate of 0.5mg/kg body weight, injected into the planter aponeurosis) and Gr-D (Drug treatment: 3.0 ml Ibuprofen at dose of l.5gm/ kg was given to each rat orally. After one hour each rat of this group was served with carrageenan solution (1%) at the rate of 0.5mg/kg body weight, injected into the planter aponeurosis and served as positive control).

TABLE 2: INHIBITION OF GRANULOMATOUS TISSUE GROWTH ON COTTON PELLETS IN RATS BY ALCOHOLIC EXTRACT OF *U. INDICA* AND STANDARD DRUG.

Groups	Dose	Mean increase in weight of pellets over original wt (mg) ± SD	% of inhibition of granuloma growth
Control	2ml	66.15±1.721	---
AEUI	4gm/Kg	51.08±1.861***	22.78
Ibuprofen	6mg/ Kg	37.46±0.99***	43.37

N = 6, Results are expressed as mean ± SEM. Unpaired t-test were performed as the test of significance. ***p<0.001

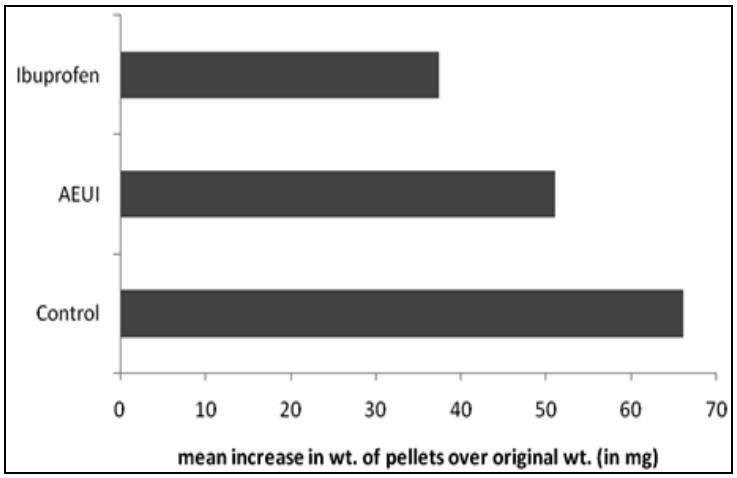

FIG. 3: DETERMINATION OF ANTI-ARTHRITIS ACTIVITY BY COTTON PELLET TEST

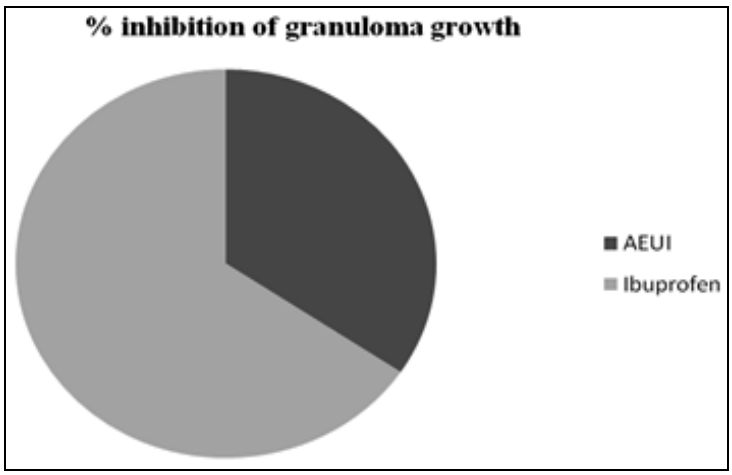

FIG. 4: DETERMINATION OF ANTI-ARTHRITIS ACTIVITY ON THE BASIS OF PERCENT OF INHIBITION

Analgesic activity test by hot plate method: Thirty rats were selected for the study and grouped into equal three classes as – Gr-A (Anti-inflammatory control: 3.0 ml of water is given orally. After one hour this group was served with carrageenan solution (1%) at the rate of 0.5mg/kg body weight, injected into the planter aponeurosis), Gr-B (Sample treatment: 3.0 ml of alcoholic extract of *Urginea indica* (at dose of 1.5gm/kg, oral) was given to each rat.

After one hour this group was served with carrageenan solution (1%) at the rate of 0.5mg/kg body weight, injected into the planter aponeurosis) and Gr-D (Drug treatment: 3.0 ml Ibuprofen at dose of l.5gm/ kg was given to each rat orally. After one hour each rat of this group was served with carrageenan solution (1%) at the rate of 0.5mg/kg body weight, injected into the planter aponeurosis and served as positive control).

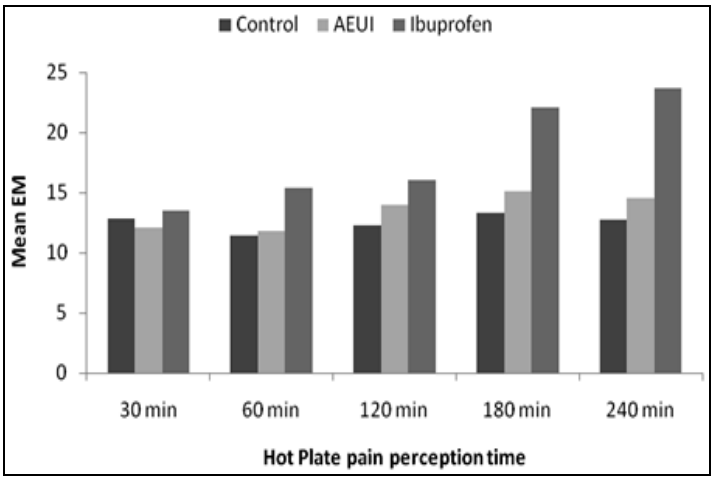

FIG. 5 DETERMINATION OF ANALGESIC EFFECT BY HOT PLATE METHOD

TABLE 3: ANALGESIC ACTIVITY STUDY ON MICE BY HOT PLATE TEST

Group	Dose	Hot Plate pain perception (In Second) Mean EM					
		0 Hr	30 min	1 Hr	2 Hr	3 Hr	4 Hr
Control	0.4 ml	11.54±0.218	12.81±0.81	11.39±0.293	12.29±0.335	1329±0.337	12.72±0.319
AEUI	1.5 gm/kg	11.96±0.483	12.12±0.485	11.81±0.392	13.96±0.435	15.13±0.287***	14.57±0.181
Ibuprofen	6mg/kg	11.65±0.168	13.51±0.275	15.37±0.424***	16.08±0.424**	22.11±0.637***	23.69±0.601***

n=10; Results are expressed as mean 11.65± SEM. Unpaired t-test was performed as the test of significance. **p<0.005, ***p<0.001. NB: As value of is greater than t0.001. Hence, difference is highly significance at level (p<0.001).

CONCLUSION: In this experiment it has been shown that the alcoholic extract of the bulb of *Urginea indica* has high anti-inflammatory activity, good anti-arthritic activity and moderately good analgesic effects. Alcohol is hydrophilic in nature so the extract easily mixed with water. The observation made by the experiment as - inhibition of carrageenan induces edema in rats and comparative result with anti-inflammatory effect shows that the root of *urginea indica*, the extract has fast onset of action and is more effective in early period after administration, the bulb of *urginea indica* (Alcoholic extract) produce significant anti-arthritic activity, in this experiment it has been shown that the analgesic effect is not better than other two effects.

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