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ANALGESIC, ANTI-INFLAMMATORY AND ANTIPYRETIC ACTIVITY OF *PISTIASTRATIOTES* L

H. K. Sundeep Kumar[1,*], M.B.V.Raju[2], S.C.Dinda[3], S. K. Sahu[1] and M.Banerjee[1]

[1]Dept.of Chemistry, Institute of Pharmacy & Technology,Salipur,Cuttack,Odisha-754202
[2]Srinivasarao College of Pharmacy,Vishakapattanm,A.P-530041
[3]School of Pharmaceutical Education & Research, Berhampur University, Berhampur, Odisha- 760007
*E-mail: rinkusundeep@yahoo.co.in

ABSTRACT

This study was intended to evaluate the analgesic, anti-inflammatory and antipyretic activity of *Pistia stratiotes*, Linn. in experimental standard models i.e. albino rats following oral administration of petroleum ether and ethanolic extract. The results showed that the ethanolic extract significantly reduce the edema induced by carrageenan within 1 to 3 hrs. post dosing and Cotton-pellet induced granuloma models than the petroleum ether extract at all the dose levels used. On the analgesic property tail immersion and acetic acid induce writhing models, ethanolic extract significantly reduce the painful stimulus than petroleum ether extract. This confirms central and peripheral effects of the drugs. It also possess antipyretic activity, ethanolic extract significantly reduces fever at higher doses within 3 hrs than petroleum ether extract on Brewer´s yeast induced pyrexia model in rats.

Keywords: analgesic, anti-inflammatory, antipyretic, pistia stratiotes, albino rats.

INTRODUCTION

Pistia stratiotes, Linn.(family-Araceae) is an aquatic, steamless, floating, stoloni feros herb found in ponds and streams almost throughout India. Leaves are sessile, ovate to obovate cuneate, densely pubescent spathe 2-4 cm long, tubular at its base, free and spreading above, slightly constricted above the middle. Flowers are minute, sessile on spadix. Fruits are ovoid to ellipsoid, green, crowned by persistent style. Seeds are few to many oblong or ovoid with a broad top[1]. The plant contains constituents like steroids, sterols, terpinoids, flavon glycosides, lipid, carbohydrates, proteins, vitamin A, vitamin B and vitamin c etc. Traditionally the leaves were used for various therapeutic purpose like antiseptic, anti tubercular and anti dysenteric. The ashes of the plant were used for the treatment of ring worm of scalp. The leaves were used in eczema, leprosy, ulcer, piles, syphilis and anthelmentic.[2]

Previously it has been reported that the plant posses antibacterial activity[3], antioxidant activity[4], bronchodilator activity[5] and antitumor activity[6]. The present study was under taken to evaluate analgesic, anti pyretic and anti inflammatory activity potential of petroleum ether and ethanolic extract of leaves of the plant on various animal models.

EXPERIMENTAL

Plant material

The *Pistia stratiotes* plants were collected from the rural belt of Salipur (Odisha) during the month of January and was authenticated by the Taxonomist of Botanical Survey of India, shibpur, Howrah. The collected plant materials were washed under running tap to remove adhered dirt, then shed dried. Then the leaf part was removed and ground in to coarse powder.

Preparation of extract

The powered plant material was extracted successively with petroleum ether (60-80°c) and then with 80% ethanol using soxhlet apparatus. The solvent was removed under reduced pressure to obtain dry extract. The extract was stored in vacuum desiccators for further use.

Animals

Albino mice of either sex (20-25gm) and male albino rats (120-150gm) were used for the study. Animals were kept in polypropylene cages and feed on standard *laboratory* diet and water *ad libitum*, exposing them to alternate cycle of 12h dark and light, temperature 25±2˚c and relative humidity 55±10%. Animals were allowed to acclimatize for 7 days to the laboratory conditions before the conduct of experiment.

Acute toxicity study

The acute toxicity of extracts was evaluated in female mice. The animals were fasted prior to the acute toxicity study. Different groups containing 6 no. of mice in each were orally administered with all extracts of 1, 2, 2.5, 3, 3.5 and 4gm/kg p.o respectively. The control group received only propylene glycol (vehicle). Drug treated and control groups were placed in propylene cage with free access of food and water. Mortality and general behavior of animals were observed continuously for initial 4h, and intermittently for 6h and then again at 24h and 48h after dosing. The parameters observed and recorded were sedation, hyperactivity, grooming, loss of rightening reflex, respiratory rate and convulsion.[7]

Evaluation of analgesic activity

Tail immersion method

Swiss Albino mice of either sex (20-25gm) were divided into 6 groups of six animals each and placed into individual cages leaving the tail hanging out freely. The animals were allowed to adopt in the cage for 30min before the experiment. Group I received sodium lauryl sulphate (SLS) 0.5% orally (3ml/kg) to the control group. Group II received standard drug Diclofenac sodium (45mg/kg) is suspended in 0.5% (w/v) SLS orally. Group III and IV received (200 and 400 mg/kg) of petroleum ether extract as suspended in 0.5% (w/v) SLS orally. Group V and VI received (200 and 400mg/kg) of ethanolic extract suspended in 0.5 % (w/v) SLS orally. The lower 5cm portion of the tail was immersed in a beaker of freshly filled hot water maintained at 55.0±1.0˚c. The time taken to withdraw the tail was noted as reaction time. A cut off time of 10sec was maintained to prevent tissue damage. The reaction time was measured at 0,15,30,45 and 60 min respectively. The mean reaction time was found out for each group and compared with the value of standard drug.[8]

Acetic acid induced writhing method

Peripheral analgesic activity was evaluated by using acetic acid induced writhing test in mice (20-25 gm) were divided into six groups of six animals each. Group I received 2% tween 80 orally (3ml/kg) which served as control. Group II served as positive control and received Aspirin (50mg/kg). Group III and IV received (200 and 400 mg/kg) of petroleum ether extract and group V and VI received (200 and 400 mg/kg) of ethanolic extract respectively. All the test doses were administered orally 1hr prior to acetic acid injection (0.1 ml of 6% v/v i.p). Ten minutes after acetic acid injection, the mice were placed in a transparent box and number of writhing movements was counted for a period of 20 min. writhing movement was accepted as contraction of the abdominal muscles accompanied by stretching with a jerk at the hind limb. Significant reduction in the number of writhes by the test treated group as compared to control is considered as positive analgesic response.[9]

Evaluation of anti-inflammatory activity

Carrageenan induced hind paw edema model

Male Albino rats (120-150gm) were selected for the study. All experimental animals were divided into 6 groups of 6 animals in each and were given the following treatment orally. Group I (control) received 1ml vehicle (propylene glycol) only. Group II received (12.5mg/kg) of Diclofenac sodium standard drug suspended in propylene glycol. Group III and IV received (200 and 400 mg/kg)of petroleum ether extract respectively suspended in propylene glycol. Group V and VI received (200 and 400 mg/kg) of ethanolic extract respectively suspended in propylene glycol. After 1h of drug treatment, the animals were administered with subcutaneous injection (0.05ml) of 2%w/v Carrageenan solution into sub plantar tissue of right hind paw. The contra lateral hind paws were injected with 0.1ml of saline as control. The paw volume was measured plethysmographically at 0, 1, 2 and 3h after injection of

odematogenic agent. The difference between initial and subsequent reading gave the actual edema volume.[10]

Cotton-pellet granuloma model

This model was employed to study the sub-chronic inflammation. Two sterilized cotton pellets weighing 10 ± 1 mg were implanted subcutaneously by incision on either side of the back of the albino rats under ether anesthesia. Drugs were administered daily orally for 7 consecutive days. Diclofenac (100mg/kg p.o.) was used as standard. After 7 days the animals were sacrificed by cervical dislocation and the pellets together with the granuloma tissues were carefully removed, dried in an oven at 60°C for 24hr, weighed and compared with control. Animals were divided into 6 groups of 6 animals in each and were given the following treatment orally. Group I (control) received 1ml vehicle (propylene glycol) only. Group II received (100 mg/kg) of Diclofenac sodium standard drug suspended in propylene glycol. Group III and IV received (200 and 400 mg/kg) of petroleum ether extract respectively suspended in propylene glycol. Group V and VI received (200 and 400 mg/kg) of ethanolic extract respectively suspended in propylene glycol.[11]

Evaluation of antipyretic activity

The antipyretic activity was evaluated by using Brewer´s yeast induced pyrexia method in Albino rats. Fever was induced by injecting 2.0ml/kg of 20% aqueous suspension of Brewer´s yeast in normal saline and 18hr after yeast injection the test drugs were administered. Rectal temperature was recorded by clinical thermometer at 0, 1, 2 and 3hr after drug administration. The animals were divided into 6 groups of 6 animals in each and were given the following treatment orally. Group I (control) received 1ml vehicle (2% gum acacia) only. Group II received Paracetamol (30mg/kg) as standard drug suspended in 2% gum acacia. Group III and IV received (200 and 400 mg/kg) of ethanolic extract suspended in 2% gum acacia. Group V and VI received (200 and 400 mg/kg) of petroleum ether extract suspended in 2% gum acacia. Before the experiment, the rats were maintained in separate cages with food ad libitum for 7 days and the animals with approximately constant rectal temperature (37.5 to 38.5˚c) were selected for the study. The mean rectal temperature was found out for each group and compared with the value of standard drug.[12]

RESULTS AND DISCUSSION

The preliminary phytochemical screening of the dried ethnolic extract showed the presence of carbohydrates, terpenoids, phenolic compounds, amino acids and proteins, flavonoids and sterols.[13&14]

Acute toxicity study

Upto 4000 mg/kg of ethanolic and petroleum ether extract do not show any toxic effect. As per the ranking system European Economic Community (EEC) for acute oral toxicity, the LD_{50} dose of 2000mg/kg and above is categorized unclassified (E C Directive 83/467/EEC, 1983).

Table-1: Analgesic activity of pet ether and ethanolic extracts of *Pistia stratiotes* by tail immersion model.

	Treatment	Dose			Average tail withdrawing time (sec)		
Group		mg/kg	0 min.	15 min.	30 min.	45 min.	60 min.
I	Control	-	2.23±0.03	2.27±0.04	2.41±0.05	2.44±0.03	2.49±0.03
II	Diclofenac sodium	45	1.64±0.04	2.33±0.09	3.14±0.10**	3.73±0.04**	4.61±0.08**
III	pet. ether extract	200	2.16±0.02	2.24±0.02	2.44±0.02	3.50±0.10**	4.73±0.07**
IV		400	2.17±0.01	2.38±0.01	2.66±0.03*	3.97±0.32**	4.98±0.24**
V	Ethanolic extract	200	2.20±0.02	2.40±0.02	3.43±0.10**	4.20±0.04**	4.56±0.05**
VI		400	2.17±0.01	2.40±0.02	3.37±0.06**	4.26±0.05**	4.89±0.06**

Values are expressed as mean±S.E. (n=6). * P<0.05 and **P<0.01 compared with vehicle control (ANOVA followed by Dunnet's t-test).

Tail immersion test

Table1shows the results of the tail immersion test. In this model, 30 min after drug administration, reaction time increased significantly, (P<0.01 and 0.05) for the test and standard when compared to the control. The test drugs produced a dose dependent increase in reaction time at various time intervals of observation.

Acetic acid induced writhing test

Dose dependent anti-nociceptive effect was noted with the extracts at the tested dose levels (Table-2). Maximum percentage of inhibition of writhing response exhibited by ethanolic extract at 400 mg/kg was 47.34% where as standard drug Aspirin at 50 mg/kg was 47.75% in comparision with control group.

Table-2:Analgesic activity of pet ether and ethanolic extracts of *Pistia stratiotes* by Acetic acid induced writhing test

Group	Treatment	Dose(mg/kg)	Number of writhings/20 min	% inhibition.
I	control	---	40.83±1.13	---
II	Aspirin	50	21.33±0.49**	47.75
III	Pet. ether extract	200	34.66±0.80**	15.11
IV		400	31.50±0.56**	22.85
V	Ethanolic extract	200	27.00±0.57**	33.87
VI		400	21.50±0.42**	47.34

Values are expressed as mean±S.E. (n=6). *P<0.05 and **P<0.01 compared with vehicle control (ANOVA followed by Dunnet's t-test).

Carrageenan induced hind paw edema test

The effect of ethnolic and petroleum ether extract on Carrageenan induced hind paw edema test in rats shown in Table 3. The result obtained indicates that the ethanolic extract possess significant anti-inflammatory activity than extract petroleum ether in rats.

Table-3: Anti-inflammatory activity of petroleum ether and ethanolic extracts of *Pistia stratiotes* by Carrageenan induced hind paw edema test.

Group	Treatment	Dose	Paw Edema Volume (ml)			
		mg/kg	0h	1h	2h	3h
I	Control	--	0.29±0.00	0.46±0.01	0.49±0.01	0.45±0.01
II	Diclofenac sodium	10	0.29±0.00	0.29±0.00**	0.29±0.00**	0.31±0.04**
III	Pet. Ether Extract	200	0.30±0.00	0.33±0.00**	0.38±0.00**	0.41±0.00**
IV		400	0.30±0.00	0.34±0.01**	0.38±0.00**	0.39±0.00**
V	Ethanolic Extract	200	0.30±0.00	0.40±0.00**	0.39±0.00**	0.39±0.01**
VI		400	0.31±0.01	0.40±0.00**	0.40±0.01**	0.41±0.01**

Values are expressed as mean±S.E. (n=6). *P<0.05 and **P<0.01 compared with vehicle control (ANOVA followed by Dunnet's t-test).

Cotton-pellet granuloma test

The effects of petroleum ether and ethanolic extract on cotton-pellet granuloma test in rats were reported in Table 4. In granuloma induced sub-chronic inflammation the ethanolic extract in dose of 200, 400 mg/kg had significant anti-inflammatory activity than petroleum ether extract (P<0.01). The percentage inhibition of granuloma after drug administration showed that 400 mg/kg of ethanolic extract had 40.33 % and Diclofenac sodium had 52.99 % when compared to control group.

Table-4: Anti-inflammatory activity of pet- ether and ethanolic extracts of *Pistia stratiotes* by Cotton-pellet granuloma test.

Group	Treatment	Dose(mg/kg)	Weight of dry cotton pellet granuloma (mg)	% Inhibition
I	Control	---	34.34±0.59**	---
II	Diclofenac-Sodium	100	16.14±0.30**	52.99
III	Pet. ether extract	200	31.18±0.51**	9.2
IV		400	28.71±0.25**	16.39
V	Ethanolic extract	200	24.65±0.42**	28.21
VI		400	20.49±0.40**	40.33

Values are expressed as mean ± S.E. (n=6). *P<0.05 and **P<0.01 compared with vehicle control (ANOVA followed by Dunnet's t-test).

Anti pyretic activity test

The results of anti pyretic activity of petroleum ether and ethanolic extracts were shown in Table 5. It was observed that ethanolic extract at 400 mg/kg showed maximum anti-pyretic activity than petroleum ether extract.

Table-5: Anti-pyretic activity of petroleum ether and ethanolic extracts of *Pistia stratiotes* by Brewer´s yeast induced pyrexia method.

Group	Treatment	Dose	Initial Temp.˚C	Rectal Temperature ˚C in mean± SEM			
				0 Hour	1 Hour	2 Hour	3 Hour
I	Control	---	37.48±0.07	38.10±0.29	39.31±0.04	38.72±0.19	38.52±0.16
II	Paracetamol	30mg/kg	37.51±0.06	38.66±0.14	38.30±0.11	38.06±0.07**	37.91±0.11
III	Pet. ether extract	200mg/kg	37.59±0.07	39.15±0.03	38.43±0.02	38.42±0.01*	38.05±0.03**
IV		400mg/kg	37.53±0.03	39.35±0.08	38.38±0.01	38.23±0.03**	38.13±0.01**
V	Ethanolic extract	200mg/kg	37.57±0.03	38.75±0.07	38.86±0.01	38.67±0.06	38.45±0.03
VI		400mg/kg	37.57±0.04	38.93±0.06	38.43±0.03	38.44±0.02*	38.22±0.03**

Values are expressed as mean ± S.E. (n=6). *P<0.05 and **P<0.01 compared with vehicle control (ANOVA followed by Dunnet's t-test).

CONCLUSION

In conclusion, this study provides evidences for the ethanolic extract of *Pistia stratiotes*, Linn.Possess significant analgesic anti-inflammatory activity than petroleum ether extract. But the petroleum ether extract posses significant antipyretic activity than ethanolic extract.

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