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Research Article

EVALUATION OF ANTHELMINTIC AND ANTI-INFLAMMATORY ACTIVITY OF *AMARANTHUS SPINOSUS* LINN

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ABSTRACT

Water extracts of whole plant of *Amaranthus spinosus* Linn was evaluated for anthelmintic on adult Indian earthworms (*Pheritima posthuma*) and *Tubifex tubifex* ,using piperazine citrate as reference standard. Aqueous extract showed anthelmintic activity in dose-dependent manner giving shortest time of paralysis(P) and death(D) with 50 mg/ml concentration, for both the worms. Extract shows more potent activity (15 mg/ml) against *Tubifex tubifex.* Extract shows more potent activity (15 mg/ml) against *Tubifex tubifex* . The petroleum ether and ethanolic extracts of whole plant of *Amaranthus spinosus* Linn. were tested for anti-inflammatory activity at the dosed of 250,500 and 750 mg/kg body weight.The extract produced dose dependent and significant inhibition of carrageenan induced paw oedema. The exhibited anti-inflammatory activity of this plant was comparable with the standard drug Ibuprofen.The presence of steroids, alkaloids & flavonoids in the extracts may be contributory to its anti-inflammatory activity.

Key words: Anthelmintic, Anti-inflammatory, Carrageenan, Ibuprofen, Piperazine citrate

INTRODUCTION

Helminth infections are most widely founds in those human beings particularly who are in low poverty level & does not maintain hygienic condition.Helminths are generally restricted to tropical regions and cause serious problem to health and contribute to the prevalence of undernourishment, anaemia, eosinophilia and pneumonia[1]. Worm diseases cause serious morbidity & affect population in endemic areas[2].The gastro-intestinal helminthes becomes resistant to currently available anthelmintic drugs so the treatment of this disease is a problem[3].The plant exhibits cooling and alexeteric properties.[4] The genus shows emollient and anthelmintic properties.[5]

The leaves and stems contains α-spinasterol and hentriacontane.The roots contain α-spinasterols octacosanoate($C_{57}H_{102}O_2$,mp 85-86^0) and saponin,viz. saponin of oleanolic acid.[6]The plant has a wide reputation among natives of being curative for intestinal-worm infections.This plant is being used by the tribals of Purulia district as an anthelmintic in the form of extract,prepared by dissolving the powdered material in water for 4-5 hours.This extract is used to treat intestinal-worm infections. The benzene and alcoholic extract shown wound healing activity using excision, incision and dead space wound models[7]. The anti-inflammatory property of methanolic extract of A. spinosus leaves was studied in different animal models[8]. As no scientific data on the anti-inflammatory activity of petroleum and ethanolic extract of whole plant has been reported, hence the present study was done to evaluate scientifically the usefulness of this whole plant parts.So an attempt has been made to evaluate the anthelmintic potential and anti-inflammatory activity of *Amaranthus spinosus* Linn.

MATERIALS AND METHODS

Plant material

The whole plants of *Amaranthus spinosus* Linn were procured from different places of Purulia, District, WB and authenticated by the Botanical survey of India, Shibpur, Howrah, WB and also by Botany Department,B.B.College,Asansol,WB, India. A voucher specimen (NO-CHN/I-I/2008/Tech. II/) was retained in our laboratory for further references. The plant material was dried in sunlight, pulverized, passed through sieve no.40 and stored in air tight container and used for further extraction.

Preparation of extract

Aqueous extract (Maceration method)

Powdered plant material (whole plant) 200 gm was macerated with 1000 ml of distilled water for 24 hrs.The extract was double filtered by using muslin cloth and Whatman no.1 filter paper and concentrated by evaporation on water bath. The extract was dried and used as a powder.The percentage yield of extract was 3.58 percent.

Petroleum ether and ethanol extract (soxhlet)

For anti-inflammatory activity freshly collected whole plant (200gm) were washed thoroughly and kept to dry in shade,were powdered to 40 mesh size with light petroleum ether(40-60^{0}C) and then with ethanol in a soxhlet extractor for 72 hrs. In both the cases the menstrum was removed and the extract was concentrated in vaccum at 40^{0}C.The percentage of petroleum ether and ethanol soluble extractives were calculated with reference to air dried plant material and the yield was found to be 4.12±0.25% w/w and 5.58±0.62%w/w respectively.

Animals

Adult earthworms(*Pheretima posthuma*) and aquarium worm (*Tubifex tubifex*) were used to evaluate anthelmintic activity in vitro .Earthworms were collected near the swampy water from santuri ,purulia and aquarium worm from aquarium fish food supplier.They all were collected and kept in normal saline solution.The average size of earthworm was 6-8 cm and aquarium worm was 1-2 cm.They all were identified from the Dept.of Zoology,B.B.College,Asansol,WB.

Albino wistar rats of either sex weighing 140-160 gm were used in the screening experiments. Selected animals were maintained under standard laboratory conditions.The animals were fed with standard pellets and water *ad libitum.* The animal experimental protocol was approved by the Institutional Animal Ethics Committee. (955/A/06/CPC SEA).

Drugs and chemicals

Piperazine citrate (Glaxo Smithkline) was used during the experimental protocol.

Carrageenan(Sigma Chemicals Co.,USA) was used for inflammation.

Ibuprofen from local medicine shop was used as standard in anti-inflammatory activity.

Methods

The anthelmintic assay was carried as per the method of Ajaiyeoba *et al*[9]. with minor modifications. The assay was performed on the aquarium worm, *Tubifex tubifex*, and *Pheretima posthuma* because they belong to same group of Annelida. They were used owing to its anatomical and physiological similarity with intestinal roundworm parasites of human beings for evaluation of anthelmintic activity.[10,11,12.] 20 ml formulations containing three different concentrations, of crude aqueous extract (15, 25 and 50 mg/ml in double distilled water) were prepared and six earthworms (same type) and a lump of *Tubifex* worms were placed in each nine cm petri dish containing 20 ml of above test solution of extracts. Piperazine citrate(10 mg/ml) was used as reference standard and distilled water as control[13,14,15,16].All the test solution and standard solution were prepared freshly before starting experiments. Time for paralysis was noted when no movement of any sort could be observed except when the worms were shaken vigorously. Time for death of worms were recorded after ascertaining that the worms neither moved when shaken vigorously nor when dipped in warm water at 50^0C. All the results were shown in Table.1 and expressed as a mean ± SEM of six worms in each group.

In anti-inflammatory activity, a dose level of 250mg/kg, 500mg/kg and 750mg/kg were prepared in both petroleum ether and ethanol extract by suspending the extract in 2 % of w/v Tween-80 and reference control ibuprofen (10mg/kg) was also suspended in same vehicle. The wistar albino rats of either sex were divided into eight groups comprising six animals in each group .Male or female albino rats with body weight of 140-160 gm were selected for the study.

Carrageenan induced paw odema[17] is the simplest and most widely used model for studying the anti-inflammatory activity of new compounds.Paw volume was measured immediately,and then at pre-determined intervals by plethysmometric method of Singh and Ghosh[18].

In all groups, acute inflammation was recorded by sub planter injection of 0.1ml of 1% w/v suspension of carrageenan(Sigma Chemicals Co.,USA) in normal saline in the right hind paw of the rats and paw volume was measured plethysmometrically at 1 , 2 and 4hr after carrageenan injection. The negative control groups were pre-treated with vehicle (2 % of w/v Tween-80) at a dose of 10ml/kg body weight . The positive control group with ibuprofen at a dose of 10mg/kg body weight .The remaining six groups received the petroleum ether and ethanolic extracts at dosage of 250,500,750 mg/kg body weight .All of the treatments were given orally 2hr before the carrageenan injection. The measurement of paw volume was accomplished immediately by displacement technique using the plethysmograph before the carrageenan injection and at 1, 2 & 4 hr after the carrageenan injection.Oedema was expressed as the increment in paw volume due to carrageenan administration. In inflammation there is an initial release of histamine and 5-hydroxytryptamine(5-HT) producing an increased vascular permeability followed by release of kinins further contributing to the increased vascular permeability and finally, the prostaglandins and slow reacting substanmce(SRS) are released to maintain the increased vascular permeability produced by histamine,5-HT and kinins[19].

RESULTS AND DISCUSSION

From the result it can be concluded that higher concentration of extract produced paralytic effect much earlier and the time to death was shorter for both the worms.Aqueous extract showed anthelmintic activity in dose-dependent manner giving shortest time of paralysis(P) and death(D) with 50 mg/ml concentration, for both the worms. Extract shows more potent activity (15 mg/ml) against *Tubifex tubifex*.Anthelmintic evaluation was compared with reference standard Piperazine citrate (Table 1).

Table 1: Result of Anthelmintic Activity - Table representing the anthelmintic activity of aqueous extract.

Group	Concentration (mg/ml)	*Pheretima posthuma*(Earthworm)		*Tubifex tubifex*	
		Time taken for paralysis(P) in min.(Mean & SEM)	Time taken for death(D) in min.(Mean & SEM)	Time taken for paralysis(P) in min.(Mean & SEM)	Time taken for death(D) in min.(Mean & SEM)
Control	___				
Aqueous extract	15	49 ± 0.43	70 ± 1.25	36 ± 1.23	49 ± 1.21
	25	33 ± 0.47	55 ± 1.19	23 ± 1.21	42 ± 0.86
	50	18 ± 0.59	33 ± 1.38	11.35 ± 0.48	35 ± 0.56
Piperazine Citrate	10	22.66 ± 1.12	45.33 ± 0.56	11 ± 1.43	31 ± 0.38

Each value represents mean ± SEM (N = 6)

Table 2: Anti-inflammatory activity of petroleum ether & ethanol extract of *Amaranthus spinosus*.Linn.

Group	Dose(mg/kg body weight)	Average volume of mercury displaced (ml)		
		1 hr	2 hr	4 hr
Group 1	---	0.35±0.60	0.64±0.05	1.00±0.03
Group II	10	0.18±0.24	0.22±0.24*	0.31±0.23*
Group III	250	0.39±0.26	0.56±0.24	0.77±0.28
Group IV	500	0.43±0.25	0.52±0.23*	0.74±0.26*
Group V	750	0.31±0.03	0.41±0.04*	0.62±0.06*
Group VI	250	0.41±0.04	0.52±0.03	0.73±0.02
Group VII	500	0.39±0.01	0.49±0.01*	0.71±0.02*
GroupVIII	750	0.21±0.07	0.37±0.01*	0.56±0.01*

Values are expressed as mean ± SEM. Number of animal used 6 in each group;*P<0.01

Preliminary phytochemical screening of extract revealed the presence of steroids,saponin,alkaloids,phenolic compounds.

In anti-inflammatory activity, it has found that both extract (Petroleum ether and ethanolic) shows significant anti-inflammatory activity at 750mg/kg dose. (P<0.01) level, which was comparable with that of ibuprofen 10mg/kg standard drug (P<0.01). Anti-inflammatory activity of both extracts against carrageenan induced inflammation is shown in Table 2. The percentage protection of both extracts is shown in Table 3. Anti-inflammatory activity of both extract can be determined by their ability to reduce or prevent edema[20].Carrageenan induced edema is biphasic, the first phase is attributed to the release of histamine,5-hydroxytryptamine and kinins[19],while the second phase is related to the release of prostaglandins. Preliminary phytochemical screening showed that the *Amaranthus spinosus* Linn contains phytosterol, alkaloid, glycoside, saponin, amino acid etc. So it may be concluded that the presence of alkaloid, flavonoid, phytosterol etc are mainly

responsible for its anti-inflammatory activity.

The oedema was produced in the paw of the rat by injecting 0.1 ml of carrageenan(1% w/v).After oral administration of suspension of petroleum ether and ethanolic extracts of *A.spinosus* at the dosages of 250,500 and 750 mg/kg body weight, the onset of reduction in paw volume was rapid.

The petroleum ether extract showed the significant reduction in the oedema volume 26% and 38% respectively, after four hours, at the dose of 500 and 750mg/kg body weight.The ethanolic extract showed the significant reduction in the oedema volume 29% and 44 % respectively,after four hours,at the dose of 500 and 750 mg/kg body weight, which is comparable to the standard drug Ibuprofen.(Figure-1).

Table 3: Percentage protection of petroleum extract & ethanol extract of Amaranthus spinosus Linn.

Group	Dose(mg/kg body weight)	Percent Inhibition of Paw Oedema		
		1 hr	2 hr	4 hr
Group 1	---	---	---	---
Group II	10	48.6	65.6	69.0
Group III	250	11.4	12.5	23.0
Group IV	500	22.8	18.8	26.0
Group V	750	11.5	36.0	38.0
Group VI	250	17.0	19.0	27.0
Group VII	500	11.4	24.0	29.0
GroupVIII	750	40.0	43.0	44.0

Values are expressed as mean ± SEM. Number of animal used 6 in each group; P<0.01

Value are presented as mean ± SEM of n= 6 animal in each group.

Animals : Wistar Albino rats

Weight: 140-160 gm, Route of administration : Oral

Group I : Control- treated with 10ml/kg body weight of Tween-80(2% w/v).

GroupII : Standard-treated with 10mg/kg body weight of Ibuprofen (in 2% w/v Tween-80).

GroupIII: treated with with 250mg/kg body weight of petroleum ether extract of *A. spinosus* (in 2% w/v Tween-80).

Group IV : treated with with 500mg/kg body weight of petroleum ether extract of *A. spinosus* (in 2% w/v Tween-80).

Group V : treated with with 750mg/kg body weight of petroleum ether extract of *A. spinosus* (in 2% w/v Tween-80).

Group VI: treated with with 250mg/kg body weight of ethanolic extract of *A. spinosus* (in 2% w/v Tween-80).

Group VII : treated with with 500mg/kg body weight of ethanolic extract of *A .spinosus* (in 2% w/v Tween-80).

Group VIII: treated with with 750mg/kg body weight of ethanolic extract of *A. spinosus* (in 2% w/v Tween-80).

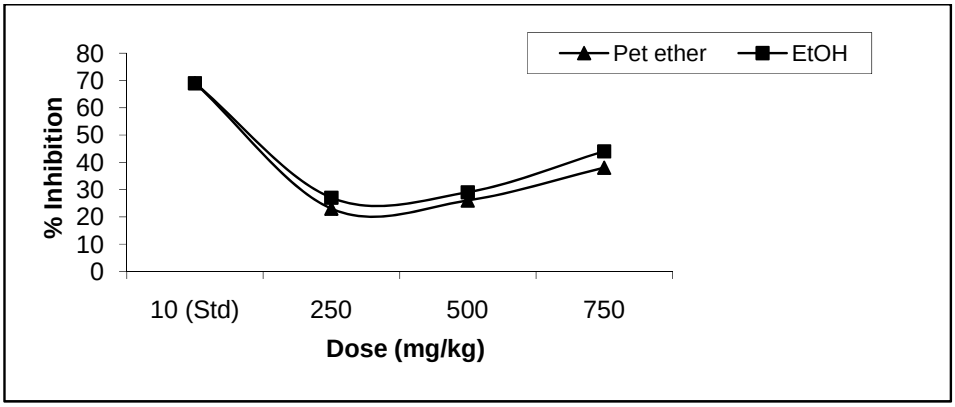

Fig.1: Percentage of inhibition Vs dose concentration of both extract in comparison to standard

However the percentage inhibition of paw volume of standard drug was 69%.The percentage decrease in paw volume was calculated by using the formula 100[1-(dt/dc)] where dt and dc are the paw volume of drug tested and inflammatory control respectively.

Ethanol extract shows much more anti-inflammatory activity than petroleum ether extract when it was compared with standard drug Ibuprofen and this is probably due to the high amount of flavonoids & alkaloid in ethanol extract.

Statistical analysis

The experimental data were calculated as mean ± SEM, evaluated by unpaired one way ANOVA, test values of P<0.01 were considered statistical significant.

CONCLUSION

From the above results, it is concluded that *Amaranthus spinosus* Linn used by tribals of purulia district to treat intestinal worm infection, showed significant anthelmintic activity. The experimental

evidence obtained in the laboratory model could provides data for being used this plant as an anthelmintic. The plant may further explored for its various pharmacological activity.

In anti-inflammatory activity ethanol extract shows much more potentiality than petroleum ether extract when it was compared with standard drug Ibuprofen and this is probably due to the high amount of flavonoids & alkaloid present in ethanol extract.

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