PHARMACOLOGICAL EVALUATION OF ROOT EXTRACTS OF TRAGIA INVOLUCRATA

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Summary

The objective of the study is to investigate the anti inflammatory, analgesic, diuretic and anthelmintic activity of Pet ether (PETI), Chloroform (CHTI) and Aqueous (AQTI) extracts of roots of *Tragia involucrata* in rodents and earth worms. Roots were successively extracted with Pet ether, Chloroform and distilled water. Preliminary phytochemical investigation was carried out to identify various phytochemical constituents present in these extracts. LD_{50} of PETI, CHTI and AQTI were conducted as per OECD guidelines No 425. LD_{50} of PETI was 500mg/kg, for CHTI 500mg/kg and for AQTI 1000mg/kgrespectively.The various activities were assessed by anti-inflammatory activity with carrageenan induced rat paw oedema, analgesic activity by radiant heat induced nociception, diuretic activity by Lipschitz model and antihelmintic activity by earthworm model. The Preliminary phytochemical investigation revealed the presence of tannins and flavonoids in PETI, alkaloids in CHTI and alkaloids and saponins in AQTI extracts. All the three extracts exhibited anti-inflammatory, analgesic, diuretic and anthelmintic activities.

Introduction

Tragia involucrata (Linn) which is popularly used for various ailments as mentioned in Ayurveda and ancient texts of ethnic medicine. Earlier the plant has been studied for its psychopharmacological [1], analgesic [2] and anti-inflammatory [3] activities using methanolic fraction of the extract. The roots of *T.involucrate* Linn (Family: Euphorbiaceae) also called as Indian stinging-nettle have been mentioned for its bitter, acrid, sweet, cooling diaphoretic, antiperiodic, depurative and alternant properties. [4].It is administered internally for suppression of urine [5] and also used as a base of an external application in leprosy [6] also used for its antipyretic and wound healing properties [7]. Presence of various chemical constituents such as alkaloids, flavonoids,

lipids, phenolic compounds, proteins, saponins and triterpenoids have been reported [8]. The objective of this study was to evaluate anti-inflammatory, analgesic, diuretic and anthelmintic activities of root extracts of *T.involucrate.* Ancient Indian medicine mentions several plants for the treatment and symptomatic relief of pain in rheumatic joints. More over by taking a clue from neuropharmacological profile from blind screening studies the extracts of *T involucrata* were tested for anti-inflammatory, analgesic, diuretic and anthelmintic activities.

Methods

Phytochemical tests:
Preliminary phytochemical investigations were carried out with petroleum ether, chloroform and aqueous extracts of *T.involucrate* by following standard methods [9] [10].

Animals:
Male albino rats of Wistar strain (140-180gm) were procured from Venkateshwara Enterprises, Bangalore and were maintained under standard husbandry conditions i.e. temperature (26 ± 2°C), relative humidity (45-55%) and 12 hours dark/light cycle. The animals were fed with standard pellet diet (Gold Mohr, Lipton India LTD. Bangalore) and water supplied ad libitum. Animal handling was performed according to good laboratory practice (GLP).

Acute toxicity (LD_{50}) *of T. involucrata:*
The acute toxicity of PETI (500mg/kg), CHTI (500mg/kg) and AQTI (1000mg/kg) of *T. involucrata* were determined in albino mice of either sex (20-30gms) by using fixed dose method (up and down method) following OECD Guidelines No: 425 of CPCSEA.1/5th of the LD_{50} dose of the respective extracts was taken for the experimental study [11].

Anti-inflammatory activity:
Five groups of rats each consisting of six animals were selected. At '0' hr left hind paw volume of all the animals were recorded plethysmographically. Group I was administered with 0.2ml of normal saline orally which served as normal control, Group II with standard Diclofenac sodium (20 mg/kg p.o), Group III, IV and V were administered with PETI (100mg/kg), CHTI (100mg/kg) and AQTI (200mg/kg) respectively which served as test groups. After 30 minutes of standard /extracts treatment 0.1 ml of 1% suspension of carrageenan was administered to all rats at sub plantar region of hind paws. Paw volume in the control, standard and test groups were measured plethysmographically at prefixed time intervals i.e. ½, 1, 2 and 3hrs [12, 13].

Analgesic activity:

The analgesic activity of different extracts of *T. involucrata* was carried out by radiant heat analgesiometer in rats. In this study rats were subjected to noxious stimuli by exposing tip of the tail (last 1-2 cm to radiant heat source) by using Radiant heat

analgesimometer and the withdrawal of the tail from the heat source is taken as the cut off time, or the end pint. The animals were divided into five groups each consisting of six animals. Initially normal reaction times in all the animals were recorded. Group I served as normal control which received with normal saline, Group II with standard pentazocine (10mg /kg i.p), Group III, IV and V were administrated with PETI (100mg/kg), CHTI (100mg/kg) and AQTI (200mg/kg) respectively which served as test groups. The reaction time was recorded at 0, 15, 30 and 45 minutes time intervals [14].

Diuretic activity:

For the assessment of diuretic activity, a group of 5 male albino rats each consisting of six animals, weighing between 140-180gm were fasted and deprived of water for 18 hours prior to the experiment. Group I was administered with normal saline (25ml/kg p.o) which served as control group. Group II, given with standard Furosemide sodium (100mg/kg), Group III, IV and V were given with PETI (100mg/kg), CHTI (100mg/kg) and AQTI (200mg/kg) extracts respectively. Immediately after extracts treatment rats were placed in metabolic cages (three in a cage) specially designed to separate urine and faeces and kept at room temperature of 25 ± 0.5°C. The urine was collected into measuring cylinders up to 3 hours after treatment. During this period no food or water was made available to animals. The total volume of urine collected was measured in control, standard and drug treated groups. The parameters taken for study were total urine volume, urine concentration of Na^+, K^+, Cl^- ions, Na^+ /K^+ ratio, P^H and specific gravity.Na^+ and K^+ ions concentration were estimated by Electrolyte Analyzer (Liston-ECS- 2000) U.S.V. and chloride concentration was estimated by Urinometer [15, 16].

Anthelmintic activity [17]:

Anthelmintic activity of PETI, CHTI and AQTI extracts were carried out using earth worm model. Piperazine hydrates was diluted with 10ml of normal saline to obtain 0.1%, 0.2% and 0.5% solutions and were poured into Petri- dishes and these served as standards. 0.1%, 0.2% and 0.5% suspensions of the PETI, CHTI and AQTI extract respectively were made with a few drops of tween-80, diluted to 10ml with normal saline, and were taken into three different petridishs. At room temperature six earth worms of nearly equal length (8 ± 1 cm) were taken and placed into the solution Control group added with normal saline only. The time taken for complete paralysis and death of earthworms were recorded. The mean paralysis and lethal time for each sample was recorded and the time taken by each earthworm without a sign of motion was noted as complete paralysis or paralysis time. To ascertain the death of immobile worms, they were frequently tested by external stimuli to stimulate and to induce movements if any in the worms, if alive.

Statistical Analysis:

Data was shown as the mean ± SEM and analysed by one way ANNOVA followed by Dunnet's't' test. The level of significance for all the experiments were P<0.05*, 0.01** and 0.001***respectively.

Results

Phytochemical investigation:

Preliminary phytochemical tests with root extracts of *T. involucrate* revealed the presence of tannins and flavanoids in PETI, alkaloids in CHTI and alkaloids and saponins in AQTI extracts All the three extracts have exhibited potent anti-inflammatory effect at different time intervals (i.e.1,2 and 3 hour) against carrageenan –induced rat paw edema model i.e., reduction in edema volume noted with all the 3 extracts were PETI (25.89%, 11.74%, 26.60%) CHTI (12.56%, 18.02%, 39.83%) and AQTI (37.42%, 41.11%, 49.86%) extracts at three time intervals respectively. The anti-inflammatory activities of the former two extracts were almost equal to standard diclofenac sodium at 3rd hour.

Anti-inflammatory activity of root extracts of Tragia involucrata (Linn)

Group	Treatment	Dose mg/kg	Paw oedema volume after					
			1st hour		2nd hour		3rd hour	
			mean ±SEM	% ROV	mean ±SEM	% ROV	mean ±SEM	% ROV
I	Normal control	0.2 ml	0.79±0.032	-	0.91±0.025	-	1.265±0.018	-
II	Standard	10	0.47±0.007***	39.57	0.51±0.010***	43.88	0.61±0.012***	51.65
III	PETI	100	0.58±0.016***	25.89	0.79±0.010***	11.74	0.93±0.018***	26.60
IV	CHTI	100	0.69±0.022*	12.56	0.74±0.018***	18.02	0.76±0.021***	39.83
V	AQTI	200	0.49±0.15***	37.42	0.53±0.015***	41.11	0.63±0.023***	49.86
0ne way ANOVA	F		44.065		107.17		205.92	
	df		25		25		25	
	P		<0.01		<0.01		<0.01	

n=6; Significance at P<0.05*, P<0.01**, P<0.001***, I-Normal control, II- Standard Diclofenac sodium (20mg/kg), III- Petroleum ether extract (100 mg/kg), IV- Chloroform extract (100gm/kg), V-Aqueous extract (200 mg/kg). All the three extracts of *T.involucrata* had exhibited significant analgesic activity at 15, 30 and 45 minute time intervals.

Analgesic activity of root extracts of Trigia involucrata (Linn)

Group	Treatment	Dose	Basal reaction time in sec at			
		mg/kg	0 min	15 min	30 min	45 min
I	Normal control	0.2 ml	3.5±0.224	3.5±0.224	3.5±0.224	3.5±0.224
II	Standard	10	3.5±0.224	7.0±0.365***	8.3±0.211***	11.17±0.401***
III	PETI	100	3.8±0.307	6.0±0.365***	9.3±0.422***	8.3±0.33***
IV	CHTI	100	3.8±0.307	5.5±0.224***	6.3±0.307***	8.0±0.365***
V	AQTI	200	3.5±0.224	6.5±0.224***	6.3±0.224***	8.3±0.211***
0ne way ANOVA	F			21.900	60.433	76.028
	df			25	25	25
	P			<0.01	<0.01	<0.01

n=6

Significance at P<0.05*, P<0.01**, P<0.001***,

I-Normal control, II- Standard Pentazocine (10mg/kg), III- Petroleum ether extract (100mg/kg), IV- Chloroform extract (100gm/kg), V-Aqueous extract (200gm/kg)

AQTI extract treated groups has shown an increase in urine volume almost equal to the group treated with standard furosemide but only a slight increase in urine volume was observed with PETI and CHTI extracts treated groups. Natriuretic effect was observed with all the three extracts, but more with AQTI and CHTI extracts than PETI extract. A significant alteration in Na^+, K^+, and Cl^- ions were also observed with all the three extracts. pH of urine recorded with AQTI extract treated group was almost equal to urine pH in furosemide treated group and specific gravity of urine in AQTI extract treated group was dragged nearer to that of furosemide treated group.

All the three extracts i.e., PETI, CHTI and AQTI extracts of roots *T.involucrata* showed dose dependent anthelmintic activity and also death. Complete paralysis of worms was readily seen at higher concentration than at lower concentration levels. There was neither paralysis nor death observed in control group. Only paralysis, but not any death occurred in the group of earthworms treated with standard Piperazine hydrate. PETI, CHTI and AQTI extracts have produced total paralysis and death in all the earthworms. The anthelmintic activity exhibited by different extracts were in the order, AQTI>CHTI>PETI with the three prefixed concentrations of 0.1%w/v, 0.2%w/v and 0.3%w/v respectively. Alkaloids, tannins and saponins were reported to posses anthelmintic activity. The difference in anthelmintic activity with the above extracts might be due to number of active phytoconstituents present with them.

Diuretic activity of root extracts of Tragia involucrata (Linn)

Treatment	**Dose mg/kg**	**Total volume of urine ml/kg**	**Na^+ µ Moles /kg**	**K^+ µ Moles /kg**	**Na^+/K^+**	**Total chloride µ Moles /kg**	**P^H**	**Specific gravity**
Normal control	25 ml	12.2 ±0.745	2091 ±7.574***	741 ±2.513	2.824	741 ±7317	7.25 ±0.076	1.017 ±0.001
Standard	4 mg/kg p.o	34.03*** ±1.255	3124 ±2.561***	2013 ±17.852***	1.553	2968 ±3.029***	7.03 ±0.071	1.005 ±0.002
PETI	100	15.93 ±0.428*	2666 ±12.511***	2078 ±7.833***	1.283	2836 ±4.651***	7.23 ±0.024	1.032 ±0.006
CHTI	100	19.67 ±0.557***	2955 ±15.847***	2827 ±3.774***	1.045	2332 ±5.785***	7.16 ±0.025	1.025 ±0.002
AQTI	200	32.23 ±0.595***	2996 ±4.088	2011 ±3.525***	1.490	3001 ±4.230***	7.02 ±0.070	1.009 ±0.002
0ne way ANOVA	F	161.48	1746.8	6824.9		33418		
	df	25	25	25		25		
	P	<0.01	<0.01	<0.01		<0.01		

n=6

Significance P<0.05*, P<0.01**, P<0.001***,

I-Normal control Saline, II- Standard Furosemide (4mg/kg), III- Petroleum ether extract (100mg/kg), IV- Chloroform extract (100gm/kg), V-Aqueous extract (200gm/kg)

Anthelmintic activity of root extracts of Tragia involucrata (Linn)

Group	Treatment	% Conc W/V	Time in minutes (Paralysis)	Time in Minutes (death)
I	Normal control	0.9		
II	Standard	0.1	104	No death
		0.2	78	
		0.5	37	
III	PETI	0.1	129	135
		0.2	109	121
		0.5	101	116
IV	CHTI	0.1	119	127
		0.2	102	111
		0.5	93	103
V	AQTI	0.1	110	121
		0.2	91	101
		0.5	72	84

n=6

Significance P<0.05*, P<0.01**, P<0.001***,

I-Normal control Saline, II- Standard Piperazine hydrate, III- Petroleum ether extract (100mg/kg), IV- Chloroform extract (100gm/kg), V-Aqueous extract (200gm/kg).

Discussion

It was observed that the *root* extracts of *T.involucrata* had significantly inhibited carrageenan- induced paw oedema in rats. It may be assumed that the anti-inflammatory effect of these extracts might be due to their possible inhibition of lipoxygenase pathway. Carrageenan induced paw edema model in rats is known to be sensitive to cyclooxygenase inhibitors and has been used to evaluate the effect of nonsteroidal anti-inflammatory type of agents which primarily inhibit the enzyme cyclooxygenase in prostaglandin synthesis [18]. Based on these reports it can be inferred that the inhibitory effect of of *T.involucrata* extracts on carrageenan- induced inflammation in rats could be due to the inhibition of the enzyme cycloxygenase leading to inhibition of prostaglandin synthesis. The root extracts of *T.involucrata* also exhibited analgesic activity in rodents. The extracts were found to significantly increase the tail flick reaction time in rats. This test is very useful for discriminating between centrally acting morphine-like analgesics and peripherally acting nonopiate type of analgesics, thus giving positive response with the former only. The analgesic response can be due to inhibition of lipoxygenase or

cyclooxgenase pathway of arachidonic acid metabolism. The different extract of *T.involucrata* have enhanced considerably the urine volume and is equivalent to standard drug furosemide treated group. Anti-inflammatory and diuretic activity of *T.involucrata* could be due to the effect of one or combination of the bioactive components in the plant.. A flaccid paralysis may also ensue when the drug causes hyperpolarization of the muscle membrane, thus preventing depolarization. Anthelmintics including organophosphates and praziquantel possibly have a neuromuscular mode of action by interfering with transmission at nerve-nerve synapse or at neuromuscular junction by inhibiting the enzyme (AChE) responsible for inactivating the neurotransmitter at the synapse. Treatment with root extracts of *T.involucrata* does cause paralysis of the worms which may be as a result of depolarizing type of neuromuscular blockade and a sustained muscle concentration.

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