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Evaluation of the analgesic and antipyretic activity of various leaf extracts of *Crotalaria pallida* Aiton.

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ABSTRACT

The present study was an attempt to investigate the analgesic & antipyretic activity of various extracts of Crotalaria pallida leaves. The analgesic activity of C.pallida extracts was evaluated on albino mice by hot plate and tail immersion methods. Where as antipyretic activity was studied on Brewer's yeast-induced pyrexia in Wister strain albino rats.all the crude extracts of C.pallida such as ethanol, ethyl acetate, n-butanol and petroleum ether were tested for analgesic & antipyretic activity at 100 and 200mg/kg body weight. Where as pentazocine (10mg/kg) and paracetamol (100 mg/kg) were used as standard drugs for analgesic and antipyretic activities respectively..Among all the extract n-butanol & ethyl-acetate shows significant action than other two extract in a dose of 200 mg/Kg body weight. Where as ethanol & petroleum ether extract in a dose of 200 mg/Kg body weight exhibited significant antipyretic activity after 90 minutes and 120 minutes as compared to standard paracetamol These findings demonstrate that C.pallida leaves have remarkable analgesic & antipyretic activities when compared with positive control and thus have great potential as a source for natural health .The data were verified as statistically significant by using one way ANOVA (analysis of variance) at 5% level of significance ($p<0.05$).

Keywords: *Crotalaria pallida;* Analgesic activity; Pentazocine; pyrexia rats

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INTRODUCTION

Analgesics are defined as the substances which decreases pain sensation by increasing painthreshold to external stimuli. Noxious pain stimuli can be developed by thermal, chemical and physical pressures.The literature survey reveals that there are no reports on the analgesic & antipyretic activity of the leaf extracts of *Crotalaria pallida*. This prompted the authors to undergo the present study.The tribal areas of Baipariguda, Koraput (District) of Eastern Orissa. due to its unique varieties geographical and climatic factors has had a rich variety of medicinal plant. *Crotalaria pallida* (family: fabaceae.) also known as jhunjhunuka (Oriya) is frecuntly distributed. And extensively used traditionally by the tribal people. *Crotalaria pallida* Aiton is a species that belongs to the Fabaceae family, popularly known as "rattle or rattlesnake" due to the sound of their fruits when dry [1]. .Crotalaria is one of the largest genera in tropical Africa. The genus includes 690 species that are mainly situated in Africa and Madagascar. the. Species have also been found throughout in India. [2] This is an erect shrub, annual short-lived perennial herb of 1.5 m or more tall. Taproot white or brown and stem grooved, solid, glabrous. Leaves trifoliolate, alternate spiral, stalked, leaflets elliptic , more than 2 cm long/ wide, hairy on upper surface, margin entire, apexobtuse base acute, pinnately veined.Flowers bisexual, grouped together in a terminal raceme, stalked, petals 5, yellow. Fruit a rounded.

This species is used in traditional medicine, the plant is used to treat urinary problems and fever, a poultice of the roots is applied to swelling of joints and fever and its leaves as vermifuge [3]. Mikirs of Assam take about 20 ml. extract of leaves in early morning to kill intestinal worms.[4]. Powder of leaf and root bark with the leaf of Wrightia tinctoria & Tragia involucrate is made to a paste with water and applied externally for skin diseases[5] .Pharmacological studies have demonstrated it also presents anti-inflammatory, antimicrobial, antioxidant, antibacterial & antifungal functions[6-9], *Crotalaria pallida* extracts as a putative HIV-protease inhibitor[10].

MATERIALS AND METHODS

Drugs and chemicals

Pentazocine and Paracetamol was procured as gift sample from Taj Pharmaceuticals Ltd, Mumbai, India. The ethanol AR and ethyl acetate AR 60-80°C (Emsure® ACS) were procured from Merck Pvt. Ltd., Navi Mumbai, Maharashtra, India. n-butanol GR 80°C, petroleum ether AR 40-60°C,Loba Chemie Pvt. Ltd., Mumbai, India. All other chemicals reagents used in present work were procured from authorized dealer.

Materials

Disposable syringe, heating mantle, stop watch, micefeeding needle, carboxy methyl cellulose were supplied by the department of Pharmacology

Collection of Plant Material

The leaves of *Crotalaria pallida* were collected from the tribal belts of the local area of Baipariguda of Koraput district.(India) in the month of November 2011.The plant was identified, confirmed and authenticated by the Biju Patnaik Medicinal Plants Garden and Research Centre, Dr. M. S. Swami Nathan Research Foundation, Jeypore, Koraput (District), Orissa (Letter No. MJ/SS/P-198/11,dated 16.12.2011).After authentification leaves were collected in bulk and washed under running tap water to remove adhering dirt. Then leaves were shade dried. The dried materials were made into coarse powder by grinding in mechanical grinder. and stored in a closed air tight container for further use.

Preparation of Extracts

The coarse powder was taken in Soxhlet apparatus and extracted successively with ethanol, ethyl acetate, n-butanol and petroleum ether as solvent. A total amount of 750 g coarse powder was extracted with 1200 ml of each solvent. For each solvent,10 cycles were run to obtain thick slurry. Each slurry was then concentrated under reduced pressure to obtain crude extract. All crude extracts were kept in closed air tight containers under cool and dark place for further study[11,12,13].

Evaluation of Analgesic Activity

Animals

Healthy albino mice of Swiss strain of either sex were used.They were housed in standard conditions of temperature (25±2 °C), 12 hours light per day cycle, relative humidity of 45-55 % in animal house of Jeypore College of Pharmacy. They were fed with standard pellets of food and water. Animals were kept and all operation on animals was done in aseptic condition.

Drugs

Pentazocine (10 mg/kg) and a dose of 100 mg/kg and 200 mg/kg of different *Crotalaria pallida* leave extracts used for activity study. The doses were prepared in 1% aqueous suspension of gum acacia and route of administration was i.p.

Experimental protocol

Animals were selected, weighed (25-30 g) and devided in to ten groups (n=6), namely control, standard drug and four groups belonging to four different extract of *C.pallida*. All the studies conducted were approved by the Institutional Animal Ethical Committee (1200/ac/08/CPCSEA), Dadhichi college of pharmacy, Vidya vihar,Cuttack, according to prescribed guide-lines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Acute toxicity studies

The acute toxicity was performed according to OECD 423, 2001.The selected female albino rats were used to determine the dose. The animals were divided into twelve groups of three in each.The animals were fasted overnight prior to the acute experimental procedure. Distilled water was used as vehicle to suspend the different leave extracts of *Clotalaria pallida* and administered orally as following doses of 100, 300,600,1000 and 2000 mg/kg body weight. Immediately after dosing, the animals were observed continuously for first four hours for behavioral changes and for mortality at the end of 24hrs and daily for 14 days respectively [14]

Tail Immersion Method

The tail immersion method was used to evaluate the central mechanism of analgesic activity. Here the painful reactions in animals were produced by thermal stimulus that is by dipping the tip of the tail in hot water Albino mice were devided in to 10 groups of six animals each. The animals were fasted for 16 hours with water adlibitum. The group-1 was served as solvent control which received the vehicle 0.5 % carboxy methyl cellulose (0.1ml/10Kg) through oral route, the group-2 was served as reference control which received Pentazocine (10 mg/Kg) and group-3 to 10 were received in a dose of 100 and 200mg/Kg each the extracts of ethanol, ethyl acetate, n-butanol.& petether .After administration of above drug, the basal reaction time was measured after in a regular interval of 30 minutes, by immersing the tail tips of the mice (Last 1-2 cm) in hotwater heated at temperature of temperature (55 ± 1) °C.The actual flick responses of mice i.e. time taken in second to withdrawn it's from hot water source was calculated and result were compared with control group[15,16]

Hot Plate Method

The hot plate method was performed for all above groups of animals (1-10) . Each mice was placed on Eddy's hot plate (55±2°C) with a cut off period of 20 s to avoid damage in the paw. The reaction time for the mice to respond the thermal pain (licked fore or hind paws or jumped) was recorded at 0,0.5, 1, 2 and 3 h after oral administration of test and standard drugs[17,18]

Antipyretic activity by Yeast induced pyrexia

For studying antipyretic activity of *Crotalaria pallida* , albino rats weighing150-200 gms were selected and divided into ten groups containing six animals in each group (Table: 3) were used for yeast induced pyrexia models**.** Group I animals received 1 ml/kg body weight of normal saline orally and served as control group. Group II animals were treated with paracetamol by intraperitoneal injection in the dose of 100 mg/kg body weight and served as standard group. The animals of group 3 two 10 received the ethanol, ethyl acetate, n-butanol and petroleum ether of leaves extract of *Crotalaria pallida* orally (100&200mg/kg body weight) to the respective groups of animals. In the beginning of the experiment normal rectal temperatures was noted by inserting 2cms of digital thermometer, lubricated with glycerine into the rectum. Pyrexia was induced by intraperitonial injection of 2ml/kg body weight of 15% brewer's yeast suspension in normal saline.The animals were then fasted for the duration of experiment (approximately 24 hours). After 18 hours of yeast injection, extracts (100&200 mg/kg body weight) are given to the respective test group animals then the basal temperatures were recorded for all the groups of animals by inserting 2cms of digital thermometer, lubricated with glycerin into the rectum. The rectal temperatures of all the animals were noted at 30 minutes of intervals till 3 hours.[19,20]

Statistical analysis

All data were calculated statistically by standard error mean (n=6) and statistically significant were verified by applying one way ANOVA at 5 % level of significance where $p<0.05$ [21]

Table 1. Analgesic activity of different leave extracts of *Crotalaria pallida* by Tail immersion method

Treatment Dose(mg/Kg)		Tail flick latency in minutes. (X ± SEM)				
		30	60	90	120	180
control	-------	3.23±0.11	2.47±0.17	2.47±0.12	2.55±0.18	2.42±0.10
Pentazocine	10	6.85±0.12	12.6±0.20	9.55±0.12	7.22±0.17	6.22±0.25
Ethanol extract	100	5.30±0.25	6.40±0.25	7.17±0.12	5.65±0.11	5.22±0.11
	200	6.27±0.30	7.32±0.22	8.67±0.17	7.13±0.11	5.33±0.12
Ethyl acetate extract	100	5.10±0.21	5.00±0.32	5.20±0.33	6.42±0.21	5.40±0.25
	200	7.00±0.17	9.27±0.21	10.2±0.22	9.10±0.16	6.43±0.24
n-butanol extract	100	5.20±0.16	6.2 8±0.33	6.47±0.26	6.23±0.12	5.32±0.20
	200	6.25±0.20	7.40±0.17	9.00±0.17	6.25±0.20	5.30±0.19
Pet. ether extract	100	4.00±0.19	5.20±0.23	5.43±0.26	5.18±0.33	3.12±0.28
	200	5.52±0.30	6.81±0.34	5.04±0.22	6.36±0.32	4.12±0.17

Table 2. Analgesic activity of different leave extracts of *Crotalaria pallida* by Eddys Hot Plate method

Treatment Dose(mg/Kg)		Reaction Time in Seconds at Time (h)				
		0	0.5	1	2	3
control	--------	7.23±0.15	8.47±0.17	8.37±0.22	9.55±0.11	9.42±0.27
Pentazocine	10	9.85±0.12	10.6±0.20	14.55±0.12	17.22±0.17	18.22±0.25
Ethanol extract	100	8.30±0.25	8.40±0.25	9.17±0.12	11.65±0.14	11.22±0.11
	200	7.27±0.20	9.32±0.22	10.67±0.11	8.13±0.18	12.33±0.27
Ethyl acetate extract	100	7.10±0.44	8.00±0.42	11.20±0.33	13.42±0.29	10.40±0.21
	200	8.00±0.37	11.27±0.23	11.2±0.12	13.10±0.16	15.43±0.34
n-butanol extract	100	7.20±0.36	10.28±0.33	11.47±0.21	14.23±0.42	13.32±0.33
	200	8.25±0.27	10.40±0.17	10.00±0.10	15.25±0.31	15.30±0.13
Pet. ether extract	100	7.00±0.18	8.20±0.13	8.43±0.27	9.18±0.33	9.12±0.34
	200	6.52±0.30	6.81±0.34	7.04±0.12	9.36±0.38	9.12±0.47

Table 3. Effect of various leave extracts of *Crotalaria pallida* against yeast induced pyrexia in rats

Group	Treatment Dose(mg/Kg)	Initial Body Temperature (ºC)	Basal Temperature (ºC)	30min	60min	90min	120min	180min
Control	----------	36.70±0.25	39.20±0.21	39.43±0.16	39.75±0.16	39.94±0.11	40.28±0.31	40.32±0.51
Paracetamol (standard)	100	37.10±0.22	39.73±0.31	38.34±0.22	38.27±0.11	37.24±0.17	37.39±0.12	37.33±0.32
Ethanol extract	100	36.82±0.18	37.36±0.27	37.75±0.25	38.23±0.27	38.43±0.32	39.33±0.38	40.26±0.28
	200	36.42±0.16	37.25±0.21	37.65±0.15	38.13±0.20	38.23±0.22	37.13±0.32	37.16±0.17
Ethyl acetate extract	100	37.44±0.22	37.36±0.11	37.77±0.32	38.22±018.	39.62±0.28	39.72±0.38	40.44±0.33
	200	37.14±0.27	37.32±0.17	37.87±0.22	38.24±028	38.40±0.23	39.40±0.17	39.24±0.23
n-butanol extract	100	36.40±0.22	37.12±0.36	38.26±0.32	38.36±0.14	39.74±0.18	40.43±0.13	40.43±0.12
	200	36.33±0.26	37.32±0.21	38.14±0.22	38.37±0.11	38.29±0.27	39.63±0.17	40.23±0.11
Pet. ether extract	100	37.15±0.22	38.50±0.11	39.32±0.23	39.76±0.13	37.51±0.36	37.31±0.44	37.36±0.18
	200	37.17±0.14	38.38±0.16	38.22±0.13	39.35±0.17	37.16±0.28	37.13±0.34	37.18±0.12

RESULTS AND DISCUSSION

In present study four extracts (ethanol, ethyl acetate, n-butanol and petroleum ether) of leaves part of *Crotalaria pallida* were studied for analgesic(by hot plate and tail flick method)& antipyretic activity (by yeast induced pyrexia method). A preliminary acute toxicity study in mice showed that all the extracts were not toxic(LD50 > 1000mg/kg).The effect of various leaf extracts of *Crotalaria pallida* shown in table no.1,2&3respectively. Among all the extracts n-butanol & ethyl-acetate shows significant analgesic effect than other two extract in a dose of 200 mg/kg body weight as compared to standard drug pentazocine in a dose of (10mg/kg). similarly pet-ether &ethanolic extract of leaf of *Crotalaria pallida* shows significant antipyretic activity than other two extract in a dose of 200 mg/kg body weight as compared to standard drug paracetamol in a dose of (100mg/kg). Decreased body temperature of yeast induced rats. The results obtained from both standards and extracts treated groups were compared with the control group. A significant reduction in the yeast elevated rectal temp. was observed in the test drug.

CONCLUSION

On the basis of present study, we may conclude that *Crotalaria pallida* leaf produces significant analgesic and antipyretic activities in dose-dependent manner on animal models. By the positive activity of *Crotalaria pallida* leaves against pyrexia, The traditional use has been pharmacologically validated. Since, *Crotalaria pallida* leaves showed remarkable activity when compared with standard drugs. Therefore, *Crotalaria pallida* leaves can be a substitute of synthetic analgesic or antipyretic drugs having adverse effects.

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