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Research Article

ANALGESIC ACTIVITY OF METHANOLIC EXTRACT FROM AERIAL PARTS OF RHYNCHOSIA CAPITATA DC

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Abstract

The methanolic extract from aerial parts of Rhynchosia capitata DC Syn R. aurea DC was evaluated for analgesic activity on Swiss albino mice by three models, viz., acetic acid induced writhing response (chemical method), hot plate reaction time (thermal method), and tail flick assay (thermal method). The study found that methanolic extract from aerial parts of Rhynchosia Capitata DC shows significant analgesic activity comparable to that of standard drug Aspirin. The methanolic extract at 100, 200 and 300 mg/kg/p.o dose inhibited the abdominal constrictions induced by acetic acid and also increased the pain threshold of mice towards the thermal source in a dose-dependent manner. The pain and writhes in mice were significantly reduced ($p < 0.001$) as compared to the control.

The study concludes that the methanolic extract from aerial parts of Rhynchosia Capitata possess analgesic activity at doses 100,200 and 300 mg/kg/p.o.

Key words: Rhynchosia Capitata, Analgesic activity, Acetic acid, Hot plate, Tail flick, Aspirin.

Introduction

"Pain" is a part of a defensive reaction against dysfunction of an organ or imbalance in its functions against potentially dangerous stimulus. Pain is usually transitory, lasting only until the noxious stimulus is removed or the underlying damage or pathology has healed, but some painful conditions, such as rheumatoid arthritis, peripheral neuropathy, cancer and idiopathic pain, may persist for years. Pain that lasts a long time is called chronic, and pain that resolves quickly is called acute. Nociceptive pain is caused by stimulation of peripheral nerve fibres that

respond only to stimuli approaching or exceeding harmful intensity (nociceptors), and may be classified as thermal, mechanical, chemical according to the mode of noxious stimulation. The ascending pathway of pain includes the contralateral spinothalamic tract, lateral pons, mid brain to thalamus and ultimately through the somatosensory cortex of the brain that determines the locations, intensity and depth of pain.

To relieve the pain, drugs like Morphine [1] and Aspirin [2] have been significantly used for the last three decades. Pain relieving commonly used drugs release chemicals which produce pronounced side-effects on the physiology of the body. In view of the fact that Medicinal herbs have been used as a form of therapy for the relief of pain throughout history [3], the present study therefore aimed to determine the antinociceptive activities of the Methanolic aerial extracts of Rhynchosia capitata.

Rhynchosia capitata DC is a prostrate or climbing herb belonging to the family Papilionaceae (fabaceae). This common weed of Kharif crop is found distributed in India, Pakistan and Ceylon with the characteristics as - stem pilose, branched; stipules 1.5-2.0 mm long, cordate, pilose. Leaf trifoliolate compound, petiole 2.5-12.5 cm long, pilose, petiolule up to 1.5 cm long, pilose; lamina 18-40 mm long, 15-41 mm broad, rhomboid, sometimes oblique, entire, obtuse, scantily pubescent. Inflorescence 4-6 flowered, axillary umbellate Peduncle 0.5-3.5 cm, pilose. Bracts c. 2.5 mm long, cordate, pilose; pedicel c. 3-4 mm long, pilose. Calyx 10-13 mm long, pilose, lower tooth longest 7-9 mm long. Vexillum 10-12 mm long, glabrous externally. Fruit 10.13 mm long, 9-12 mm broad, mucronate transversely striated, pilose [4]. The Rhynchisia capitata DC Syn R. aurea DC plants appear through the seeds just after the rains and are annual prostrate twinners spreading all around the root stock. The plants start flowering as early in one month and the seeds mature within 3 months [5].

Till now, we have studies to show that decoction of the roots of Rhynchosia capitata roots have stomach cleaning action. Different specifies of the plant like R. minima roots [6] and R. nulbilis seeds [7] have been reported to be used in Ingestion. The broad spectrum antibacterial, antifungal and antioxidant activity have been reported in R. minima [8]. The anti-inflammatory effect against cotton pellet induced sub- acute inflammation in rats have been reported in the methanolic extracts of flowers of R. cana [9]. In addition, studies have shown the occurrence of C-glycosides, o-glycosides, prenylated flavonoids and aglycones in these species of the plant [10]. However, there is no scientific report available in the literature on the antinociceptive and anti-inflammatory effect of Rhynchosia

capitata. The present study therefore aimed to determine the analgesic activity of the methanolic extract from aerial parts of Rhynchosia capitata in relation with their folklore medicinal properties.

Materials and Methods

Plant Material and Preparation of Methanolic extract

The aerial parts of Rhynchosia capitata were procured from Nirankar Herbs; 2211/4344/Aggarwal market, Tilak Bazar, khari bawri market, Delhi-6 (India) locally in the month of February 2009. The authentication of aerial parts was done by Dr. H.B. Singh, Head, Raw material, Herbarium and Museum division, National Institute of Science Communication and Information Resources (NISCAIR), PUSA New Delhi, India.

The air-dried aerial parts were made in to a coarse powder and macerated with 900ml of methanol for 48 hrs. Extraction was done through Soxhlet apparatus. After extraction, the solvent was distilled off and the extract was concentrated under reduced pressure on a water bath at a temperature below 50°C to give a semisolid syrupy consistency residue of 64.8g (yield 3%, w/w) which was stored in a closed bottle and refrigerated at temperature below 4°C until tested.

Preliminary Phytochemical Analysis

Preliminary phyto-chemical analysis of extract was performed for analysis of alkaloids, carbohydrates, tannins, saponins, terpenes, coumarins; anthraquinones according to Khandelwal, 2006 [11].The extract was solubilized in normal saline (0.9%w/v sodium chloride) for use in in-vivo experimental animals. Results of analysis are given in the [Table–1] and [Table-2].

Table-1: Result of Preliminary Phytochemical Analysis.

Extract	Steroid	Alkaloid	Tannin	Carbohydrate	Gum Glycoside	Flavonoid	Saponin
Rhynchosia Capitata	-	-	+	-		+	+

(+) Methanolic extract of Rhynchosia capitata *Present.*
(-) Methanolic extract of Rhynchosia capitata *Absent.*

Table-2: Result of Preliminary Phytochemical Analysis.

S. NO.	PARAMETER	RESULT
1	Loss on drying	10.22% w/w
2	Total Ash	6.92% w/w
3	Acid Ash insoluble	1.10% w/w
4	Water extractable matter	10.94% w/w

Phytochemical Analysis

Animals used for the test

Swiss albino mice (25-30 g) of either sex were procured from the central animal house of the Delhi institute of Pharmaceutical Sciences and Research, New Delhi, India. They were housed in standard polypropylene cages and kept under controlled room temperature (24 ± 20 ° C; relative humidity 60-70 %) in a 12 hr. light-dark cycle. The animals were given a standard laboratory diet and water ad libdum. Food was withdrawn 12 hr. before and during the experimental hours. All experimental protocols were approved by the Institutional animal ethics committee, according to the Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) guidelines.

The mice were randomly allocated into five groups of six mice each for the three different experimental animal models. Group I (control) received 10 ml/kg,p.o of normal saline, Group II received standard drug aspirin 100 mg/kg, p.o [12], Group III received Rhynchosia capitata (RC) 100 mg/kg,p.o, Group IV received Rhynchosia capitata(RC) 200 mg/kg, p.o and Group V received(RC) 300 mg/kg,p.o.

Drugs used for the test

The Analytical grade drugs used for the test were Normal Saline, Acetic acid (CDH) and Aspirin (SigmaAldrich).

Test results

Peripheral Analgesic Activity – Abdominal Constriction

Acetic acid induced writhing test was used for detecting peripheral analgesic activity using the method of Seighmund, E et.al, 1957[13]. The effect of different doses of the methanolic extracts from aerial parts of Rhynchosia capitata on the number of Writhes/stretches movement induced by 0.6%v/v of acetic acid (1ml/100g body weight) in mice are shown in [Table-3]. Plant extract and reference substance (aspirin) or solvent was administered 30 minutes before the intra peritoneal administration of acetic acid. Control animals received 0.9%

NaCl solution under the same experimental conditions. Number of writhes per animal was counted during a 15 min. series beginning 5min after the injection of acetic acid. The number of writhing's and stretching's were recorded and permitted to express the percentage of protection using the formula ratio.[14] [Table-4]

Table-3: Effect of Rhynchosia capitata extract on acetic acid induced writhing in mice.

	Number of Wriths in 20 mins					Number of Wriths reduced			
	Normal Saline	Aspirin	Rc(100 mg/kg)	Rc(200 mg/kg)	Rc(300 mg/kg)	Aspirin	Rc(100 mg/kg)	Rc(200 mg/kg)	Rc(300 mg/kg)
	23	10	15	12	10	13	8	11	13
	19	9	16	14	7	10	3	5	12
	20	8	17	12	7	12	3	8	13
	21	10	17	13	9	11	4	8	12
	21	8	19	12	6	13	2	9	15
	18	7	15	10	6	11	3	8	12
Mean	20.330000000	8.666666667	16.500000000	12.166666670	7.500000000	11.666666670	3.833333333	8.166666667	12.833333330

Table-4: % Inhibition of RC extract on acetic acid induced writhing.

S.No	Treatment	Number of wriths reduced	Number of wriths	% inhibition
1	Normal Saline (10 ml/kg)	20.333333330	20.330 ± 0.70047	-
2	Aspirin (100 mg/kg)	11.66666667 **	8.666 ± 0.48442 **	57.374 **
3	Rc (100 mg/Kg)	3.833333333 *	16.500 ± 0.06066 *	18.851 *
4	Rc (200 mg/Kg)	8.166666667 **	12.166 ± 0.53166 **	40.161 **
5	Rc (300 mg/kg)	12.83333333 **	7.500 ± 0.65726	51.890 **
Each value represents the mean ± SEM, n=6				
* p<0.05 , p<0.001 **				

$$\text{Percent protection} = (1 - V_c/V_t) \times 100$$

Where:

V_t = Mean number of writhing in test animals

V_c = Mean number of writhing in control

Administration of extracts (100–300 mg/kg) significantly ($p < 0.05$, $P<0.001$) reduced the number of writhes induced by the injection of acetic acid in mice as compared to control group. Rhynchosia capitata 300 mg/kg showed better results of inhibitory effect as compared to control, which were also comparable to the reference drug.

Central Analgesic Activity (Analgesic effect by Tail Flick Method)

The central analgesic activity was determined by radiant heat tail-flick model in mice using analgesiometer (Inco, India)[15,16,17].Experimental animals of either sex were randomly selected and divided into five groups

designated as group-I, group-II, group-III, group-IV and group-V consisting of six mice in each group for control, positive control and test samples. Each group received a particular treatment i.e. control (Normal Saline0.9%w/v, 10 ml/kg), positive control (Aspirin 100mg/kg,p.o) and the test sample (Methanolic extract of Rhynchosia capitata 100 mg/kg,p.o, 200 mg/kg,p.o, 300 mg/kg,p.o respectively). The tail flick latency was obtained thrice before drug administration and mean was used as pre drug latency. A cut off time of 10 sec was observed to prevent any tissue damage to the animal. The animal which failed to withdraw its tail in 3-5 sec was rejected from the study. The instrument's nichrome wire was heated to the required temperature and maintained by means of heat regulators. The strength of the current passing through the naked nichrome wire was kept constant at 4 Amps. The mice were kept in a holder with only the tail portion protruding out. The tail was placed on the platform in such a way that the middle portion of the tail remained just above the hot wire but without touching it. The latency period (reaction time) was noted when the animal responded with a sudden and characteristic flick or tail lifting. The reaction time for each group was measured at 15, 30, 45, 60, minutes.

Table-5: Effect of crude extract of Rhynchosia capitata on latency to tail flick test in mice.

Group	Treatment	Dose	Basal reaction time(sec)	Reaction time in sec			
				After 15 min	After 30 min	After 45 min	After 60 min
1	Normal Saline	10 ml/kg	2.50 ± 0.0408	2.70 ± 0.0500	2.80 ± 0.0484	3.00 ± 0.0680	3.60 ± 0.0565
2	Aspirin	100 mg/kg	3.00 ± 0.0816	4.20 ± 0.1658 **	5.00 ± 0.1784 **	6.20 ± 0.3166 **	8.40 ± 0.5724 **
3	Rc	100 mg/kg	2.60 ± 0.0565	3.40 ± 0.1093 *	3.80 ± 1256 *	4.00 ± 0.1324 *	4.20 ± .0.1445 *
4	Rc	200 mg/kg	2.80 ± 0.0512	3.60 ± 0.1177 **	4.00 ± 60.1093 **	4.80 ± 0.0993 **	5.00 ± 0.1408 **
5	Rc	300 mg/kg	2.70 ± 0.0688	3.90 ± 1275 **	5.00 ± 0.0938 **	5.50 ± 0.1417 **	6.80 ± 0.1538 **
Groups treated with the Methanolic leaf extracts of R (100–300 mg/kg) elicited an increase in the latency response by tail flick method							
Results are presented as mean ± SEM (n=6)							
* p<0.05 , ** p<0.001							

Analgesic Effect by Hot Plate Method

Animals were individually placed on a hot plate maintained at a constant temperature (55^0C) and the reaction of animals, such as paw licking or jump response was taken at the end point [18]. Experimental animals of either sex were randomly selected and divided into five groups designated as group-I, group-II, group-III, group-IV and group-V consisting of six mice in each group for control, positive control and test samples. Each group received a particular treatment i.e. control, positive control (Aspirin 100 mg/kg,p.o) and the test sample (Methanolic extract of Rhynchosia capitata 100 mg/kg, p.o, 200 mg/kg,p.o and 300 mg/kg,p.o respectively).The animals were positioned on Eddy's hot Plate kept at a temperature of 55 ± 0.5^0C. A cut off period of 15s was observed to avoid

damage to the paw. The reaction time in control and treated animals was recorded at 0, 15, 30, 45, 60 min after the treatment [19]. Data is represented in [Table-6].

Table-6: Effect of Rhynchisia capitata extract on latency to hotplate test in mice.

S.No	Group	Treatment	Dose(mg/kg)	Basal Reaction Time(Sec)	Reaction time in sec			
					After 15 min	After 30 min	After 45 min	After 60 min
1	Control	Normal Saline	10 ml/kg	2.30 ± 0.02981424	2.40 ± 0.02190000	2.50 ± 0.06572671	2.40 ± 0.08640988	2.34 ± 0.10327956
2	Standard	Aspirin	100 mg/kg	2.75 ± 0.18710000	4.12 ± 0.29940000 **	5.43 ± 0.46760000 **	6.68 ± 0.59470000 **	8.25 ± 0.98130000 **
3	Test Drug	Rc	100 mg/kg	2.73 ± 0.09545000	3.60 ± 0.11250000 *	4.80 ± 0.18070000 *	5.78 ± 0.11670000 *	6.81 ± 0.10460000 *
4	Test Drug	Rc	200 mg/kg	2.50 ± 0.06022181	3.72 ± 0.11977757 **	5.00 ± 0.14895189 **	5.30 ± 0.15933000 **	6.88 ± 0.18875020 **
5	Test Drug	Rc	300 mg/kg	2.53 ± 0.08640000	3.87 ± 0.23955500 **	5.17 ± 0.23787952 **	5.83 ± 0.93333300 **	6.97 ± 1.11200000 **
Animal groups treated with the Methanolic leaf extracts of Rhynchisia capitata[RC] (100–300 mg/kg) elicited an increase in the latency response in the hot plate test								
Results are presented as mean ± SEM, (n=6)								
* p<0.05 , p<0.001 **								

Statistical Analysis

The results were presented as mean± SEM. “One- way ANOVA” with Dunnett’s post-test was performed using Graph Pad Prism version 3.00 for Windows (Graph pad software, San Diego California, USA). P<0.05 and P<0.001 were considered to be statistically significant.

Results and Discussion

It is well established that chemical mediators are responsible for the inflammatory pain. Acetic acid produces nociception by liberating endogenous substances including serotonin bradykinin, histamine and prostaglandin. Inflammatory mediators are released sequentially with histamine and serotonin released within the first 30min, followed by kinins released approx. 1 hr. and prostaglandins released approx. 2 hr. after induction of inflammation [20].It is known that the NSAIDs reduce pain by inhibiting synthesis and release of prostaglandins. Aspirin, the reference in the current study, offers relief from pain by suppressing the formation of pain substances in the peripheral tissues where prostaglandins and bradykinins are suggested to play an important role.

Taking these points in view, the methanolic extract from aerial parts of Rhynchosia capitata was studied using chemical and thermal methods. Acetic acid induced writhing test was used to detect both central and peripheral analgesic effect, whereas hot plate and tail flick tests were used mainly to study central analgesics effect. From this study, it is established that methanolic extract from aerial parts of Rhynchosia capitata possesses significant

analgesic effects in a dose dependent manner. The analgesic property of Rhynchosia capitata in the dose of 300 mg/kg is similar to aspirin, a standard NSAID.

The observed analgesic activity in the methanolic extract of of Rhynchosia capitata is attributed to the presence of compounds including flavonoids, tannins and saponins. The presence of these compounds was confirmed by the phytochemical screening conducted during the study. Flavonoids have been reported to have a role in analgesic activity by targeting prostaglandins and inhibiting prostaglandin synthetase, more specifically the endoperoxidase. The role of tannins in anti-nociceptive activity has also been reported [21].

In the peripheral analgesic test, Rhynchosia capitata extract worked to manage the pain by inhibiting the synthesis and/or release of endogenous substances like serotonin, bradykinin, histamine and prostaglandin that would have been liberated by acetic acid to stimulate sensory nerve endings [22, 23]. In the Central Analgesic test, Rhynchosia capitata (100 mg/kg, 200 mg/kg, 300 mg/kg) significantly increased the reaction time implying its central analgesic activity. Also, based on the observation that the methanolic extract of Rhynchosia capitata and aspirin dose-dependently increased the pain threshold in hot-plate and tail-flick models, the study concludes the extract possessing analgesic property similar to aspirin, a standard NSAID.

Fig-1: Effect of Rhynchosia capitata on acetic acid induced writhing in mice.

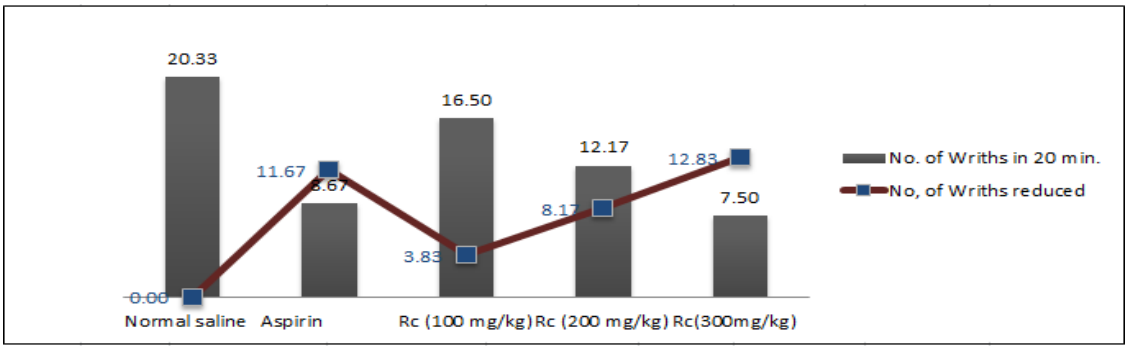


Fig-2: % Inhibition of RC extract on acetic acid induced writhing.

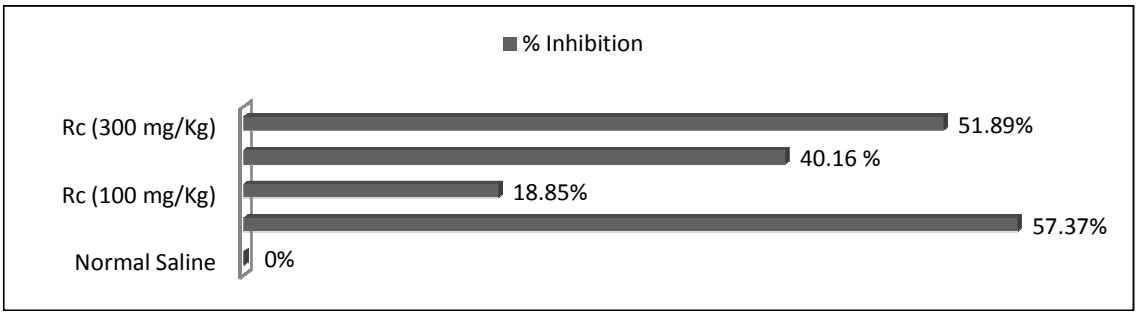


Fig-3: Effects of RC on latency time of mice exposed to tail flick test.

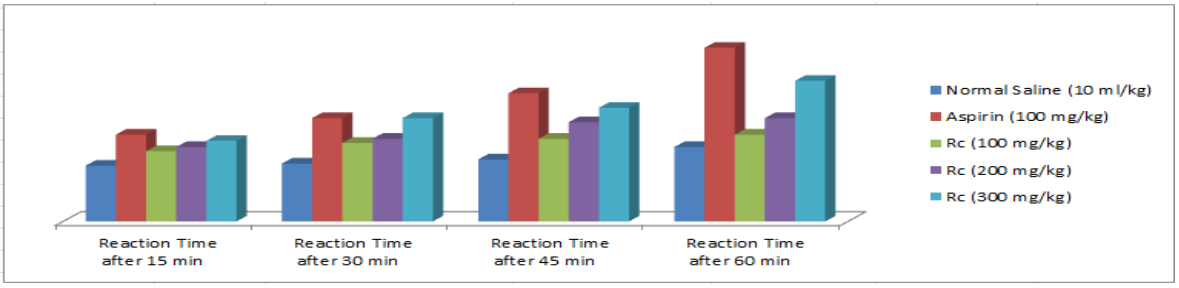
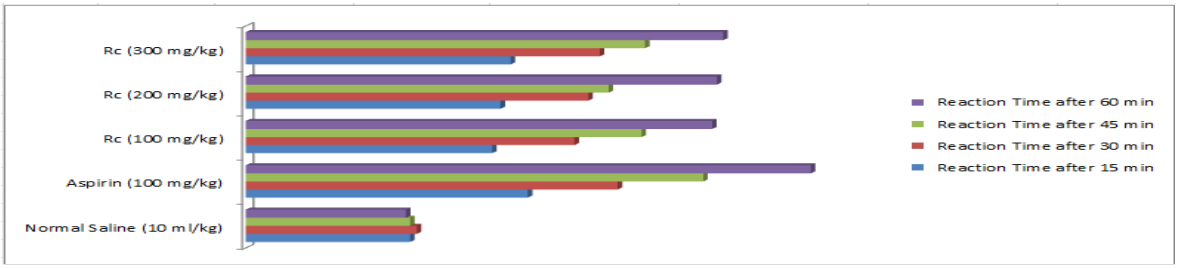


Fig-4: Effect of Rhynchisia capitata on latency time of mice exposed to hot plate test.



Conclusion

The present study concludes that methanolic extract from aerial parts of Rhynchosia capitata possess potent peripheral and central analgesic activity against different stimuli. The phyto-constituents in the plant produce peripheral analgesic effect by acting with the prostaglandin pathways. Also the plant produces central analgesic effect by acting on central opioid receptors.

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