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Evaluation of analgesic activity of ethanolic extract of *Sphaeranthus indicus*

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ABSTRACT

The *Sphaeranthus indicus* has been widely used for its reported biological activity in indigenous system of medicine. The present investigation was carried out to found the analgesic effect of ethanolic extract of *Sphaeranthus indicus* in experimental animal models of pain. The analgesic activity was evaluated by acetic acid induced writhing method in albino mice and by tail flick latency method in albino rats respectively. The percentage protection against writhing showed by ethanolic extract of SI in doses of 100, 200 and 400 mg/kg was 14.22, 36.90 and 62.20 respectively. In the tail flick model, the ethanolic extract of SI in the above doses increased the pain threshold significantly after 30 min, 1, 2 and 4 h of administration. The ethanolic extract *Sphaeranthus indicus* in different doses (100,200, and 400mg/kg, p.o) exhibited dose dependent and significant analgesic activity in both models of pain.

Key Words: Analgesic, *Sphaeranthus indicus*, Acetic induced writhing, TFL.

INTRODUCTION

Since time immemorial indigenous plants have been a major source of medicine because of the different components they contain have immense therapeutic value. Though considerable progress has been made in medical science in last few years, Management of pain still remains a challenge for medical community. The presently available analgesic (opiod and non-opioid) though effective are not free from various undesirable side effects. As a result more and more people are turning to herbal medicines for alternative treatment of pain. Herbal drugs have lesser side effects and are largely replacing synthetic drugs. .

Sphaeranthus indicus (Hindi-Gorakhmundi) is a much branched herb widely distributed in India and belongs to family asteraceae [1]. It is widely used in the treatment of diverse disease conditions namely convulsion, bronchial asthma and dysentery [2].Essential oil obtained from leaves possess antifungal activity [3] and ethanolic extract showed an anxiolytic activity [4]. There is paucity of study available for its analgesic activity, Hence the present study is an

attempt to assess the analgesic activity of ethanolic extract of *Sphaeranthus indicus* using various models of pain.

MATERIALS AND METHODS

Chemicals and drugs

Aspirin, pentazocine, acetic acid were used in the study.

Collection of Plants

The leaves of *Sphaeranthus indicus* were collected from rural area of Sambalpur, Orissa. They were authenticated by faculty of botany of Sambalpur University.

Preparation of the extract

The powdered plant material was extracted with ethanol in a Soxhlet apparatus for 48 hrs. The extracts were filtered through Whatman filter paper (No.1) and concentrated by vacuum evaporation. The yield of extract as per solvent used was 4.25% w/w. The dried extracts were suspended in 2% gum acacia and used for experiments.

Phytochemical studies

Preliminary phytochemical study was performed [5]. The presence of phytoconstituents such as flavonoids, terpenoids, carbohydrates and glycosides were confirmed.

Test Animals

Adult Swiss male albino rats (150-200gms) and albino mice (20-30gms) were obtained from animal house, Dept. of pharmacology, VSS Medical College, Burla and used throughout the study. They were housed in microton boxes in a controlled environment (temperature $25 \pm 2^\circ\text{C}$ and 12hr dark/light cycle) with standard laboratory diet and water *adlibitum*. All experimental procedures and protocol used in this study were reviewed and approved by institutional animal ethical committee, V.S.S. Medical College, Burla

Analgesic activity

Acetic acid-induced writhing test.

The prescreened animals were divided into seven groups with six albino mice in each group. Each group were treated with vehicle, test drug (EESI, 100,200 and 400mg/kg, p. o.), standard drug (Aspirin, 50 and 100mg/kg, p. o.) and combination of EESI (100 mg/kg, p. o.) and Aspirin (50mg/kg) respectively. Writhing was induced 30 min later by intraperitoneal injection of 10 ml/kg of 0.6% acetic acid in distilled water [6] [7]. The number of writhes was counted for 30 min immediately after the acetic acid injection. The percentage protection was calculated. Percentage protection against writhing was taken as an index of analgesia. It is calculated as

$$\frac{X1-X2}{X1} \times 100$$

X1 = No. of writhing in control group

X2 = No. of writhing in treated group

Tail flick method.

The prescreened animals (reaction time: 3-4 sec) were divided into seven groups with six albino rats in each group. Groups were treated with vehicle, test drug, (EESI 100,200 and 400mg/kg, p. o.), standard drug (pentazocine, 5 and 10mg/kg, i. p.) and combination of EESI (100 mg/kg, p. o.)

and pentazocine (5mg/kg ,i.p) respectively . After administration of drugs, tail flick latency was assessed by the analgesiometer (Inco, India) at 30 min, 1hr, 2hr and 4hr. The strength of the current passing through the naked nichrome wire was kept constant at 6 Amps. The distance between the heat source and the tail skin was 1.5 cm. The site of application of the radiant heat in the tail was maintained at 2.5 cm, measured from the root of the tail. The cut-off reaction time was fixed at 10 sec to avoid tissue damage [8][9].

Statistical Analysis

All values were shown as mean±SEM. Statistical analysis was performed using one-way analysis of variance (ANOVA) followed by Dun net's t test. $P < 0.05$ was considered statistically significant.

RESULTS

Table-1 Analgesic activity (Acetic induced writhing method)

Groups	Treatment(mg/kg,p.o)	Number of writhing	%protection
I	Control	29.5 ± 1.20	
II	EESI(100)	25.6 ± 1.42*	14.22%
III	EESI(200)	18.6 ± 0.99**	36.90%
IV	EESI(400)	11.1 ± 0.63**	62.20%
V	Aspirin(50)	20.5 ± 0.88**	32.20%
VI	Aspirin(100)	7.5 ± 0.69**	74.23%
VII	EESI(100)+Aspirin(50)	17.8 ± 0.75**	42.20%
One way ANOVA		F	32.72
		df	6, 35
N=6 in each group $p^* < 0.01$, $p^{**} < 0.001$ as compared to control.			

The readings are expressed as mean±SEM. $P < 0.05$ as compared to control was considered significant.

Table-2 Analgesic activity (Tail flick latency method)

Group	Treatment(mg/kg)	Predrug reaction time in sec {mean+ SEM}	Reaction time in sec (mean + SEM)			
			30 min.	1hr.	2hr.	4hr.
I	Control	3.41±0.08	4.00±0.08	3.12±0.08	3.64±0.04	3.53±0.06
II	EESI(100, p.o)	3.56±0.08	5.82±0.04*	6.44±0.04*	6.20±0.06*	6.05±0.04*
III	EESI(200, p.o)	3.62±0.04	6.47±0.05*	6.41±0.05*	7.23±0.05*	7.01±0.04*
IV	EESI(200, p.o)	3.53±0.05	7.63±0.04*	7.85±0.02*	8.21±0.03*	8.15±0.03*
V	Pentazocine (10,i.p)	3.85±0.04	7.86±0.04*	7.87±0.04*	7.76±0.03*	7.71±0.03*
VI	Pentazocine (5,i.p)	3.81±0.04	9.07±0.04*	9.12±0.02*	9.17±0.02*	9.22±0.04*
VII	EESI(100,p.o) + Pentazocine(5,i.p)	3.65±0.05	8.13 ± 0.05*	8.30±0.04*	8.51±0.03*	8.38±0.04*
One way ANOVA		F	0.96	9.23	12.21	11.29
		P	> 0.05	< 0.05	< 0.05	< 0.05
		N=6 in each group	df 6,35		* $p < 0.01$ compared to control	

The results obtained as %age protection against writhing are shown in table -1. The results show that ethanolic extract of SI (100, 200 and 400 mg/kg, p.o), standard drug, aspirin, (50 and 100mg/kg) and combination (EESI 100mg/kg, p.o and aspirin 50mg/kg, p.o) suppressed the acetic acid-induced writhing response significantly in a dose-dependent manner. The results were found to be highly significant in comparison to the control.

The results for tail flick model are shown in table-2. It shows that there was no significant difference in the mean predrug reaction time between the different groups. Thirty min after drug administration, reaction time increased significantly for the test and standard groups when

compared to the predrug reaction time. The test drug (EESI, 100,200, 400mg/kg p.o), standard drug (pentazocine, 5 and 10mg/kg i.p) and combination (EESI 100mg/kg p.o and pentazocine 5mg/kg i.p) produced a dose-dependent increase in the reaction time at various time intervals of observation. The results were found to be highly significant in comparison to the control.

DISCUSSION

The abdominal constriction response induced by acetic acid is a sensitive procedure to establish peripherally acting analgesics. This response is thought to involve local peritoneal receptors. PGE2 and PGF2 levels were increased in the peritoneal fluid of acetic acid induced mice [10]. In the tail flick model, the test drug in different doses increased the pain threshold significantly during the period of observation and this indicates the involvement of a higher centre [11]. The results of the present study suggest that the ethanolic extract of SI in doses of 100, 200 and 400 mg/kg demonstrated significant analgesic activity in acetic acid-induced writhing and tail flick models. However, the analgesic activity of EESI was found to be more significant on the acetic acid-induced model ($P < 0.001$) than the tail flick model ($P < 0.01$) and thus it appears that the test drug inhibits predominantly the peripheral pain mechanism.

CONCLUSION

The present experimental study protocol showed that ethanolic extract of *Sphaeranthus indicus* elicited significant analgesic activity in acetic induced writhing model and tail flick latency model. In both models they exhibited analgesic effect in a dose dependent manner which can be comparable with that of aspirin and pentazocine respectively. On preliminary phytochemical screening the ethanolic extract of EESI was found to contain flavonoid compounds. Flavonoids are known to target prostaglandins which are involved in the late phase of acute inflammation and pain perception [12]. Hence, the presence of flavonoids may be contributory to the analgesic activities of EESI. Further studies may reveal the exact mechanisms of action responsible for the analgesic activities of EESI.

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