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Neuropharmacological Screening of *Alangium salvifolium* (Linn.f.) Stem Bark Extract in Rats

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The neuropharmacological activities of the aqueous extract of *Alangium salvifolium* (Linn.f.) stem bark extract were screened in rat. The extract effect on pentobarbital-induced sleeping time, pentylenetetrazole induced seizure, spontaneous motor activity (SMA), exploratory behaviour and rota-rod performance (motor coordination) were evaluated. The extract (100 and 200 mg/kg p.o.) produced a significant ($p < 0.05$) prolongation of pentobarbital-induced sleeping time and reduced the spontaneous motor activity and exploratory behaviour. The extract prolonged onset of the phases of seizure activity but did not protect rats against lethality induced by pentylenetetrazole. It also failed to affect the motor coordination test. These results suggest that the extract contained an agent with neuropharmacological activity that may be sedative in nature.

Key Words: *Alangium salvifolium*, Neurophamacology, Rat.

INTRODUCTION

Alangium salvifolium (Linn.f.) Wang. (Alangiaceae) small bushy tree, often thorny, dark light coloured, distributed through out India from NW. Himalayas to Srilanka. Africa, China, Vietnam and Thailand. Flower white or cream, fragrant, 1.2-3.0 cm long, axillary fascicles from the axils of fallen leaves. Fruits subglobose, or ellipsoid, 0.8-2.0 cm long, black, succulent with bony endocarp common in waste ground, waysides and mixed forests. In Oriya it is known as Aisa, Dhala, Ankol, Ankula, in Hindi it is Akola Angol, Dhera, Dhela, in English it is Sage-leaved alangium. The roots are used as astringent, emollient, anthelmintic, thermogenic, diuretic and purgative. Root bark is an antidote for several poisons[1]. The roots are useful for external application in acute case of rheumatism, leprosy and inflammation and for external and internal application in case of bites of rabid dogs. Fruits are sweet, cooling and purgative and are useful in treating burning sensation and haemorrhages. In present studies, the effect of the aqueous extract of the plant on pentobarbital induced sleeping time, pentylenetetrazole-induced seizure, spontaneous motor activity (SMA), exploratory behaviour and rota-rod performance (motor coordination) were evaluated in rats.

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EXPERIMENTAL

N.K. Parida of the Department of Pharmacology, IGIPS, Bhubaneswar collected the plant materials in Phulbani district of Orissa State, India in November 2007 by help of the local tribal villagers. The identity of the plant was authenticated by Dr. S.P. Rath, Taxonomist, Department of Botany, Utkal University, India and a voucher specimen (number 19/07) was deposited at the herbarium unit of IGIPS. The dried material was powdered and 500 g were macerated in 2 L of cold distilled water for 24 h with occasional shaking, then filtered through Whatman number 1 filter paper and freeze-dried Using Lyovac GT2 (Germany). This gave a yield of 8.67 % w/w.

Sodium pentobarbital, pentylenetetrazole and diazepam (Sigma Chemical Co., USA) were used. Normal saline (10 mL/kg p.o.) was used as control in all the experiments.

Healthy swiss albino rats weighing 150-200 g of either sex were used in the present study. They were housed in standard conditions of temperature (25 ± 2 ºC) relative humidity of 45-55 % in plastic cages with sawdust as bedding in animal house of IGIPS. They were fed with a standard pellet diet and water *ad libitum* and all operations on animals were done under aseptic condition. The experimental protocol was subjected to the scrutiny of the Institutional Animal Ethical Committee of IGIPS and was cleared by the same before starting.

Neuropharmacological evaluation

Pentobarbital-induced sleeping time[2]: The test was carried out in a total of 24 rats grouped into four (n = 6) and treated as follows; Group 1 receive normal saline, groups 2 and 3 received the extract. (100 and 200 mg/kg p.o., respectively), while group 4 received diazepam (I mg/kg i.p.). The animals were all given sodium pentobarbital (25 mg/kg i.p.) 0.5 h after treatment. The index of hypnotic effect was recorded as follows; time elapsed between the administrations of pentobarbital until the loss of righting reflex was recorded as the onset of sleep. The time from the loss righting reflex to recovery was recorded as the sleeping time

Effect against pentylenetetrazole (PTZ)-induced seizure: This technique was used to evaluate the extract activity against pentylenetetrazole-induced seizure[3]. Rats were grouped into three (n = 6) and treated with either extract (100 and 200 mg/kg p.o.) or saline. They were all given pentylenetetrazole (70 mg/kg i.p.) 0.5 h later and observed for occurrence of seizures. Latency to first convulsion (onset of tonic), generalized seizure (tonic clonic) and lethal time were all noted. Falling and jerking were considered as beginning of seizure[4]. Animals devoid of seizures were considered protected.

Spontaneous motor activity (SMA): Three groups of rats were treated with either the extract (100 and 200 mg/kg p.o.) or saline. They were singly placed in Letica (Spain) activity cage (LE 886) consisting of four ventilated motor cages connected to a multicounter (LE 3806) 0.5 h after treatment. Activity was automatically recorded for 6 min and at an interval of 0.5 h, for a total of 2 h. Results of the treated groups were compared with those of the saline control at each time interval[5].

Hole board test for exploratory behaviour pattern: Letica (Spain) board measuring 40 cm × 40 cm with 16 evenly distributed holes and a multicounter (LE 3333) was used for the study. The method used was similar to those as described previously[6]. A total of 24 rats were used and treated with saline, extract (100 and 200 mg/kg p.o., respectively) or diazepam (1 mg/kg i.p.). Each rat was placed on the board and the instrument automatically counted the number of times they dipped their heads into the holes during the 5 min trial.

Test for motor coordination (rota-rod performance): This test was performed using Ugo Basile (Italy) No. 7600 horizontal rotation rod (Tread mill) device. The instrument was set at a rate of 16 rpm and rats that were able to remain on the rod longer than 3 min were selected and grouped into three (n = 6). Group 1 was given saline, while groups 2 and 3 received the extract (100 and 200 mg/kg p.o, respectively). They were again placed on the rod 0.5 h after treatment. The test was considered positive if a rat is unable to remain on the rod during the 3 min trial. The time it falls were recorded in seconds[7,8].

Statistical analysis: Results were expressed as mean ± SEM. Treated groups were compared with the saline control and differences were estimated by means of ANOVA followed by Dunnet's test for multiple comparison. Effects were considered significant at $p < 0.05$.

RESULTS AND DISCUSSION

The extract (100 and 200 mg/kg p.o.) prolonged the duration of pentobarbital induced sleeping time as can be seen in Table-l but did not affect the onset significantly. The effect was more pronounced among the group that received 200 mg/kg of the extract, which was in similar pattern with the group that received diazepam.

TABLE-1
EFFECT OF AQUEOUS EXTRACT OF *A. salvifolium* BARK ON PENTOBARBITAL-INDUCED SLEEPING TIME

Treatment	Onset of sleep (min)	Duration on sleep (min.)
Saline (10 mL/kg p.o.)	5.13 ± 1.02	40.43 ± 2.24
Extract (100 mg/kg p.o.)	4.53 ± 0.32	43.66 ± 1.27*
Extract (200 mg/kg p.o.)	4.11 ± 0.36	52.36 ± 3.14*
Diazepam (1 mg/kg i.p.)	3.88 ± 0.21*	61.48 ± 5.12*

Values are mean ± SEM (n = 6).
*Denotes significant difference from saline group $F[(3, 23) = 6.08; p < 0.05]$.

The extract alter the time of onset of clonic phases induced by pentylenetetrazole, but did not give any protection to rats against all the phases of seizure activity as defined by tonic clonic, hind paw extension and lethality (Table-2).

The spontaneous motor activity was gradually inhibited by the extract after 0.5 h, with a gradual time dependent inhibition that was more or less maintained throughout the 2 h duration of the experiment (Fig. 1).

TABLE-2
EFFECT OF AQUEOUS EXTRACT OF *A. salvifolium* BARK ON PENTYLENETETRAZOLE INDUCED SEIZURE IN RATS

Treatment	Time(s)			
	Onset of tonic	Tonic clonic	Tonic clonic with hind limp extension	Death
Saline (10 mL/kg)	12.34 ± 1.45	44.00 ± 3.80	79.67 ± 4.42	79.00 ± 3.62
Extract (100 mg/kg)	23.33 ± 4.41	179.67 ± 4.41*	393.33 ± 97.96*	384.00 ± 93.0
Extract (200 mg/kg)	245.33 ± 12.59*	336.67 ± 6.68*	368.33 ± 63.47*	376.68 ± 0.8

Values are mean ± SEM (n = 6).
*Significantly different from saline group F [(2,17) = 4.07; p < 0.05].

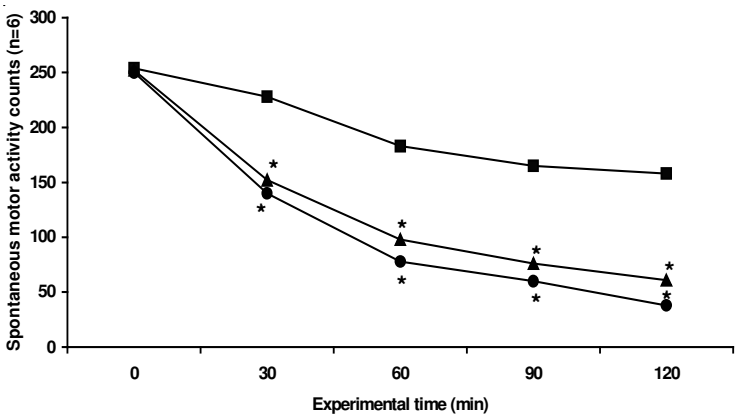

Fig. 1. Effect of aqueous extract of *A. salvifolium* on SMA in rats; (–■–)saline (10 mL/kg p.o.); (–▲–) extract (100 mg/kg p.o.); (–●–) extract (200 mg/kg p.o.), Asterisks denotes significant difference (n = 6)

The extract caused dose dependent reduction in head dip responses in the rats after the administration of the extract. The observed effect in the treated groups was significantly different ($p < 0.05$) from that of the control group (Table-3). The extract did not exhibit profound effect on motor coordination as determined by the rota-rod performance in rats (Table-4).

TABLE-3
EFFECT OF AQUEOUS EXTRACT OF *A. salvifolium* BARK ON EXPLORATORY BEHAVIOUR IN RATS

Treatment	Mean head-dips in 5 min	
	Pre-dose	0.5 h post-treatment
Saline (10 mL/kg p.o.)	75.6 ± 9.42	62.24 ± 8.01
Extract (100 mg/kg p.o.)	77.47 ± 4.12	23.72 ± 2.57*
Extract (200 mg/kg p.o.)	69.35 ± 6.19	12.32 ± 1.56*
Diazepam (1 mg/kg i.p.)	76.13 ± 3.42	2.13 ± 0.94*

Values are mean ± SEM (n = 6).
*Significantly different from saline group F [(3, 23) = 5.21; p < 0.05].

TABLE-4
EFFECT OF THE AQUEOUS EXTRACT OF *A. salvifolium* BARK ON ROTA-ROD PERFORMANCE

Time (min)	Cut-off time (s)	Endurance time (s)		
		Saline	100 mg/kg	200 mg/kg
30	180	180	181	178
60	180	180	178	174
90	180	176	176	175
120	180	172	173	169

This study is aimed at screening the CNS activity of the aqueous extract of *A. salvifolium*. The result of the investigation showed that the extract has some potent neuropharmacological activity. The activity is depressant in nature as evident by the ability of the extract to potentiate pentobarbital-induced sleep. Prolongations of barbital hypnosis were due to sedative and/or hypnotic property[8] attributed to inhibition of pentobarbital metabolism[9] or central mechanisms involved in the regulation of sleep[10]. This assertion is furthermore collaborated by the activity of the extract, which significantly reduced spontaneous motor activity. Reduction in spontaneous motor activity leads to sedation[11] as a result of reduced excitability of the CNS[12]. The potentiation of pentobarbital sleep and the decrese in spontaneous motor activity strongly suggests central depressant effect[6]. This is in conformity with the significant reduction in the exploratory behaviour. Hole-Board test is a measure of exploratory behaviour[13] and an agent that decreases this behaviour reveals sedative[14,15] and anxiolytic[16] activities. The failure of the extract in the motor coordination test is suggestive that the activity of the extract might not be exerted through peripheral neuromuscular blockage but rather, elicited centrally[6] thus acting as a neurosedative[17]. The result of this study generally tends to point towards sedative properties. The extract significantly prolonged onset of, but did not protect rats against lethality induced by pentylenetetrazol. Its inability to exhibit such potency points to fact that neither can the extract be useful in cases of increase threshold in the brain, nor was it capable of augmenting the functional role of γ-amino butyric acid[18,19]. The significant effect on the onset time and severity of seizure, however, suggests that further purification of the extract might increase its anticonvulsant property. The plant can also be further investigated to yield agents of better sedative effect having satisfied standard regulations on sedative phytomedicine[20]. On the other hand, the sedative activity observed may be attributed to the phytochemical constituents reported in the plant[21] especially the saponins. Saponins have been implicated in opioid receptor mechanism[22] through antagonistic activity[23] by binding on the sensory nerve terminals.

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