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Antidepressant Activity of Bark of *Saraca indica* Linn

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Antidepressant effects of ethanolic extract of the bark of *Saraca indica* was studied on reserpine induced hypothermia and forced swim test to assess the antidepressant activity. The study revealed that the bark extract at the dose levels of 100, 200 and 400 mg/kg showed significant antidepressant effect in a dose dependent manner.

Key Words: *Saraca indica*, Antidepressant activity, Reserpine, Imipramine, VMA.

INTRODUCTION

Saraca indica is also known as 'Ashoka' which is one of the sacred trees of the Hindus and is found plentifully along the roadside in Eastern Bengal, South India. Various parts of the plant such as bark, fruit and flowers are used both in Ayurvedic and Homeopathic system of medicine in the treatment of many diseases. *Saraca indica* is widely used both in Ayurvedic and Unani system of medicine in the treatment of dyspepsia, diseases of blood, biliousness, tumours, enlargement of abdomen, colic, piles, ulcers, bloody discharges from the uterus, menorrhagia, useful in fractures of bones, *etc*. However no specific study has been carried out so far to check the antidepressant activity of bark extract of *Saraca indica*[1].

EXPERIMENTAL

Extraction of stem bark of *Saraca indica*: Fresh bark of *Saraca indica* was collected from the local areas near to Mangalore, Karnataka. The plant of *Saraca indica* had been authenticated by botanist Mr. Vishwanath Shetty, Head of Botany Department, Mangalagangothri and Mangalore. The bark was shade dried and powdered. The powdered plant

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material of *Saraca indica* was packed in thimble and extracted with 80 % v/v ethyl alcohol in soxhlet extractor exhaustively for 20-24 h (40 cycles each batch). The extract was concentrated to dryness under reduced pressure and controlled temperature using flash evaporator.

Acute toxicity studies: Acute toxicity study was carried out in female albino rats as per staircase method[2] and OECD guidelines 425[3]. The animals tested with oral dose starting from 100 mg/kg body weight upto 2000 mg/kg body weight of ethanolic extract of bark of *Saraca indica*. The animals were continuously observed for 2-3 h for general behavioural, neurological, autonomic profile and death for a period of 24 h and for 14 d, after administration of the leaf extract. There was no mortality and no signs of toxicity were found upto 2000 mg/kg body weight and found to be safe up to 2000 mg/kg body weight.

Assessment of antidepressant activity

By forced swim test: Rats and mice, when forced to swim in a cylinder from which there is no escape will rapidly adopt a characteristic immobile posture after initial period of vigorous activity and make no further attempts to escape apart from the movement necessary to keep their heads above water. It was suggested[4] that this escape is impossible and resigns itself to the experimental conditions.

Assessment of antidepressant activity was carried out in Swiss albino mice. The animals of either sex were used and the animals were segregated into five groups each of four mice and maintained on normal pellet and water *ad libitum*.

- Group I. Vehicle (0.5 % w/v CMC in distilled water)
- Group II. Imipramine HCl (8 mg/kg body wt)
- Group III, IV and V received the different doses of ethanolic extract of *Saraca indica* 100, 200 and 400 mg/kg body wt p.o.

Alcoholic extract was suspended in 0.5 % w/v sodium CMC, suspension was administered orally in the doses of 100, 200 and 400 mg/kg body weight, once daily for five consecutive days[5]. (Fourth day dose was administered immediately after the pretest session and fifth day dose was administered 1 h prior to the test session). In the similar way, control animals were treated once daily for five consecutive days, with vehicle (p.o). In case of standard drugs imipramine HCl (8 mg/kg) were administered intraperitoneally, a single dose just 0.5 h prior to the test session.

This test was performed essentially as described by Porsolt *et al.*[6]. Groups of mice (N = 4/dose) were individually introduced into a transparent glass vessel (25 cm high, 10 cm diameter) containing fresh water upto a height of 10 cm, maintained at 25 ºC and left there for a minute. Mice were then removed and allowed to dry for 15 min in a heated enclosure (32 ºC) before being returned to homecage (pre test session). 24 h later, mice

were replaced into the vessel containing water (10 cm deep, 25 ºC) and left for 6 min. After 2 min of habituation, total duration of immobility was measured during the next 4 min (test session)[5]. Mice were judged to be immobile when they ceased struggling and remained floating motionless in the water, making only those movements necessary to keep their heads above water.

Statistical evaluation: Time of immobility in seconds was measured during 4 min and all groups were compared statistically with the control. Data were evaluated by non-parametric methods due to a non-normal distribution. Analysis was done by Dunnett's comparison test.

By reserpine induced hypothermia: This model is widely used in experimental pharmacology for the detection of the mechanism of action of antidepressant drugs. Interest in the psychotropic effects of reserpine, a depletor of central monoamine, arose from clinical observations that some patients treated with reserpine for hypertension show clear signs of clinical depression[7]. This model found to be useful in evaluating possible mechanism of drug depending on adrenergic/serotonergic/dopaminergic activity[8]. In rodents, subcutaneous administration of 2 mg/kg reserpine leads to a decrease in core-temperature after 18 h and also causes behavioural changes like ptosis and akinesia[9].

Assessment of antidepressant activity by reserpine induced hypothermia was carried out in albino rats. The rats of either sex were used and the animals were segregated into five groups each of four rats and maintained on normal pellet and water *ad libitum*.

Group I: Reserpine + Vehicle [0.35 % w/v CMC in distilled water (control)]
Group II: Reserpine (2 mg/kg s.c) + 100 mg/kg body wt. p.o
Group III:Reserpine(2 mg/kg s.c) + 200 mg/kg body wt.p.o
Group IV: Reserpine (2 mg/kg s.c) + 400 mg/kg body wt. p.o
Group V: Imipramine HCl (15 mg/kg body wt.i.p)

Alcoholic extract was suspended in 0.35 % w/v sodium CMC suspension, was administered orally in the doses of 100, 200 and 400 mg/kg body weight once daily for seven consecutive days. In the similar way, control animals were treated once daily for seven consecutive days with vehicle (p.o). In case of standard drugs, imipramine HCl (15 mg/kg) was administered intraperitoneally as a single dose.

Procedure: On the day before testing, animals were dosed with 2 mg/kg reserpine s.c, they are housed in a climate-controlled (temperature and environment) and were allowed free to access food and water. After 18 h reserpine administration, the animals were placed into individual cages. The initial rectal temperature was determined by insertion of digital thermometer (telethermometer) to a constant depth of 2 cm. Following the administration of the drugs (p.o) and imipramine HCl (i.p) the rectal temperature was measured again at 1 h intervals for 7 h.

Evaluation: Rectal temperature was recorded every hour. The difference in the temperature from "0" time was calculated for each time and the difference was scored.

Estimation of VMA in urine: This metabolite of epinephrine and norepinephrine is extracted into an organic solvent from acidified urine. It is reextracted into basic aqueous solution, oxidized by means of periodate to vanillin and read directly at 360 nm.

Procedure: Collect a 24 h specimen in a jar containing 10 to 20 mL of concentrated hydrochloric acid, measure the volume and transfer 2 or 3 mL aliquot to a glass-stoppered tube. Dilute to 5.5 mL with water. In a second tube place a volume of water equal to the urine. Add 0.5 mL of 6 N hydrocholide acid and mix well. Add 2.5 to 3 g of sodium chloride and mix until most is dissolved and only a small amount of solid remains at the bottom of the tube, add 30 mL of ethyl acetate, shake well and centrifuge if required to separate the two layers. Pipette 25 mL of the ethyl acetate (top) layer into a second glass- stoppered tube and add 1.5 mL of 1 M potassium carbonate. Shake well and again centrifuge if necessary to separate the layers. Transfer 1 mL of the aqueous (bottom) layer to a third tube, add 0.1 mL of sodium metaperiodate solution, mix well and incubate the mixture for 0.5 h at 45 to 55 ºC and cool down to room temperature. Add 0.1 mL of sodium metabisulfite solution and then adjust to a pH below 8.8 by the addition of 0.3 mL of 10 per cent acetic acid and 0.6 mL of the phosphate buffer. If required add more acetic acid.

Add 20 mL of toluene, stopper, shake well and centrifuge, if required, to separable the layers. Transfer 15 mL of the toluene layer to another stoppered tube, add 4 mL of potassium carbonate, shake well, centrifuge and transfer the aqueous layer to cuvette. Read at 360 mμ, setting the photometer to read zero with a reagent blank prepared by using water in place of the urine and carrying through the entire procedure. Prepare a standard by mixing 0.1 mL of vanillin standard with 3.9 mL of potassium carbonate solution. Read against water blank.

RESULTS AND DISCUSSION

Range of doses of alcoholic extract of *Saraca indica* (100, 200 and 400 mg) was studied in the forced swim test to measure the immobility time. Doses of imipramine are also studied for comparison. Alcoholic extract of *Saraca indica* (100, 200 and 400 mg/kg) after 5 d consecutive oral administration, immobility time in mice were reduced significantly. Alcoholic extract of *Saraca indica* (100, 200 and 400 mg/kg) after 5 d consecutive oral administration, immobility time in mice were reduced significantly.

• Dose of imipramine (8 mg/kg) after single dose i.p, administration, immobility time in mice is reduced significantly.

• Alcoholic extract of *Saraca indica* 400 mg (p.o) significantly reduced the immobility time indicating that it may have antidepressant action and the results are comparable with antidepressant like imipramine (8 mg/kg, i.p) (Table-1).

TABLE-1
COMPARISON OF DECREASED IMMOBILITY TIME BY ALCOHOLIC EXTRACT OF *Saraca indica* AND IMIPRAMINE HCl v/s CONTROL IN MICE

Group	Drug	Route (mg/kg)	Immobility time (s) Mean ± SEM
I	Control (CMC)	0.5 (p.o)	197.25 ± 1.650
II	Imipramine HCl	8 (i.p)	133.75 ± 3.986*
III	Drug ext 1	100 (p.o)	168.50 ± 4.645*
IV	Drug ext 2	200 (p.o)	166.80 ± 0.663*
V	Drug ext 3	400 (p.o)	148.25 ± 2.212*

*The mean difference is significant at the 0.01 level, when compared to the control group.

Range of doses of alcoholic extract of *Saraca indica* (100, 200, 400 mg) was studied in the reserpine induced hypothermia to measure the difference in temperature at various time intervals. Doses of Imipramine are also studied for comparison. Alcoholic extract of *S. indica* (100, 200 and 400 mg/kg), after 7 d consecutive oral administration (Table-2). There is

TABLE-2
DIFFERENCE IN RECTAL TEMPERATURE AT VARIOUS TIME INTERVALS

	1 h Mean ± SEM	2 h Mean ± SEM	3 h Mean ± SEM	4 h Mean ± SEM
Reserpine	4.800 ± 0.313	5.150 ± 0.480	5.675 ± 0.480	6.025 ± 0.466
100 mg/kg	2.630 ± 1.561	2.150 ± 0.096*	3.100 ± 0.310*	3.850 ± 0.357*
400 mg/kg	1.275 ± 0.170	2.070 ± 0.160*	2.575 ± 0.246*	2.950 ± 0.433*
200 mg/kg	1.970 ± 0.870	2.275 ± 0.250*	2.925 ± 0.193*	3.400 ± 0.349*
Imipramine	0.750 ± 0.150	1.450 ± 0.185*	1.975 ± 0.246*	2.250 ± 0.433*
	5 h Mean ± SEM	6 h Mean ± SEM	7 h Mean ± SEM	
Reserpine	6.325 ± 0.439	6.625 ± 0.390	6.700 ± 0.625	
100 mg/kg	4.775 ± 0.317	4.975 ± 0.193	4.000 ± 0.141*	
400 mg/kg	3.600 ± 0.466*	4.050 ± 0.437*	4.075 ± 0.322*	
200 mg/kg	4.225 ± 0.301	4.600 ± 0.245*	4.150 ± 0.194*	
Imipramine	2.775 ± 0.585*	3.650 ± 0.477*	3.550 ± 0.301*	

significant decrease in tempt, after the first hour with 400 mg/kg, 200 mg/kg and imipramine. There is significant decrease in tempt, after the second hour with 100, 200, 400 mg/kg drug extract and imipramine (Table-3).

TABLE-3
SHOWING URINE VMA LEVELS

	Reserpine	100 mg	200 mg	400 mg	Imipramine
	0.00028	0.000059*	0.000058*	0.000057*	0.000055
	0.00027	0.000058*	0.000057*	0.000056*	0.000054
	0.00028	0.000060*	0.000059*	0.000059*	0.000054
	0.00028	0.000059*	0.000058*	0.000058*	0.000056
Mean ±	0.00028 ±	5.9×10^{-5} ±	5.8×10^{-5} ±	5.75×10^{-5} ±	5.48×10^{-5} ±
SEM	2.5×10^{-6}	4.08×10^{-7}	4.08×10^{-7}	6.45×10^{-7}	4.78×10^{-7}

*The mean difference is significant at the 0.01 level, when compared to the control group.

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