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Pharmacological Evaluation of *Saraca indica* Leaves for Central Nervous System Depressant Activity in Mice

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Abstact:
Saraca indica is popularly known as Ashoka and one of the most legendary and sacred trees of India. *Saraca indica* is a medicinal plant widely used in Ayurveda to treat painful conditions, improves complexion of the body, improves digestion and assimilation, alleviates excessive thirst, to kills all infectious agents, in blood disease, inflammation and also as CNS depressant. In view of alleged CNS depressant potential, the present study was carried out to evaluate the central nervous system depressant activity of different extracts of *Saraca indica* leaves. *Saraca indica* leaves extracted successively with petroleum ether, chloroform, methanol and water respectively depending upon their polarity. CNS depressant activity was evaluated using pentobarbitone induced sleeping time and by determining locomotor activity using actophotometer. Methanolic extract of *Saraca indica* leaves (400 mg/kg) produced highest activity as it significantly (P<0.01) reduced the onset and prolonged of sleep duration induced by pentobarbitone, extract also decreased locomotor activity by 67.33%. Water, chloroform and petroleum ether extract also produced dose dependent CNS depressant activity. Though petroleum ether extract does not produce significant CNS depressant activity in both models. The results indicate that different extracts of *Saraca indica* leaves possesses CNS depressant activity.
Keywords: *Saraca indica, Pentobarbitone, Locomotor activity, Methanolic extract.*

Introduction:

Saraca indica L. (Family: Leguminosae) is a medium sized evergreen tree upto 9 m in height with numerous spreading and drooping glabrous branches commonly known as asoka. The bark of the plant is dark brown to grey or black; flowers are fragrant, numerous, dance and orange or red color; leaves are pinnate, 15-25 cm long having 4-6 pairs of oblong-lanceolate leaflets [1]. The plant is popularly known as Asok or Asoka (Hindi, Oriya, Bengali, Gujrati, Assamese, Marathi, Punjabi), Ashokadamara (Kannada), Asogam (Tamil), Asokam (Malayalam), Ashokapatta (Telugu). *Saraca indica* is one of the important indigenous medicinal plants and found throughout India. Bark of the plant is bitter and traditionally used as astringent, anthelmintic, demulcent, emollient, stomachic and in blood disease, biliousness, colic, piles, ulcers, fractures, menorrhagia, metropathy, dyspepsia, visceromegaly. Leaves are usefull in stomachalgia and flower are use in vitiated condition of pitta, syphilis, hyperdipsia, inflammation, dysentery, haemorrhoids and scabies in children [2–4]. Stem bark of *Saraca indica* is astringent, antileucorrhoeic, antibilious and uterine sedative; flowers are used as uterine tonic, antidiabetic and antisyphilitic traditionally. Plant is also important for CNS depressant activity as aerial part is important for its CNS active, hypothermic, CNS depressant and diuretic activity [5,6].

Chemical investigation found the presence of β-sitosterol, flavonoids, flavone glycosides, anthocyanins, fixed oil in flower; bark contain different catechols, sterols, tannins, flavonoids, glycosides, leucopelargonidin and leucocyanidin. Seed and pod contains oleic, linoleic, palmitic and

stearic acids, catechol, epicatechol and leucocyanidin; leaves and stem found to contain quercetin, quercetin-3-O-α-L-rhamnoside, kaempferol 3-O- α-L-rhamnoside, amyrin, ceryl alcohol and β-sitosterol [7–9]. The antidiabetic, oxytocic, anticancer, peptic ulcer, antimicrobial, antibacterial and antioxidant activities of the plant have been reported [10–16].

Advance in modern science and technology has contributed to an enormous development in the quality of human life. Though, stress in modern life responsible for the surge in incidence of variety of psychiatric disorders. Drugs currently used in treatment of different neuropsychiatric and neurological disorders like anxiety, depression, schizophrenia, epilepsy, parkinsonism either refractory or have serious side effects or posses unfavorable drug-drug/drug-food interactions. Psychoneural drugs like benzodiazepines commonly employed in anxiety, depression, epilepsy and insomnia but possess side effects like cognitive function, physical dependence and tolerance[17,18]. Plants are used as medicine since time immemorial. Drugs from plant sources are being used by about 80% of the world population. Herbal medicines have stood the test of time for their safety, efficacy, acceptability and lesser side effects [19, 20].

Taking into account these findings and in view of alleged CNS depressant activity of the *Saraca indica*, it was decided to evaluate the CNS depressant activity of *Saraca indica* leaves using various experimental models.

Meterials and Methods:

Plant material

The leaves of *Saraca indica* L. were collected from Gonda region of Uttar Pradesh, India in the month of August, 2009. Kamala Nehru Krishi Vigyan Kentra, Sultanpur, Uttar Pradesh authenticated the botanical identity of the plant. The herbarium was prepared and a voucher specimen (Sample No 01, Ref no KVK/Gen/2009-10/3012) was deposited to the Department of Pharmacognosy, AND College of Pharmacy, Gonda, Uttar Pradesh, India.

Animals

Albino mice (18-25 g) of either sex were used in these experiments. Animals were provided with standard food and water *ad libitum* and were maintained at a temperature of 25±2°C, humidity of 55±5% and with 12 h light - dark cycle. All animal procedures have been approved and prior permission from the Institutional Animal Ethical Committee was obtained as per the prescribed guidelines.

Preparation of extracts

The leaves of *Saraca indica* were washed thoroughly and dried under shade and then made into a coarse powder using dry grinder. The powder leaves was passed through sieve no. 40 and stored in an air tight container at 25°C, used for further study. Powdered plant material (1.2 kg) were successively extracted using Soxhlet apparatus using the solvents in order of increasing polarity viz., petroleum ether (60-80°C), chloroform, methanol and water. Each time the marc was dried and later extracted with other solvents. All the extract were concentrated by distilling the solvent in a rotary vacuum evaporator and evaporated to dryness. The yield was found to be 7.99, 1.46, 12.15 and 12.90% w/w respectively with reference to the dried plant material.

Preliminary phytochemical investigation

The individual leaf extracts like petroleum ether (PSI), chloroform (CSI), methanol (MSI) and water (WSI) were subjected to qualitative chemical investigation for the identification of different phytoconstituents like sterols, glycosides, saponins, carbohydrates, alkaloids, flavonoids, tannins, proteins, triterpenoids. Phytochemical screening of the extracts was

performed using the standard procedures [21, 22].

Acute toxicity test

Acute oral toxicity was performed as per OECD-423 guidelines [23]. Mice's were fasted overnight with free excess of water. Chloroform, methanol and water extracts were administered to the different groups orally at the dose level of 5 mg/kg body weight and mortality was observed for 14 days. If mortality was not observed for any animal then the procedure was repeated again with higher doses such as 50, 300 and 2000 mg/kg. The animals were observed for toxic symptoms such as behavioral changes, locomotion, convulsions and mortality for 72 hours.

Determination of pentobarbitone induces sleeping time

CNS depressant activity was performed as describe by Sivaraman and Muralidaran [24]. In this method, mice of either sex were randomly taken and divided into control, standard and different test groups, each group contain six animals. Group I served as control and treated with normal saline (10 ml/kg, i.p.), group II (standard) treated with standard drug chlorpromazine hydrochloride (1mg/kg, i.m.) 15 min before the administration of pentobarbitone (40mg/kg, i.p.). Test groups III-VIII were treated with CSI (200 and 400 mg/kg), MSI (200 and 400 mg/kg) and WSI (200 and 400 mg/kg) respectively. Pentobarbitone (40mg/kg, i.p.) was administered 30 min later. Onset of sleep and duration of sleep measured for all the group. Onset of action was recorded by noting the time of loss of reflex for three consecutive trials, duration of sleep recorded by time difference between loss of righting reflex and recovery time.

Locomotor activity using actophotometer

The CNS depressant activity of the various extracts of *Saraca indica* was evaluated by studying locomotor activity of mice using actophotometer [25]. Briefly, Albino mice of either sex (20 - 25 g) were randomly divided into eight groups of six animals. The mice were placed individually inside the chamber of actophotometer for 10 min and basal activity score was noted. Group I was treated with vehicle (0.5% sod. CMC) and standard drug chlorpromazine (3 mg/kg, i.p.) administered to group II. The animals of the group III-VIII were treated with CESI (200 and 400 mg/kg), MESI (200 and 400 mg/kg) and WESI (200 and 400 mg/kg) respectively and after 30 min of mice are placed again in actophotometer for 10 min and the activity was monitored. Percent decrease in activities were calculated for each group using the formula,

Percent decrease in activity=(1-Wa/Wb)X100, where Wa and Wb are average activity scores after and before drug administration respectively and average decrease in activity was calculated for all groups.

Statistical analysis

The results have been expressed as mean ± standard error mean (S.E.M) and analyzed using statistical package for social science (SPSS) version 10.0 using ANOVA followed by Dunnett's test.

Results:

Results of phytochemical screening

Preliminary phytochemical screening of methanolic, water, chloroform and petroleum ether was investigated. Primarily methanolic extract showed the presence of tannins, triterpenoids, saponin, flavonoids and glycosides; aqueous extract contain alkaloids, saponin, tannins, flavonoids and glycosides; chloroform extract contains alkaloids, glycosides, tannin and steroids; petroleum ether contain glycosides, steroids and triterpenoids.

Acute toxicity studies

Different extracts like PSI, CSI, MSI and WSI administered separately up to 2000 mg/kg body weight, none of the extracts produced any toxic symptoms of mortality,

Table 1: Effect of *Saraca indica* leaf extract on pentobarbitone induced sleeping time

Treatment	Onset of action (min)	Duration of action (min)
Control (Normal saline)	8.81±0.91	34.34±3.15
Chlorpromazine (3 mg/kg i.p.)	3.10±0.65**	54.99±0.62**
PSI (200 mg/kg, p.o)	8.59±0.88	35.44±2.59
PSI (400 mg/kg, p.o)	7.33±0.90	37.04±3.77*
CSI (200 mg/kg, p.o)	7.81±0.83	38.44±3.50
CSI (400 mg/kg, p.o)	5.32±0.71*	44.74±4.87*
MSI (200 mg/kg, p.o)	5.85±0.44*	43.80±5.21*
MSI (400 mg/kg, p.o)	4.01±0.59**	50.09±4.22**
WSI (200 mg/kg, p.o)	6.10±0.65*	41.81±3.08*
WSI (400 mg/kg, p.o)	4.25±0.40**	48.91±5.05**

All values are mean ± SEM; Statistical analysis by one-way ANOVA followed by Dunnet's multiple comparison test; *$P < 0.05$ **$P < 0.01$; N =6).

Table 2: Effect of *Saraca indica* leaf extracts on locomotor activity, accessed using actophotometer

	Locomotor activity for 10 min.	
Treatment	Before treatment	After treatment
Control (Normal saline)	426.11±42.90	401.94±39.25
Chlorpromazine (3 mg/kg i.p.)	453.77±40.36	129.91±19.06**
PSI (200 mg/kg, p.o)	403.23±34.08	330.78±31.56
PSI (400 mg/kg, p.o)	387.91±42.49	276.32±27.01
CSI (200 mg/kg, p.o)	398.32±32.89	266.45±15.77*
CSI (400 mg/kg, p.o)	446.91±48.12	201.59±20.42**
MSI (200 mg/kg, p.o)	459.05±41.47	247.21±25.71*
MSI (400 mg/kg, p.o)	384.81±32.09	125.73±21.66**
WSI (200 mg/kg, p.o)	429.91±30.08	248.65±22.34*
WSI (400 mg/kg, p.o)	441.20±39.14	165.43±18.62**

All values are mean ± SEM; Statistical analysis by one-way ANOVA followed by Dunnet's multiple comparison test; *$P < 0.05$ **$P < 0.01$; N =6.

hence the drugs were considered safe for further pharmacological screening. So, according to the OECD-423 guidelines for acute oral toxicity, the LD_{50} dose of 2000 mg/kg and above is categorized as unclassified.

Results of pentobarbitone induced sleeping time

The results showed that *Saraca indica* leaf possess CNS depressant activity. Table 1 shows the effect of different extracts of *Saraca indica* leaf. MSI (200 and 400 mg/kg), WSI (200 and 400 mg/kg), CSI (400 mg/kg) produced significant reduction in the onset and prolongation of sleep duration induced by pentobarbitone. Methanolic extract (400 mg/kg) showed most potent effect ($P<0.01$) as it followed by the effect of water and chloroform extracts. Effects of MSI and WSI at a dose of 400 mg/kg are comparable with the effect produced by standard drug chlorpromazine. But CSI (200 mg/kg) and PSI (200 and 400 mg/kg) did not produce significant decrease in onset of action and increase in duration of action.

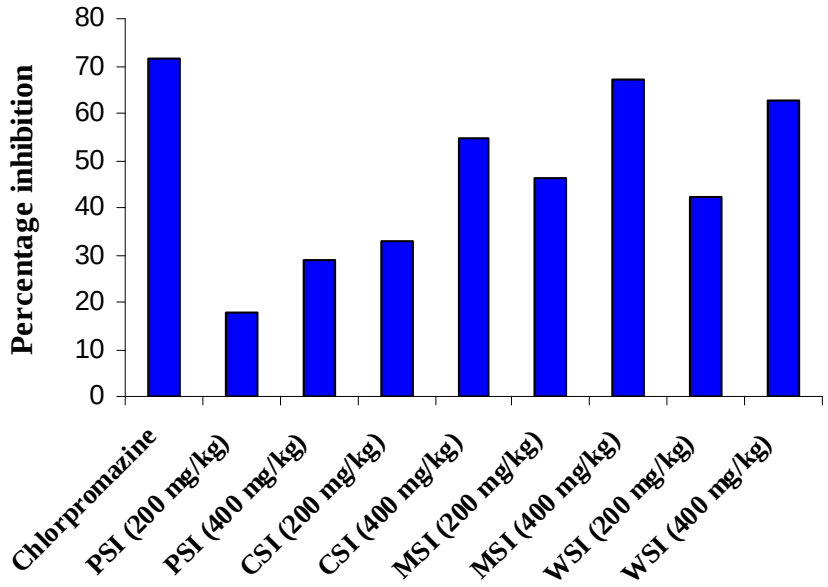

Figure 1: Percentage inhibition of locomotor activity by different extracts of Saraca *indica* *l*eaves

Results of locomotor activity by actophotometer

Results of locomotor activity were tabulated in Table 2. Extracts of *Saraca indica* significantly decreased the locomotor activity in mice. The activity was found to be maximum for methanolic extract at a dose of 400 mg/kg, PSI (200 mg/kg) did not produced significant reduction in locomotor activity. MSI (200 and 400 mg/kg), WSI (200 and 400 mg/kg), CSI (200 and 400 mg/kg) and PSI (200 and 400 mg/kg) produced 67.33, 46.15, 62.50, 42.16, 54.88, 33.10, 31.09, 17.96% decreased in locomotor activity (Fig. 1), where standard drug chlorpromazine produced71.37% decreased in activity.

Discussion and Conclusion:

This study has established the central nervous system depressant properties of *Saraca indica* leaf. The study demonstrated that different extracts of *Saraca indica* leaves caused an earlier onset of the effect of phenobarbitone (sleep latency) when compared with the control and it also increased the duration of action of pentobarbitone (sleeping time) significantly (P<0.01). Locomotor activity considered as an increase in alertness and decrease in locomotor activity indicated sedative effect [26]. Extracts of *Saraca indica* leaf decreased locomotor activity indicates its CNS depressant activity.

Gamma-aminobutyric acid (GABA) is the major inhibitory neurotransmitter in the central nervous system. Different anxiolytic, muscle relaxant, sedative-hypnotic drugs are elucidation their action through $GABA_A$, therefore it is possible that extracts of *Saraca indica* may acts by potentiating GABAergic inhibition in the CNS via membrane hyperpolarization which leads to a decrease in the firing rate of critical neurons in the brain or may be due to direct activation of GABA receptor by the extracts [27].

Many research showed that plant containing flavonoids, saponins and tannins are useful in many CNS disorders [28]. Earlier investigation on phytoconstituents and

plants suggests that many flavonoids and neuroactive steroids were found to be ligands for the $GABA_A$ receptors in the central nervous system; which led to the assume that they can act as benzodiazepine-like molecules [29]. Phytochemical investigations also showed the presence of alkaloids, flavonoids, saponins and tannins in the extract, so might be this phytoconstituents are responsible for its CNS depressant activity.

Therefore, this plant merits further attention. Search on the most active principle as well as elucidation of the exact mechanism of its action is needed. Thus, we conclude that *Saraca indica* leaf possess CNS depressant activity and successive studies are mandatory to establish the precise nature of active constituents as well as their mechanism of action.

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