Anxiolytic and Antiseizure Effects of *Sida tiagii* Bhandri

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The central nervous system (CNS) pharmacological effects of ethylacetate extract (EAS) of *Sida tiagii* Bhandri collected from northern province (Rajasthan) of India were assessed by elevated plus-maze, pentobarbitone induced sleeping time, spontaneous motor activity, pentylenetetrazole-induced seizure, forced swim test and rotarod tests. The results of the present study demonstrated the CNS depressant potential, *i.e.*, anxiolytic, antiseizure, reduction in spontaneous locomotion and potentiation of pentobarbital-induced hypnosis of the plant under investigation.

Key words —— *Sida tigaii* Bhandari, ethylacetate extract, central nervous system depressant

INTRODUCTION

Sida tiagii Bhandari (*Sida pakistanica* B.; Family- Malvaceae), a native species of the Indian and Pakistan desert area, popularly known as "Kharinti" in India; is used in the folk medicine as blood purifier, tonic and muscle strengthener.[1)] The other species from *Sida* genus like *Sida cordifolia* (*S. cordifolia*), *Sida acuta*, *Sida rhombhofolia* and *Sida spinosa* are traditionally used as/in febrifuge, abortifacient, diuretic, dysentery, vomiting, gastric disorders, asthma, fever, pain, ulcer, skin disease, diarrhea during pregnancy, rheumatism, neurological disorder and anti-worm medication.[2)] These species have been known to possess anti-inflammatory, analgesic,[3,4)] antimalarial,[5)] antiplasmodial,[6)] antibacteria,[7)] antidiabetic,[8)] antihypertensive,[9)] diuretic and tonic properties.[10)] Various extracts of roots and leafs of these species was reported pharmacologically but still no work is reported on the fruit. The aim of the present study was to evaluate the effects of *Sida tiagii* (*S. tiagii*) fruit extract in psychopharmacological animal models.

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MATERIALS AND METHODS

Plant Material and Preparation of Extract —— *S. tiagii* was collected from the local field of Rajasthan (India) and identified by Dr. H. B. Singh, Head, Raw Materials Herbarium and Museum, National Institute of Science Communication and Information Resources (NISCAIR), India; specimen was deposited to Herbarium, Department of Pharmaceutical Sciences, Guru Jambheshwar University of Science and Technology, Hisar (Voucher number PHARM/25/2007). The fruits were dried at $40 \pm 1°C$, grounded into a granulated powder and defatted with petroleum ether. The ethanolic extract was obtained by extracting 4 kg of defatted seed powder with ethanol (95%) at 50°C for 72 hr in soxhelet apparatus followed by filtration and concentrated in rotaevaporator at $50 \pm 5°C$ to its one third volume. The filtrate was partitioned with *n*-hexane (*n*-Hexane Extract) and ethylacetate (Ethylacetate Extract; EAS) and the respective layers were separated out and dried on water bath at 30°C till dryness (*n*-Hexane Extract 32.23 gm, EAS, 26.68 gm). The residual ethanolic fraction (Residual Ethanolic Extract) was dried on water bath separately (104.10 gm) and the extracts were stored at temperature below 10°C. In the present study we hereby explored the antidepressants potential of different extracts of fruits of *S. tiagii*. The extracts were freshly prepared with 2% Tween 80 for pharmacological experiments.

Phytochemical Analysis —— Freshly prepared organic extracts were tested for the presence of alkaloids, steroids, triterpenoids, glycosides, tannins, flavonoids, carbohydrates and cardiac glycosides using standard procedures.[11)]

Animals —— Male Swiss mice (weighing 25–35 g, 90 days old) obtained from Disease Free Small Animal House, Chaudhary Charan Singh Haryana Agriculture University (CCSHAU), Hisar, India, were maintained at controlled room temperature ($21 \pm 2°C$) on a 12 hr light/dark cycle with free access to food and water. All experiments were conducted between 8:00 and 13:00. Procedures were approved by the Institutional Animal Ethical Committee, GJUS&T, Hisar, India.

Drugs and Chemicals —— Sodium pentobarbita (Hi-media), diazepam (Hi-media), tween 80 (Hi-media), imipramine (Ranbaxy, New Delhi, India), fluoxetine (Ranbaxy). All drugs were administered in a volume of 1ml/100 g body weight (mice). 2% Tween 80 solution was used as a vehicle.

Spontaneous Locomotion Test —— This experimental model was described by Carlini to evaluate the interference of a substance in the motor activity of the animals.[12)] Groups of 10 mice were treated with EAS of *S. tiagii* with dose of 500 mg/kg (i. p.), 500 mg/kg (p. o.), or vehicle. The animals were placed in the activity cage (with a square area of 48 cm, 30 cm in height and demarcation squares of 12 cm × 12 cm). After 30, 60 and 120 min of treatment, the number of squares invaded within a period of 3 min were counted.[13)] The invasion criterion adopted was the presence of all paws of the animal within the square.[14)]

Forced Swim Test —— This test is the most widely used and recognized pharmacological model for assessing antidepressant activity.[15)] The development of immobility when mice are placed in an inescapable cylinder filled with water reflects the cessation of persistent escape-directed behavior. The apparatus consisted of a clear plexiglass cylinder (20 cm high × 12 cm diameter) filled to 15 cm depth with water ($24 \pm 1°C$). The mice were treated with different doses of extract (100, 200 and 500 mg/kg, p. o.; $n = 6$), imipramine and fluoxetine (positive control, $n = 6$) and 2% Tween 80 (control group, $n = 6$). In the pre-test session, every animal was placed individually into the cylinder for 15 min, 24 hr prior to the 5 min swimming test. During the test session (30 minute after treatment) the following behavioral responses were recorded by a trained observer: climbing behavior, which is defined as an upward directed movements of the forepaws along the side of the swimming chamber; swimming behavior, defined as movement throughout the swimming chamber, which includes crossing into another quadrant; and immobility time, when the mice made no further attempts to escape, and makes only movements to keep its head above the water.

Elevated Plus-maze Test —— The elevated plus-maze test has been widely validated for measuring anxiolytic and anxiogenic-like activities in rodents.[16)] This apparatus was made of plexiglass and consisted of two open arms (30 cm × 5 cm) and two closed arms (30 cm × 5 cm) with 25 cm walls. The arms extended from a central platform (5 cm × 5 cm). The maze was elevated 38.5 cm from the room's floor. The mice were treated, 30 min before the test, with different doses of EAS of *S. tiagii* (100, 200 and 500 mg/kg, p. o.; $n = 8$), diazepam (positive control, 5 mg/kg; i. p.; $n = 8$) and 2% tween 80 (control group, p. o.; $n = 8$). Each animal was placed at the center of the maze, facing one of the enclosed arms. The number of entries and the time spent in enclosed and open arms (entry in open arm; EOA, time spent in open arm; TOA) were recorded for 5 min. Entry into an arm was defined as the animal placing all four paws onto the arm. Total exploratory activity (number of entries) and other ethologically derived measures (grooming, rearing, stretched attend postures and head dipping) were also registered.

Rotarod Test —— This method was described by Dunham and Miya.[17)] Mice were placed on a rotating rod (2.5 cm diameter rotating at 5 rpm) for a pre-selection and those able to remain on the rod for 3 or more min in two successive trials were selected for testing.[18)] After 24 hr of pre-selection, groups of 6 mice were treated with EAS of *S. tiagii* at dose of 500 mg/kg (p. o.), 500 mg/kg (i. p.) or vehicle. After 30, 60 and 120 min of treatments the animals were placed on a rotative bar of the rotarod apparatus for 5 min and the time spent by each animal on the rotarod was recorded.[18,19)]

Pentylenetetrazole-induced Seizures —— Pentylenetetrazole (75.0 mg/kg) was injected i. p., 30 min after administration of EAS of *S. tiagii*. The mice were treated with different doses of extract (50, 100, 200 and 500 mg/kg, p. o.; $n = 6$). The control group ($n = 6$) was treated with a TW solution and positive control group ($n = 6$) was administrated with Diazepam 1.0 mg/kg i. p. in the same conditions. After pentylenetetrazole injection mice were placed separately into transparent plexiglass cages

(25 cm × 15 cm × 10 cm) and observed for 30 min for the occurrence of seizures. The time taken before the onset of clonic convulsions and the percentage of mortality protection were recorded.[20)]

Pentobarbital-induced Hypnosis —— Sodium pentobarbital (a sub-hypnotic dose, 30.0 mg/kg) was injected i. p. 30 min after administration of EAS of *S. tiagii*. The mice were treated with different doses of extract (100, 200 and 500 mg/kg, p. o.; $n = 6$), the control group ($n = 6$) was treated with 2% tween water solution and positive control group ($n = 6$) was administrated with Diazepam 1.0 mg/kg in the same conditions. The effect was recorded for disappearance (latency) and reappearance (duration) of the righting reflex. Hypnotic sleeping time was considered to be the time interval between disappearance an reappearance of the righting reflex.[20)]

Statistical Analysis —— Values reported are mean ± Standard error of mean (SEM) (number of mice). Differences between group mean were assessed by a one-way analysis of variance (ANOVA), followed by Dunnett's test to assess the significance of the difference between individual group. p value higher then 0.05 was considered insignificant.

RESULTS

Acute Toxicity

The EAS of *S. tiagii* was found to be toxic at high doses when administered intraperitonially. The LD_{50} values were 2100 mg/kg with 95% confidence limits of 1500–2500 mg/kg for i. p. administration. Deaths were not observed among orally treated animals.

Effect of Extract on Spontaneous Motor Activity in Mice

The mice treated with extract at a dose of 500 mg/kg (i. p.) caused a significant reduction ($p < 0.01$) of the spontaneous locomotor activity in comparison with the control group at 30 and 60 min whereas the animals treated with 500 mg/kg (p. o.) showed a decrease ($p < 0.05$) of ambulation at 60 min (Fig. 1).

Forced Swimming Test

Mice treated with extract 100 and 500 mg/kg (p. o.) showed no significant effect ($p > 0.05$) on immobility period while extract 200 mg/kg (p. o.) increased immobility period ($p < 0.05$) compared to control and showed depressant effect. On the other hand fluoxetine and imipramine significantly ($p < 0.01$) reduced immobility period (Fig. 2).

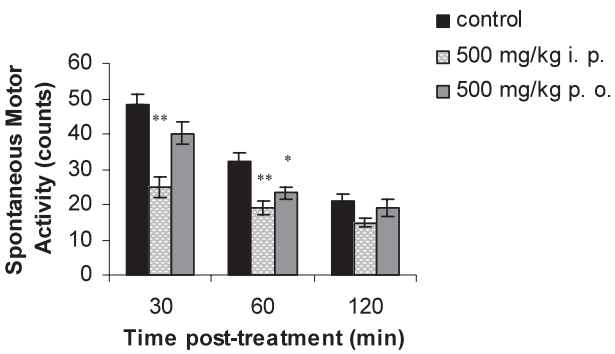

Fig. 1. Effect of Extract on Spontaneous Locomotion in Mice

The values represent mean ± SEM ($n = 10$); $^{*}p < 0.05$, $^{**}p < 0.01$ significantly different from control.

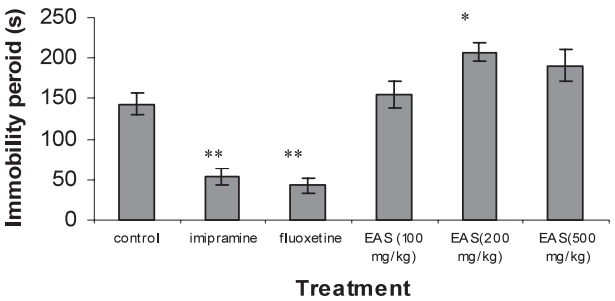

Fig. 2. Effect of Extract on Immobility Period

The values represent mean ± SEM ($n = 6$); imipramine (15 mg/kg; p. o.); fluoxetine (20 mg/kg; p. o.). (One-way ANOVA followed by Dunnett's test); $^{*}p < 0.05$, $^{**}p < 0.01$ significantly different from control.

Elevated Plus-maze Test

Diazepam significantly increased ($p < 0.01$) the number of entries and the time spent by mice in the open arms, as well as the percentage of these same parameters. The administration of 100 and 200 mg/kg (p. o.) of extract increased significantly ($p < 0.01$) the percentage of number of entries and the percentage of time spent on the open arms whereas 500 mg/kg (p. o.) showed no significant effect as compared to the control group. Lower doses showed the higher effect (Fig. 3).

Effect on Rotarod Test

The EAS of *S. tiagii* at a dose of 500 mg/kg (p. o. and i. p.) did not cause a significant difference in the motor coordination of the treated animals in comparison with the control group (Fig. 4).

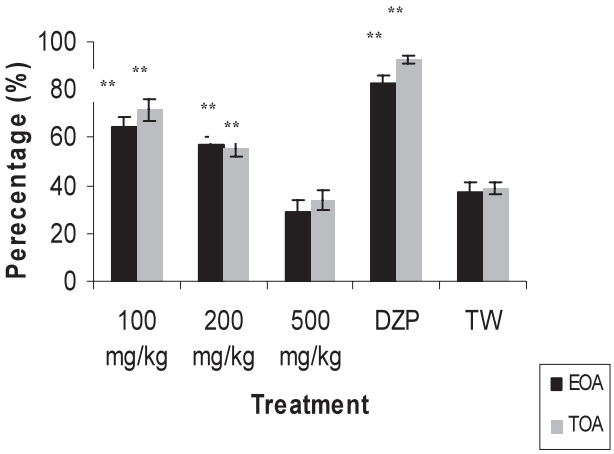

Fig. 3. Effect Produced by Different Doses (100, 200 and 500 mg/kg, p. o.) of Extract on Percentage of Entries and Time Spent by Mice in Open Arms on the Elevated Plus-maze Test

The results are presented as mean ± SEM (**) $p < 0.01$ as compared with the control group. (One-way ANOVA followed by Dunnett's test); $n = 8$ mice per group. EOA = entry in open arm, TOA = time spent in open arm, DZP = diazepam (1 mg/k i. p.); TW = 2% Tween 80.

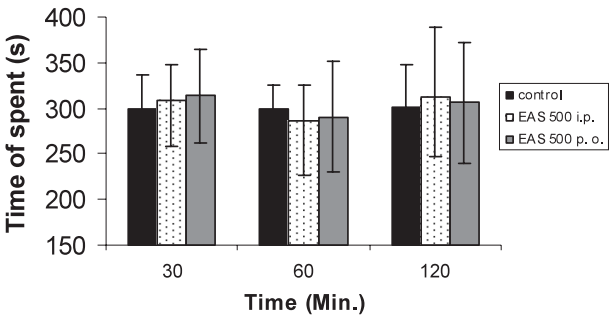

Fig. 4. Effect of EAS on the Motor Coordination

The values represent mean ± SEM; ($n = 6$).

Pentylenetetrazole-induced Seizure

The animals treated with diazepam (1.0 mg/kg) did not show any convulsion after pentylenetetrazole injection ($p < 0.001$). The oral administration of 50 and 100 mg/kg of extract induced 100% protection and significantly ($p < 0.01$) increased onset of seizure whereas 200 mg/kg dose induced 85.3% protection and significantly ($p < 0.05$) increased the onset of seizure compare to control. Where as dose 500 mg/kg induced a protection of 69.13%, but it did not change the onset of seizures as compared to control (Fig. 5).

Pentobarbital-induced Hypnosis

The absolute values of sleep latency and sleeping time showed in Fig. 6 demonstrated that animals treated with EAS of *S. tiagii* (100, 200 and 500 mg/kg; p. o.) or diazepam (1 mg/kg; i. p.), 30 min before injection of pentobarbital, presented a significant decrease in the sleep latency and prolongation of pentobarbital-induced sleeping time. Re-

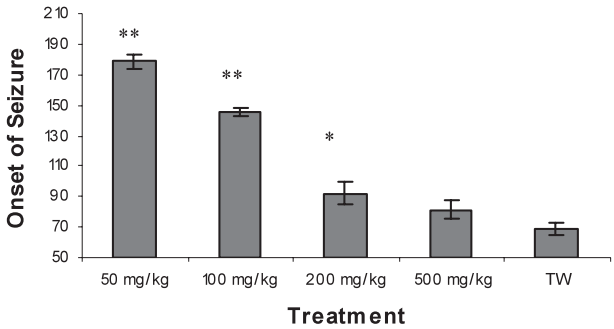

Fig. 5. Values are Mean ± SEM for the Onset of Seizure, $n = 8$; ** $p < 0.01$, Compared to Control Using ANOVA and Dunnett's Test. TW = Tween 80.

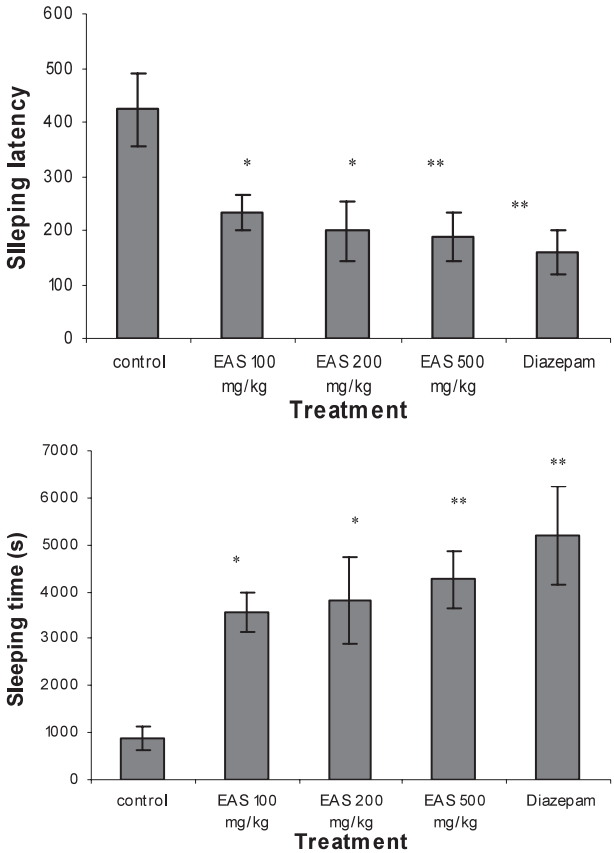

Fig. 6. Effect Produced by Different Doses (100, 200 and 500 mg/kg, p. o.) of Extract on the Latency and duration of hypnosis Induced by Sodium Pentobarbital (30 mg/kg, Sub-Hypnotic Dose)

The results are presented as mean ± SEM; * $p < 0.05$; (**) $p < 0.01$ as compared with the control group. (One-way ANOVA followed by Dunnett's test); $n = 6$ mice per group. Diazepam (1 mg/kg i. p.).

sults were showed a dose dependent potentions of pentobarbital-induced hypnosis.

DISCUSSION

In the present study we evaluated the central effects of the EAS of *S. tiagii* fruits in several behavioral animal models viz. spontaneous locomotion, forced swim, elevated plus-maze, pentylenetetrazole-induced seizure, pentobarbital-induced hypnosis and rotarod test. These are classical animal models for preliminary pharmacological activities on central nervous system (CNS), which provide information about psychomotor performance and motor behavior. Phytochemical screening for active constituent(s) of the extract indicated the presence of several classes of compounds like triterpinoids, steroids, glycosides, tannins and flavanoids. However, more work is needed to determine the fractions or the compounds that are responsible for the biological actions observed.

In preliminary experiments, the toxicity of the extract was tested, and judging from the high doses (5 g/kg p. o.) that were tolerated without significant mortality or overt signs of toxicity, it seemed that the plant extract is relatively less toxic. The high doses of the extract used in the present pharmacological experiments demonstrated the presence of low concentration of active compound in it.

In pharmacological behavioral screening, the animals treated with EAS of *S. tiagii* showed decrease of response to the touch and reduction of motor activity. These data are indicative of depressive activity of the CNS.[21] The general depressive activity was confirmed in the spontaneous locomotion test where extract significantly reduced spontaneous motor activity. The decrease in motor activity gives an indication of the level of excitability of the CNS.[21] The decrease may be related to sedation resulting from depression of CNS not to the muscle relaxant activity as the time taken by the animal to fall from the rotarod dosen't decreased appreciably.[22,23] EAS of *S. tiagii* caused a decrease in spontaneous motor activity at 500 mg/kg, i. p. and it may be the behavioral toxic dose. The decrease in spontaneous motar activity observed in case of EAS of *S. tiagii* is similar to the earlier reports.[24,25] The forced swim test has been validated as a suitable tool to evaluate drugs with putative antidepressant effect.[26] When rodents are forced to swim in a confined space, they tends to become immobile after vigorous activity (struggling). This inescapable stressful situation can be evaluated by assessing different behavioral strategies and immobility during the test could be an efficient adaptive response to this stress.[26,27] EAS of *S. tiagii* (200 mg/kg; p. o.) significantly increased the immobility period and showed CNS depressant activity. The increase in immobility period by extract may be attributed to non-flavanoid constituents of the extract as flavanoids are responsible for decrease in immobility period.[28] Further the aforementioned results confirmed, the absence of alkaloid content in as it is responsible for the increase in immobility period.[29] EAS of *S. tiagii* also elicited anxiolytic-like effects when evaluated in the elevated plus-maze. Anxiety, a symptom accompanying various central nervous system disorders and a disorder by itself, is characterized in humans by a tense and exhaustive physical alertness.[30] Rodents demonstrate anxiety, fear and curiosity when placed in a new environment, and an overall assessment of behavior could be determined through the observation of freezing, grooming (fear), rearing, head-dips (curiosity) and the number of fecal boluses.

The elevated plus-maze has been frequently used to detect and evaluate anxiolytic/anxiogenic properties of drugs.[31,32] The frequency and time spent in the open arms is the major index of the anxiety in the plus-maze model, given the fact that an open area is extremely aversive to rodents.[33] EAS of *S. tiagii* (100 and 200 mg/kg) increased the percentage of open arms entries and the time spent in those arms. In elevated plus-maze as well in case of forced swim test EAS at a dose of 200 mg/kg p. o. produced significant difference from the control which supports the CNS depressant activity of *S. tiagii*. So it may not be a behavioural toxic dose. At a dose of 500 mg/kg p. o. in both elevated plus-maze and forced swim test, wehave not observed significant CNS depressant activity. So it may be possible that the behavioural toxic dose may lies in between 200–500 mg/kg of oral dose. The potentiation of pentobarbital sleep and decrease in spontaneous motor activity strongly indicated the central depressant effect (Perez et al., 1998) of extract. The pentylenetetrazole test represents a valid model for human generalized myoclonic seizures.[34] Pentylenetetrazole induces seizures in rodents by blocking the Cl^- channel of Gamma amino butyric acid (subtype A) ($GABA_A$) receptors. It has been reported that GABAergic neurotransmission plays an important role in stress, anx-

iety,[35] pain[36] and epilepsy.[37] The non significant results observed at higher doses may be attributed to the fact that the concentration of constituents in the plant with such biological activities could be attributed to their activity with antagonist compounds whose concentrations increase with doses. Also those substances with possible toxic effects may be increasing when the dose of EAS of *S. tiagii* is higher.[38] Our results indicated that EAS of *S. tiagii* may act on the benzodiazepine site of the GABA receptor in the mouse's brain. Thus, the anxiolytic, hypnotic effects of EAS of *S. tiagii* may be caused by its combined action on several neurotransmitter receptor systems, including $GABA_A$ receptors. It is important to note that this is the first report on CNS depressant activity of *S. tiagii.* But similar CNS depressant activity was observed with plant of same genous *S. cordifolia* leaf extract[39] which supports the present results.

In conclusion the EAS of *S. tiagii* possess sedative, depressant, anxiolytic and anti-seizure activity. The sedative action may be due to CNS depression. The anxiolytic and anti-seizure properties may be through modulation of GABAergic transmission. The importance of identifying CNS depressing agents of plants orign has recently been highlighted.[13] Further studies are needed to determine the exact mechanism(s) of action of various compounds in the extract.

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