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Research Article

EFFECT OF *STREBLUS ASPER* LEAVES ON LOCOMOTION, ANXIETY AND COGNITION IN RATS

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ABSTRACT

Objective: The current study deals with the evaluation of neuropharmacological activities of hydroalcoholic extract of the plant *Streblus asper.*

Methods: Hydroalcoholic extract of *S. asper* leaves was administered to animals at the dose of 200 and 400 mg/kg p.o., respectively. The neuropharmacological activities, namely, anxiolytic, muscle-relaxant, nootropic, and locomotor activities of hydroalcoholic extract of *S. asper* leaves were evaluated. The antioxidant activity of the hydroalcoholic extract of *S. asper* leaves was also investigated.

Results: The dose 400 mg/kg p.o. of hydroalcoholic extract indicated significant variation with control group on neuropharmacological activity, especially nootropic and locomotion, whereas the mentioned dose did not show a significant effect on anxiolytic and muscle-relaxant activities. Percentage scavenging activities and inhibition concentration (IC_{50}) were reported as 63.132 at 100 µg/ml and 35.33, respectively.

Conclusion: It was found that hydroalcoholic extract of *S. asper* leaves can treat central nervous system disorders caused by oxidative stress.

Keywords: Neuropharmacological, Antioxidant, *Streblus asper*, Nootropic activity, Locomotion

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INTRODUCTION

In today's life of stress and strain, there is a direct need of agent having neuroprotective and neuropharmacological activity which can protect learning, memory, and behavior performance of the brain [1]. Stress involves complex immunological, neural, and biochemical mechanisms and plays a vital role in the genesis and progression of different kinds of diseased states [2]. Importantly, stress also affects cognitive functions leading to a delay in the progress of development and also affects the memory engram rather than the learning skill. Therapeutic use of psychoactive drugs was increasingly recognized as most effective in the treatment of stress, anxiety, and psychosomatic disorders during the past two decades, but these drugs have various adverse effects. Thus, herbal drugs primarily relieve the symptoms and give a palliative relief of a temporary nature.

Streblus asper is a gnarled tree known by several common names mostly *Sihor, Siamese* rough bush. It is geographically present in the drier portion of India, Malaysia, Thailand, and Sri Lanka [3]. *S. asper* contains myricetin, naringenin, kaempferol, quercetin, and ginkgetin flavonoids [4].

Flavonoids are a large group of plant secondary metabolites that share a basic phenyl benzopyrone feature and are found in all vascular plants where they occur in several structurally and biosynthetically related classes [5,6]. They are important components of the human dietary product. Among the variety of biological and pharmacological properties of these agents, we find a series of the literature review on their activity in the central nervous system (CNS) [7]. Some of the discovered flavonoid derivatives with a flavone-like structure such as quercetin and kaempferol have been therapeutically reported for neuropharmacological activity. *S. asper* possesses anticancer [8], anti-Parkinson [9], antidiabetic [10], anti-inflammatory [11], antifilarial [12], and anti-viral activities [13]. Therefore, we explored the effect of hydroalcoholic extract of *S. asper* leave on locomotion, anxiety, and cognition activity.

METHODS

Animal

All conditions were maintained according to the CPCSEA guideline. The animals of either sex were selected randomly from animal house having uniform weight of 160±20 g. The room temperature was kept at 22±2°C with free access to water and food. 1 h before the beginning of the experiment, animals were shifted to the laboratory and food and water were removed. The study protocol was approved by the institutional animal ethical committee (Approve Ref No. SRGI/COP/A/29/2016, CPCSEA Reg No. 1624/PO/a/CPCSEA).

Plant material

The leaves of *S. asper* collected from forest region of Lakhimpur Kheri district, Uttar Pradesh, India, were authenticated by taxonomist of CSIR-NBRI, Lucknow, India. The voucher specimen of the plant was deposited in herbarium for future reference (NBRI/CIF/526/2016).

Preparation of extraction

The powdered drug (*S. asper)* was taken and subjected to solvent extraction. The extraction of *S. asper* was carried out for 16 h with the hydroalcoholic solvent. A 1: 5 w/v ratio of drug and solvent was maintained.

The *S. asper* hydroalcoholic extract was concentrated by distilling the solvent and evaporated it until dry at low temperature, then weighed, and calculated the percentage of different extractive values.

Phytochemical screening

The hydroalcoholic extract of *S. asper* leaves was tested for various classes of chemical constituents by phytochemical tests for alkaloids, glycosides, flavonoids, phenolic compounds, saponins, and carbohydrates [14].

Antioxidant activity

Free radical scavenging activity (antioxidant activity) of *S. asper* hydroalcoholic extract was analyzed by 2,2-diphenyl-1-picrylhydrazyl

(DPPH) assay. The stock solution (20–100 μg/mL) of *S. asper* hydroalcoholic extract was prepared in methanol. DPPH (0.3 mM) reagent was added to the methanolic stock solutions of the sample (20–100 μL) and kept in the dark at room temperature for 30 min [15]. The absorbance (A) of each sample extract was recorded at 517 nm using UV spectrophotometer (Shimadzu, Japan). A control sample was also prepared to approximate the radical scavenging followed by an equation:

$$\text{Scavenging active}(\%)=\left(1-\frac{\text{Asample}}{\text{Acontrol}}\right)\times 100$$

All tests were run in triplicates (n=3), and the average values were calculated.

IC_{50} value

DPPH method indicated inhibition concentration (IC_{50}) which calculated by the elucidation of the data results. The color reaction of the sample (absorbance) was plotted against the sample extract concentration to calculate IC_{50} value. It is defined as the volume of sample necessary to reduce the absorbance of DPPH by 50%.

Behavioral changes and toxicity studies (LD_{50})

The doses (200 and 400 mg/kg) of the hydroalcoholic extract of *S. asper* leaves were administered intraperitoneally to mice (n=5) for 7 days, and behavioral changes were observed at 30-min interval up to 2 h on 7^{th} day after 1 h of drug administration [16].

For LD_{50} study, male mice (n=5) were received *S. asper* hydroalcoholic extract at 2000 mg/kg dose, and the mortality was recorded after 24 h.

Spontaneous locomotor activity

The actophotometer is an instrument which measures locomotor activity by counting number of the steps of animal (movement of animals) by cutting of a beam of light dropping on the photocell. In the actophotometer, rat was positioned individually for 10 min and basal activity score was recorded. Rat was divided into four groups (n=5). Group 1 received vehicle (DMSO), Groups 2 and 3 received *S. asper* hydroalcoholic extract (200 and 400 mg/kg), and Group 4 received diazepam 2 mg/kg (standard drug group) for 7 consecutive days. Rat was positioned in the actophotometer for measuring the activity score after 60 min of treatment [17].

Motor coordination

The rats were skilled to sustain balance for 2 min on the revolving rod at the speed of 20 rpm. Only those rats which could balance themselves were qualified for the study. Each rat was positioned individually on the rotarod, and the total number of falls within 2 min was counted which was referred as the basal reading. Subsequently, the rats were separated into four groups each consisting of five animals. All the treatments of vehicle (dimethyl sulfoxide [DMSO]), *S. asper* hydroalcoholic extract (200 and 400 mg/kg), and diazepam (2 mg/kg) were administered to rat for 7 consecutive days [18]. The rat was placed on Rota-Rod for recording the activity after 60 min of drug administration.

Open-field test

Open-field test is used to measure exploratory behavior range of anxiety-induced, locomotion activity. In this test, there was a novel and bright area. Rat was placed in the bright area that tends to stay away from brightly illuminated areas [19]. The wall was circular (33" diameter) with high dimension (30 cm) and composed of plywood. The black color was painted on the entire apparatus except for line (6 mm) that separated each floor. During the experiment, the place was kept in the dark except open-field apparatus. The doses of all treatment including vehicle (DMSO), *S. asper* hydroalcoholic extract (200 and 400 mg/kg), and diazepam (2 mg/kg) were administered to rat for 7 consecutive days. Each rat was centrally positioned in the open-field apparatus for 5 min after 1 h of treatments, and the certain behavioral parameters were counted [20].

1. Ambulation: This was calculated by the number of spaces crossed by the animal.
2. Rearings: The number of times the animal stood on its hind limbs.
3. Immobility: Duration of time (s) the animal stayed immobile.

Elevated plus maze (EPM)

Anxiolytic activity (exploratory behavior) of rodents was evaluated by EPM model. In this model, two open arms (50×10 cm) and two closed arms (50×10×40 cm) were positioned perpendicularly and elevated 50 cm above the floor. Group 1 served as control received vehicle (DMSO) and Groups 2, 3, and 4 served as *S. asper* hydroalcoholic extract

Table 1: Phyto-chemical screening of hydro-alcoholic extract of *Streblus nigra*

S. No.	Class of compounds	Hydroalcoholic extract of *Streblus asper*
1	Alkaloids	Present
2	Glycoside	Present
3	Flavonoids	Present
4	Carbohydrates	Present
5	Saponins	Present
6	Phenolic compound	Present

Table 2: Estimation of anti-oxidant activity of hydro-alcoholic extract of *Streblus nigra* using DPPH test

S. No.	Concentration of hydroalcoholic extract (μg/ml)	Abs. of extract	Abs. of control	SCV (%) extract	IC_{50} (μg/ml) Extract
1	20	0.342	0.632	45.88	35.33
2	40	0.302	0.632	52.21	
3	60	0.285	0.632	54.90	
4	80	0.251	0.632	60.28	
5	100	0.233	0.632	63.13	

Values are mean±SEM (n=5) One-way ANOVA followed by Dunnett's multiple comparison tests. *Significant variation as compared to vehicle control treated group (p<0.5). **Significant variation as compared to standard drug-treated group (p<0.05). SEM: Standard error of the mean. ANOVA: Analysis of varianace

Table 3: Effect of hydro- alcoholic extract of *Streblus asper* in locomotion test

S. No.	Treatment	Dose	Number of steps (scores) in 10 min		
			Before treatment	After treatment	Reduction in activity (%)
1	Control		196.2±4.532	132.2±2.596	36.053±5.050
2	Hydroalcoholic extract of *Streblus asper*	200 mg/kg p.o.	121.8±3.56	71.8±2.08	40.83±2.4865
3	Hydroalcoholic extract of *Streblus asper*	400 mg/kg p.o.	242.8±3.823	135.6±5.25	44.206±1.469
4	Diazepam	2 mg/kg i.p.	236.2±4.71	113.2±3.435	55.44±6.220*

Table 4: Effect of hydro- alcoholic extract of *Streblus asper* in rota-rod test

S. No.	Treatment	Dose	Number of falls in 2 min	
			Basal reading	After reading
1	Control		5.2±0.584	5.6±0.511
2	*Streblus asper* hydroalcoholic extract	200 mg/kg p.o.	6±0.708	8±0.838
3	*Streblus asper* Hydroalcoholic extract	400 mg/kg p.o.	8.2±0.584	9.2±0.582
4	Diazepam	2 mg/kg i.p.	8.2±0.86	12.2±0.801*

Values are mean±SEM (n=5) One-way ANOVA followed by Dunnett's multiple comparison tests. *Significant variation as compared to vehicle control treated group (p<0.5). SEM: Standard error of the mean. ANOVA: Analysis of varianace

Table 5: Effect of hydro- alcoholic extract of *Streblus asper* in Open field test

S. No.	Treatment	Dose	Ambulation	Immobilization	Rearing
1	Control		71.2±2.381	5.99±125.8	4.8±0.862
2	*Streblus asper* hydroalcoholic extract	200 mg/kg p.o.	88.2±1.719*	80.2±1.36	8.2±0.736
3	*Streblus asper* hydroalcoholic extract	400 mg/kg p.o.	93.6±4.213**	69.6±0.929**	9.8±0.66**
4	Diazepam	2.5 mg/kg i.p.	104.6±5.368**	61.4±1.507**	11±1.227**

Values are mean±SEM (n=5). One-way ANOVA followed by Dunnett's multiple comparison tests. *Significant variation as compared to vehicle control treated group (p<0.5). **Significant variation as compared to standard drug-treated group (p<0.05). SEM: Standard error of the mean. ANOVA: Analysis of varianace

Table 6: Effect of hydro-alcoholic extract of *Streblus asper* in Elevated plus maze test (anti-anxiety activity)

S.No.	Treatment	Dose	% Preference open arm	Number of entries in the open arm	Time spent in open arm (s)
1	Control		28.59	3.4±0.511	85.8±2.402
2	*Streblus asper* hydroalcoholic extract	200 mg/kg p.o.	30.802	5.2±0.586	92.4±4.97
3	*Streblus asper* hydroalcoholic extract	400 mg/kg p.o.	33.01	4.6±0.245	99±3.67
4	Diazepam	2 mg/kg i.p.	52.9*	6.8±0.4912*	159±6.81*

Values are mean±SEM (n=5) One-way ANOVA followed by Dunnett's multiple comparison tests. *Significant variation as compared to vehicle control treated group (p<0.5). EPM: Elevated plus maze, SEM: Standard error of the mean. ANOVA: Analysis of varianace

Table 7: Effect of hydro-alcoholic extract of *Streblus asper* in Elevated plus maze test (nootropic activity)

S. No.	Treatments	Dose	Transfer latency		
			Day 1	Day 2	Day 7
1	Control		43.4±2.881	26.20±1.56	22.4±1.96
2	*Streblus asper* hydroalcoholic extract	200 mg/kg p.o.	33.6±1.21	21.20±1.24*	18.2±1.15*
3	*Streblus asper* hydroalcoholic extract	400 mg/kg p.o.	25.2±1.63	17.80±1.11**	15.4±0.92**
4	Paracetamol	100 mg/kg i.p.	21.2±0.971	15.40±1.07**	12.2±1.06**

Values are mean±SEM (n=5) One-way ANOVA followed by Dunnett's multiple comparison tests. *Significant variation as compared to vehicle control treated group (p<0.5). **Significant variation as compared to standard drug-treated group (p<0.05). SEM: Standard error of the mean. ANOVA: Analysis of varianace, EPM: Elevated plus maze

(200 and 400 mg/kg) and the standard group received diazepam (2 mg/kg), respectively, for 7 consecutive days. On the 7th day, the rats were individually positioned on the center of the maze after 1 h of oral administration. During 5 min of the observation period, we noted the time spent in opened arms and the number of entries [21].

EPM

Recently, EPM model is utilized for the evaluation of nootropic activity [22] (learning and acquisition of memory). The EPM consisting of two open arms (50×10 cm) and two covered arms (50×10×40 cm) were positioned perpendicularly and elevated 50 cm above the floor. Each rat was placed at the terminal site of an open arm facing away from the central platform on the 1st day. The rat was moved from the open arm into closed arms which counted as time domain (second) called as transfer latency (TL). The vehicle (DMSO) and treated drug were administered for 7 consecutive days. On the 1st day, TL was noted for each animal after 1 h of oral administration. If the animal could not reach one of the covered arms within 90 s, it was mildly pushed into one of the two closed arms and TL considered as 90 s. For another 2 min, the rat was allowed to stand on the maze. Then, rat was shifted to its home cages. It was again noted TL on 2 and 7 days which animal exhibit for this learned-task (acquisition of memory) after the 1st time trial [23].

Body temperature

Body temperature was noted after or prior administering of control vehicle, test, and standard treatment dose. It was recorded by inserting about 2 cm into the rectum of the animals which enabled with a thermoelectric probe linked to digital thermometer.

Statistical analysis

The results are representing as a mean±SEM. The statistical significance was analyzed using one-way analysis of variance followed by Dunnett's test. Significant of P value was to be considered when the difference of P value was less than 0.05.

RESULTS AND DISCUSSION

Acute toxicity and general behavior studies-

S. asper hydroalcoholic extract (2000 mg/kg) wasadministered to mice for conduction of acute toxicity studies (LD_{50}). During conducting LD_{50}, it was regularly observed for any general behavioral changes. It was resulted that The significant reductions in drowsiness, spontaneous locomotor motility were observed.

Hydroalcoholic extract of *S. asper* leaves results as changing the general behavior patterns and reducing normal body temperature. All of the two results concluded that a CNS-depressant action of hydroalcoholic extract was validated by this model.

The results obtained demonstrated that hydroalcoholic extract at a dose of 200 and 400 mg/kg, respectively, indicated that increase in the TL showed significant improvement in the acquisition and retention of memory of the learned task, thus resulting in nootropic activity.

It is probable that the building of memory was amplified on the involvement of neurotransmitters when the repeated administration of the drug was achieved. There was prediction that the serotonergic, noradrenaline, and central cholinergic transmission play an important role in the cognitive function of the brain [24,25].

In our study, the hydroalcoholic extract of *S. asper* leaves did not produce any significant change in the anxiolytic activity using the EPM model. Anxiolytic compounds, by decreasing anxiety, increased the open arm exposing time as well as the number of entries into the open arm. The hydroalcoholic extract of the plant failed to demonstrate any such effect in the rats, and hence, we can conclude that extract does not possess anxiolytic activity. In generaL, most of the anxiolytic agents have an adverse effect on memory as seen with the benzodiazepines, commonly used as anxiolytics [26]. In the current situation, EPM model is also being used to analyze learning and acquisition of memory in rodents. Scopolamine, an anticholinergic agent inducing the impairment of learning and memory, is revealed by extended transfer of latency from the open arm to the closed arm [27]. With respect to our results, in contrast to that of diazepam, hydroalcoholic extract did not cause any improvement in the number of entries into the open arm.

Locomotor activity is reflected as an index of alertness and a decline in it would observe sedative activity. The hydroalcoholic extract showed results which had no influence on the locomotor activity (index of alertness and a decline in it would observe sedative activity). Therefore, the insignificance of effect on locomotor activity was indicated which referred to the advantage of the plant indicating nootropic activity.

It was observed that hydroalcoholic extract possess neurobiological activity. It was reflected by the presence of flavonoids.

CONCLUSION

It was concluded that *S. asper* hydroalcoholic extract exhibits a potential role in memory enhancing but haS no effect on locomotion, motor coordination, and anxiolytics activity.

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CONFLICTS OF INTEREST

All authors have none to declare.

AUTHORS' CONTRIBUTIONS

Himani awasthi and DCP singh contributed equally to this work.

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