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Pharmaceutical Sciences **Research Article……!!!**

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CENTRAL NERVOUS SYSTEM STIMULANT EFFECT OF EXTRACTS OBTAINED FROM THE BARKS OF *SESBANIA SESBAN*

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ABSTRACT

Sesbania sesban is a short lived shrub which belongs to family *Fabaceae*. Bark is traditionally used as a bitter tonic ,used in debility nervous disorders. This study was intended to evaluate the CNS stimulant activity of crude drug extract. The activity was carried out on albino mice. Caffeine was used as a reference drug. The crude extract showed significant CNS stimulant activity in comparison to control group and result were comparable to the activity shown by reference drug.

INTRODUCTION

Herbal medicines are in great demand in the developed world for primary health care because of their efficacy, safety and lesser side effects. They also offer therapeutics for age-related disorders like memory loss, osteoporosis, immune disorders, etc. for which no modern medicine is available. India despite its rich traditional knowledge, heritage of herbal medicines and large biodiversity has a dismal share of the world market due to export of crude extracts and drugs. WHO has not systematically evaluated traditional medicines despite the fact that it is used for primary health care by about 80% of the world population. However, in 1991 WHO developed guidelines for the assessment of herbal medicine. CNS disorders can affect either brain or the spinal cord resulting in neurological or psychiatric disorders. Causes of CNS diseases are truma, infection, degeneration, autoimmune disorders, structural defects, tumours and stroke, neurodegenerative diseases, mood disorders, schizophrenia and autism.[2]

Central nervous system (CNS) stimulants are medicines that speed up physical and mental processes. Central nervous system stimulants are used to treat conditions characterized by lack of adrenergic stimulation, including narcolepsy and neonatal apnea. Additionally, methylphenidate (Ritalin) and dextroamphetamine sulfate (Dexedrine) are used for their paradoxical effect in attention-deficit hyperactivity disorder(ADHD).The majority of CNS stimulants are chemically similar to the neurohormone norepinephrine and simulate the traditional "fight or flight" syndrome associated with sympathetic nervous system arousal. Caffeine is more closely related to the xanthines, such as theophylline. A small number of additional members of the CNS stimulant class do not fall into specific chemical groups.[9]

The present study is directed towards the potential CNS stimulant effect of *Sesbania sesban* in an experimental animals.

Sesbania sesban has a long history of use in India, primarily as a green manure and a source of cut and carry forage. Planted or assisted to establish as a volunteer as an improved fallow in maize fields in Southern and East Africa because it improves crop yields and provides fuel wood. It can be intercropped with corn, beans, cotton and many other field crops. Harvested leaves make a rich compost. Its leaves are a good source of

protein for cattle and sheep. These are used as a grazed forage in sub-tropical Australia and Kenya. It has been used as a reclamation species of saline spoils in southern China. It produces a light fuel wood suitable for cooking and charcoal production. It has been used as a live support for black pepper, grapes, cucurbits and betel vine and as a shade tree for coffee and turmeric. Root and bark used as bitter tonic used in debility nervous disorders, CNS stimulant. Root of plant used as dysuria, retention of urine, hepatoprotective activity. Leaves are used as anthelmintic activity**.**

Materials and Method

Plant collection and authentication:

Plant *Sesbania sesban* L. was collected from Junnar district of Maharashatra. The plant was authenticated from Botanical Survey of India, Pune.

Animals:

Male Swiss albino mice weighing 20-25 gm were obtained from Lakshmi Biopharms Pvt.Ltd., Alephata, Tal-Junnar, Dist-Pune. They were housed in an environmentally regulated room on a 12:12 hr. Light: Dark cycle with 25^{o}C (±2^{o}C) and had free access to food and water.

The experimental protocol was approved by the Institutional Animal Ethical Committee and experiments conducted according to the CPCSEA. Indian guidelines on the use and care of experimental animals. Experiments were carried out between 9:00 and 18:00 hr.

Table no.1 Groups of Animals receiving test drug, Reference drug and vehicle[10]

Sr.No	Group	Animals
1	Control	6
2	Caffeine treated	6
3	Aqueous extract of *Sesbania sesban*	6

Chemicals:

All the Chemicals (LR Grade) from Merck Ltd. were used for extraction, Caffeine (Standard) was obtained from Yucca Enterprises, Mumbai. Normal saline solution was used as a vehicle.

Equipments:

1. Elevated plus maze.

2. Light – Dark apparatus.

Experimental Work

Extraction method

The active constituents from bark of *Sesbania sesban* were extracted by successive extraction in soxhlet apparatus using petroleum ether ,chloroform ,alcohol and water as a solvent for 12 to 13 hrs. Solvents were separated from extracts by means of vacuum distillation. The obtained extracts were dried and stored in glass container till further use.

Pharmacological Activity

A) Preparation of dosage forms

Aqueous extract of *Sesbania sesban* Linn. (bark) were used for studies & their dosage form is was prepared as follows:

The dried powders of the extracts were mixed with the gradual addition of normal saline to make up the required volume. Vehicle to be administered to respective control groups, were prepared using the same procedure without addition of extract.

The dosage form of all the extracts were prepared freshly on the day of experiment and kept at room temperature in air tight container. The standard drug dose i.e. caffeine was prepared by using normal saline.

B) Acute toxicity study [7]

Acute oral toxicity study of aqueous extract of *Sesbania sesban* Linn. was carried out in Swiss albino mice of either sex (20-22 g) according to OECD guidelines no 423.

Extract at different doses up to 4000 mg/kg, *p.o*. was administered and animals were observed for behavioral changes, toxicity and mortality up to 48 hrs. So 1/10 dose (i.e. 400mg/kg) was selected as safe dose for (50%v/v) *Sesbania sesban* extracts.

C) Neuropharmacological Evaluation[1][4][7][10]

1. Elevated plus maze(EPM)

This test has been widely validated to measure anxiety in rodents. This apparatus was made of Plexiglas and consisted of two open arms (30cm × 5cm) and two closed arms (30cm × 5cm)with 25cm walls. The arms extended from a central platform (5cm × 5cm). The maze was elevated 38.5cm from the room floor.

Each animal was placed at the center of the maze, facing one of the enclosed arms. Number of entries and the time spent in enclosed and open arms was recorded for 5 min test. Entry into an arm was defined as the animal placing all four paws onto the arm. All tests were taped by a video camera. After each test, the maze was carefully cleaned up with a wet tissue paper (10% ethanol solution).

The animals received the treatment as per the following schedule.

Group I - served as control and treated orally with vehicle (Normal saline)

Group II - served as standard and caffeine 30mg/ kg was given i.p.

Group III - received aq. extract 400 mg/kg, p.o.

The animals received the treatment 45 min before the start of session. At the beginning of the session, a mouse was placed at the centre of the maze, its head facing the closed arm. It was allowed to explore the maze for 5 min. The time spent in open arm, percent entries in the open and closed arms and total entries were recorded. An entry was defined as the presence of all four paws in the arm. The EPM was carefully wiped, with 10 % ethanol after each trial, to eliminate the possible bias due to the odour of the previous animals.

$$\text{Percent time spent in open arm} = \frac{\text{Time in the open arm}}{\text{Time in the open arm} + \text{time in the closed arm}} \text{x}100$$

2.Light-Dark Test (LDT)

The apparatus consisted of a Plexiglas box with two compartments (20cm × 20cm each), one of which was illuminated with a white light while the other remained dark. Each animal was placed at the center of the illuminated compartment, facing one of the dark areas. The time spent in illuminated and dark places, as well as the number of entries in each space, was recorded for 5 min(Crawley and Goodwin,1980).

The animals received the treatment as per the following schedule.

Group I - served as control and treated orally with vehicle (Normal saline).

Group II - served as standard and caffeine 30mg/ kg was given i.p.

Group III - received pet. ether extract 400 mg/kg, p.o.

D) Statistical analysis

The statistical tests used to compare the different doses of the administration of *Sesbania sesban* in the Elevated plus maze and Light – Dark Apparatus were one way analysis of variance (ANOVA) followed, when significant, by the Dunnett´s test. A p-value less than 0.05 was considered statistically significant. Data were analyzed by Graph Pad prism version – 4.0 software and presented as mean ±S.E.M. values.

Results

Table no.2 Qualitative chemical evaluation of *Sesbania sesban* L.

Sr.No	Test / Reagents used	Pet ether extract	Chlorofom extract	Ethanol extract	Aqueous extract
1	Carbohydrate Molisch' test a)Fehling test	-	-	+	+
2	Glycosides a) Legal's Test b) Borntrager's Test	- -	- -	- -	- -

3	Alkaloids a) Wagner's Test b) Dragondorff's Test c) Mayers Test d) Hanger's reagent.	 - + - -	 - + - -	 - + - -	 - + - +
4	Phytosterols a) Salkowaski Test	 +	 +	 +	 -
5	Saponins glycosides a)Foam test	 +	 +	 +	 +
6	Tannins a) Gelatin Test	 -	 -	 -	 -
7	Saponins a) Shinoda's Test	 -	 -	 -	 -
8	Proteins a) Biuret Test	 -	 -	 -	 -
9	Phenolic compound a)Lead acetate solution	 -	 -	 -	 +

Table No. 3

LD_{50} determination for aq. extract of *Sesbanian sesban* L.

Group	No. of animals	Dose	No. of animal dead
I	6	1000 mg/kg	0
II	6	2000 mg/kg	0
III	6	3000 mg/kg	0
IV	6	4000 mg/kg	3

Determination of CNS stimulant activity

1. Elevated Plus Maze

Table No. 4 Effect of *Sesbania sesban* on percentage time spent in open and closed arm

Animal No.	Control		Caffeine (30 mg/kg b.wt.)		Aq. SS (400 mg/kg b.wt.)	
	Close Arm	Open arm	Close Arm	Open arm	Close Arm	Open arm
01	95.33	4.66	31.00	69.00	38.33	61.66
02	95.66	4.33	31.66	71.66	35.00	65.00
03	95.33	4.66	32.33	71.66	34.00	66.00
04	96.00	4.00	32.00	68.00	37.33	62.66
05	95.00	5.00	32.66	70.33	34.33	63.33
06	95.66	4.33	31.66	69.66	34.00	62.33
MEAN ± SEM	95.50 ± 0.1425	4.497 ± 0.1425	31.27** ± 0.2378	70.05** ± 0.5978	33.54** ± 0.7633	63.50** ± 0.6829

Analysis: One-Way ANOVA Followed by Dunnett's Test

**P < 0.01 compared with Control Group

Table No. 5 Percentage time spent in Open Arm

	Control	Caffeine (30 mg/kg b.wt.)	Aq. SS (400 mg/kg b.wt.)
Mean	4.497	70.05	63.50
Std. Deviation	0.3490	1.464	1.673
Std. Error	0.1425	0.5978	0.6829

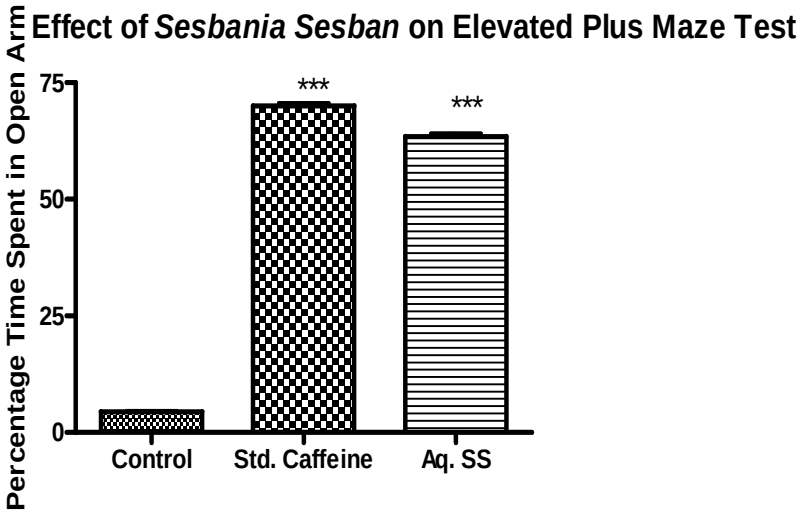

FIGURE NO.2

Analysis: One-Way ANOVA Followed by Dunnett's Test

***P < 0.01 compared with Control Group

Table no. 6 Percentage time spent in Close Arm

	Control	Caffeine (30 mg/kg b.wt.)	Aq. SS (400 mg/kg b.wt.)
Mean	95.50	31.27	33.54
Std. Deviation	0.3490	0.5825	1.870
Std. Error	0.1425	0.2378	0.7633

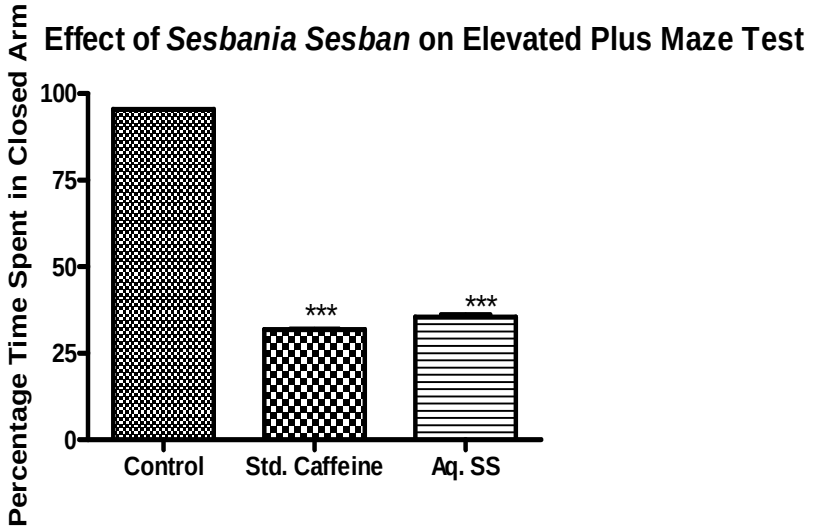

FIGURE NO.3

Analysis: One-Way ANOVA Followed by Dunnett's Test

**P < 0.01 compared with Control Group

2. LIGHT-DARK METHOD

Table No: 7 Effect of *Sesbania sesban* in Light arm before and after treatment on average time spent.

Animal No.	Control		Caffeine (30 mg/kg b.wt.)		Aq. SS (400 mg/kg b.wt.)	
	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment
01	115	113	114	192	112	182
02	114	124	112	191	118	178
03	120	121	115	193	119	190
04	118	118	119	195	114	187
05	120	122	118	182	113	181
06	119	119	123	194	121	182
MEAN ± SEM	117.7 ± 1.054	119.5 ± 1.565	118.5 ± 1.478	191.2** ± 1.922	116.2 ± 1.493	183.3** ± 1.783

Analysis: One-Way ANOVA Followed by Dunnett's Test

**P < 0.01 compared with control.

Table no. 8 LIGHT ARM RESULT BEFORE TREATMENT

	Control	Caffeine (30 mg/kg b.wt.)	Aq. SS (400 mg/kg b.wt.)
Mean	117.7	118.5	116.2
Std. Deviation	2.582	3.619	3.656
Std. Error	1.054	1.478	1.493

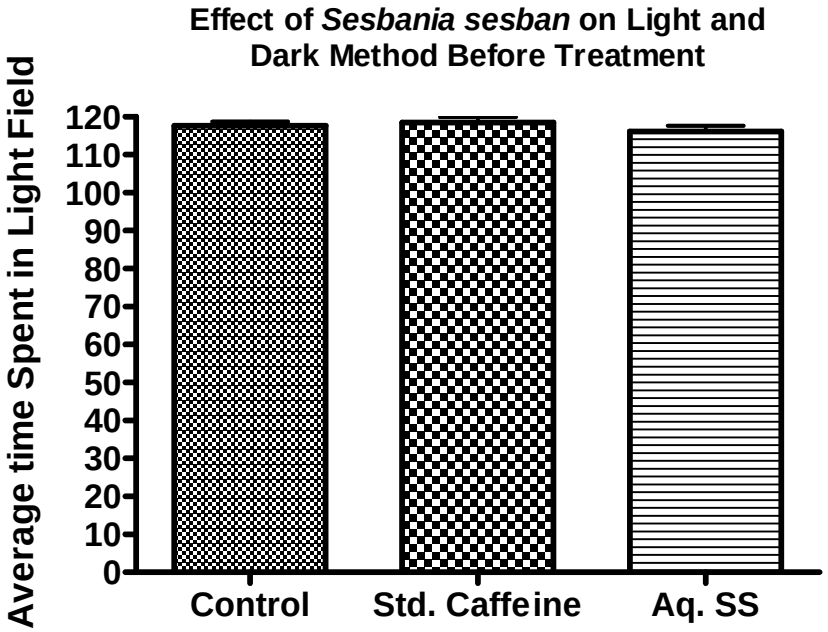

FIGURE NO.4

Analysis: One-Way ANOVA Followed by Dunnett's Test

P > 0.05 compared with Control Group

Table no. 9 LIGHT ARM RESULT AFTER TREATMENT

	Control	Caffeine (30 mg/kg b.wt.)	Aq. SS (400 mg/kg b.wt.)
Mean	119.5	191.2	183.3
Std. deviation	3.834	4.708	4.367
Std. Error	1.565	1.922	1.783

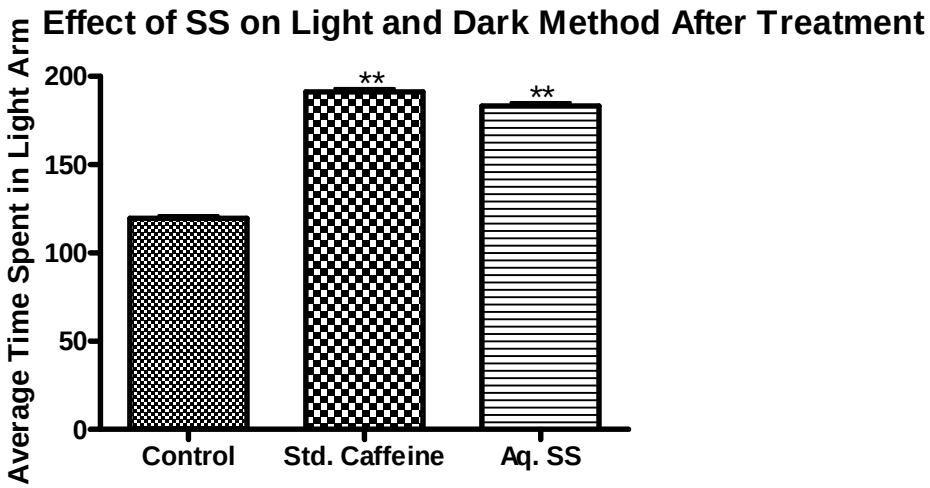

FIGURE NO.5

Analysis: One-Way ANOVA Followed by Dunnett's Test

**P < 0.01 compared with Control Group

Table No: 10 Effect of *Sesbania sesban* in Dark arm before and after treatment on average time spent.

Animal No.	Control		Caffeine (30 mg/kg b.wt.)		Aq. SS (400 mg/kg b.wt.)	
	Before Treatment	After Treatment	Before Treatment	After Treatment	Before Treatment	After Treatment
01	190	188	184	113	185	124
02	173	178	180	110	171	126
03	178	179	179	114	179	119
04	175	175	185	120	180	123
05	193	171	175	109	182	119
06	182	179	181	121	183	122
MEAN ± SEM	181.8 ± 3.321	178.3 ± 2.305	180.7 ± 1.476	114.5** ± 2.045	180.0 ± 2.00	122.2** ± 1.138

Analysis: One-Way ANOVA Followed by Dunnett's Test

**P < 0.01 compared with Control Group

Table no. 11 DARK ARM RESULT BEFORE TREATMENT

	Control	Caffeine (30 mg/kg b.wt.)	Aq. SS (400 mg/kg b.wt.)
Mean	181.8	180.7	180.0
Std. deviation	8.134	3.615	4.899
Std.S Error	3.321	1.476	2.00

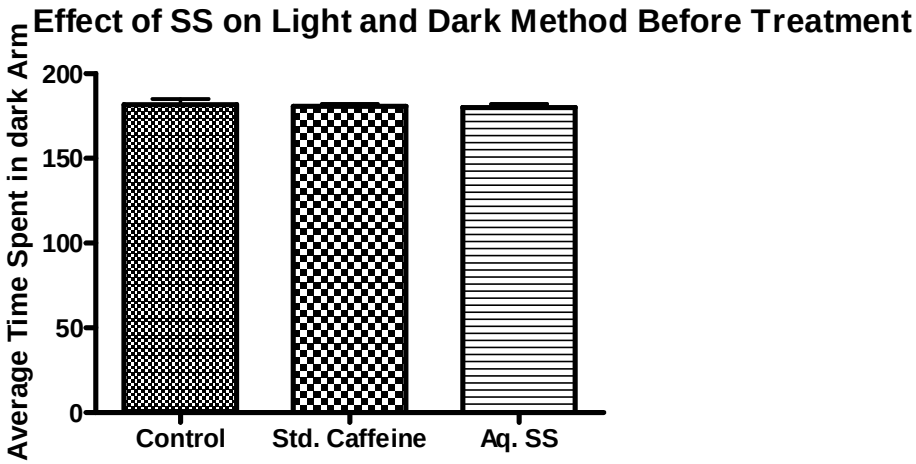

FIGURE NO.6

Analysis: One-Way ANOVA Followed by Dunnett's Test

P = 0.8619

Table no. 12 DARK ARM RESULT AFTER TREATMENT

	Control	Caffeine (30 mg/kg b.wt.)	Aq. SS (400 mg/kg b.wt.)
Mean	178.3	114.5	122.2
Std. deviation	5.645	5.010	2.787
Std. Error	2.305	2.045	1.138

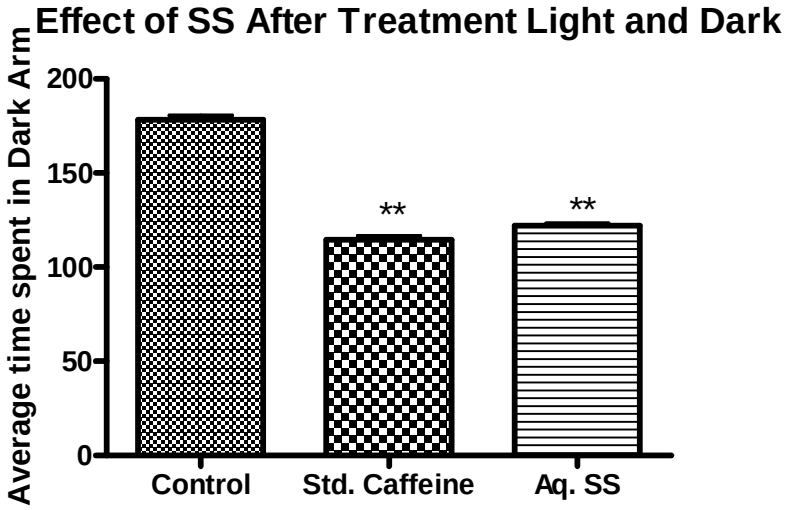

FIGURE NO. 7

Analysis: One-Way ANOVA Followed by Dunnett's Test

**P < 0.01 compared with Control Group

The aqueous extract of *Sesbania sesban* (400 mg/kg) and caffeine (300 mg//kg) showed significant effect (** P < 0.05) as compared to control.

Discussion

The medicinal plants are suggestive of high therapeutic potency in disease conditions especially in CNS disorders, etc. Caffeine, a mild stimulant is the widely used psycoactive drug in the world. It increases nor-epinephrine secretion and enhance neural activity in numerous brain areas. Many of its effect are believed to occur by means of competitive antagonism at adenosine receptors. Tolerance occurs rapidly to the stimulating effects of caffeine, thus a mild withdrawal syndrome has been produced. Many CNS stimulant drugs have been used clinically for treatment of drowsiness. Therapies with these drugs are effective but sometimes there are adverse effects and compliance can be low.

In view of this *Sesbania sesban* plant growing in Maharashtra has been selected for current study. The aqueous extract of *Sesbania sesban* exhibited the CNS stimulant activity compared to that of caffeine .

Some physiological active molecules which were responsible for the CNS stimulant activity further work on the types of phytoconstituents isolation of bioactive compound can reveal the exact potential the plant to encourage in developing novel new CNS stimulant drug in future. After screening of bark extract of *Sesbania sesban* has revealed that the bark possesses potent CNS stimulant effect in the models like Elevated plus maze, Dark and light.

More studies are required to achieve the proper role of *Sesbania sesban* extract to find out more specific biochemical, pharmacological and molecular aspects of the targated molecules within that may have broadest implication to society.

Conclusion

The results suggest that the aqueous extract of *Sesbania sesban* has a potential CNS stimulant effect that can be explored for therapeutic advantage as an alternative treatment in medical conditions associated with dizziness and sedative.

Further work is in progress to monitor activity in a familiar and novel environment where the activity of the animals is minimal to further confirm its stimulant effect on the central nervous system. Thus, these preliminary studies confirm the CNS stimulant effect of *Sesbania sesban* , further it will be interesting not only to isolate the active chemical constituents that are responsible for the CNS stimulant effect but also to predict the mechanism of action.

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