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NEUROPHARMACOLOGICAL EVALUATION OF *SESBANIA SESBAN* USING EXPERIMENTAL ANIMALS

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ABSTRACT

The extension of the expectancy of life and ageing of populations on a global level in recent times are expected to cause a huge rise in the occurrence of many chronic, progressive, non-communicable conditions inclusive of neurological disorders. This has stimulated the use of a variety of medicinal plants containing numerous chemical constituents to provide therapeutic effects similar to those drugs obtained from other sources, with lesser or no side effects. The current study includes the use of ethanolic extract of *Sesbania sesban* which are evaluated for their neuropharmacological activities.The different types of animals models used for neuropharmacological evaluation consisted of elevated plus maze test, radial arm maze test and conditioned place preference chamber test. The estimation of dopamine in rat brains was also carried out using UV-visible spectroscopy. The extract was found to possess anxiolytic, partially nootropic and non-addictive properties from the results obtained.

KEYWORDS: Conditioned place preference, dopamine, elevated plus maze, neuropharmacological, radial arm maze.

INTRODUCTION

The increasing capacity of modern medicine to prevent death has also increased the frequency and severity of impairment attributable to neurological disorders. This has led to the rise of the issue of restoring or creating a quality of life acceptable for people who suffer from the sequelae of neurological/mental/psychiatric disorders. [World Health Organization; 2006; Neurological Disorders: Public Health Challenges].[1]

Neurological/Mental/Psychiatric Disorder limits itself to the classification of the disturbances of mental functioning. It does not comprise of diagnoses of intracranial pathology or neurologic diagnoses, per se. Conditions like these should be separately diagnosed, whether or not a mental disturbance is associated with them. It is the mental disorder which is diagnosed in situations where an intracranial lesion is accompanied by a mental disorder.

Psychiatric disorders are divided into two major groups

(1) Those in which a primary impairment of the function of the brain precipitates/leads to a disturbance of mental function, generally due to diffuse impairment of brain tissue; and

(2) Those in which any associated brain function disturbance is secondary to the psychiatric disorder, and which are the result of a more general difficulty in adaptation of the individual.

[The Committee on Nomenclature and Statistics of the American Psychiatric Association; 1952; Diagnostic And Statistical Manual: Mental Disorders (DSM-1)].[2]

Table I: Types Of Neurotransmitters Involved In Neuropharmacology.[3,4,5]

Type	Neurotransmitter
Amino Acids	γ-aminobutyric acid (GABA); Glycine; β-alanine; taurine; Glutamic acid; Aspartic acid
Amines	Serotonin (5-HT); Dopamine (DA); Norepinephrine (NE); Epinephrine (EPI); Acetylcholine (ACH)
Pituitary peptides	Corticotropin (ACTH); Growth hormone (GH); Lipotropin; Oxytocin;Vasopressin; Melanocyte stimulating hormone
Gastrointestinal Peptides	Cholecystokinin; Gastrin; Motilin; Secretin; Vasoactive Intestinal Peptide; Substance P
Circulating hormones	Angiotensin; Calcitonin; Glucagons; Insulin
Opioid Peptides	Dynorphin; β-endorphin; Met-enkephalin; Leu-enkephalin; Kyotorphin

(* Bloom, 2006; Rang et al., 2003; Satoskar et al., 2007)

Steps in Neurochemical Transmission

The sequence of events involved in neurotransmission is of particular importance because pharmacologically active agents modulate the individual steps.

Steps involved are as follows

Uptake of Precursors
Synthesis of transmitter (T)
Storage of transmitter in vesicles
Degradation of surplus transmitter
Depolarization by propagated action potential
Influx of Ca^{++} in response to depolarization

Release of transmitter by exocytosis
Diffusion to postsynaptic membrane
Interaction with postsynaptic receptors
Inactivation of transmitter
Reuptake of transmitter or degradation products
Interaction with presynaptic receptors
[Rang et al., 2003].[4]

Characteristics of Central Nervous System Disorders

Clinical neuropharmacology or 'neuropsychopharmacology' includes the basic principles of neuroscience that underlie neuropharmacology and the basic pathological processes encountered in neurology. The disorders are characterized as:

Psychotic disorders include the manic phase of bipolar (manic-depressive) illness, acute idiopathic psychotic illnesses, schizophrenia, and other conditions marked by severe agitation All these exhibit disturbances in reasoning, often with delusions and hallucinations to a major extent. The imbalance in the dopamine level is mainly responsible for these disorders (Baldessarini and Tarazi, 2006).[6,7]

Anxiety disorders may be acute and transient, or recurrent or persistent, more commonly. Symptoms may include mood changes (like fear, panic or dysphoria), or limited abnormalities of thought (like obsessions, irrational fears, or phobias), or of behaviour (like avoidance, rituals or compulsions). The major cause of these disorders is the imbalance in one or more neurotransmitters eg. dopamine or serotonin (Baldessarini and Tarazi, 2006).[6,7]

Major depressive disorders commonly include persistent abnormalities of mood caused mainly due to an imbalance in the noradrenaline level, as well as disordered autonomic functioning (eg. altered rhythms of activity, appetite and sleep) and behaviour (Baldessarini, 2006).[6]

Some features of depressive disorders overlap those of the anxiety disorders, including generalized anxiety disorder, panic-agoraphobia syndrome, social anxiety disorder, severe phobias, post-traumatic stress disorder, and obsessive-compulsive disorder (Kessler et al., 1994).[8]

Neurological disorders, which include movement disorders, seizure disorders, and headaches. A seizure is a transient alteration of behaviour due to the disordered, synchronous, and rhythmic firing of populations of brain neurons. Epilepsy refers to a disorder of brain

function that is characterized by periodic and unpredictable occurrence of seizures. Seizures, when evoked in a normal brain by treatments such as electroshock or chemical convulsants, can be 'non-epileptic', whereas, when occurring without evident provocation, are 'epileptic' (McNamara, 2006).[9]

Neurodegenerative disorders include Alzheimer's disease (AD), Huntington's disease (HD), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS) (Standaert and Young, 2006).[10] The cause of these diseases is a fluctuation in neurotransmitter levels.

Herbal Therapy in Neurological Disorders

Medicinal plants are a local heritage with global importance and always have been the principal form of medicine in India. They are becoming popular presently as people are constantly striving to stay healthy in the face of chronic stress, with medicines that work in synergism with the body's own defences to treat illnesses. Medicinal plants are particularly a boon in remote parts of developing countries with few health facilities, thereby playing an important role in the lives of the rural population as well (Prajapati et al., 2003).[11] Around 70,000 plant species have been used for medicinal purposes, according to a rough estimation. Herbs provide the starting material for the isolation or synthesis of conventional drugs. In Ayurveda, about 2,000 plant species are considered to have medicinal value, while the Chinese Pharmacopoeia lists over 5,700 traditional medicines, most of which are of plant origin (Prajapati et al., 2003).[11]

One of the categories of drugs that have long been essential to medical practice are those which act to influence brain functions. As the importance of brain to normal physiological and psychological function is quite high, centrally acting drugs have diverse actions. CNS-acting drugs can induce anaesthesia, relieve fever and pain, prevent or modify seizures, induce sleep, reduce anxiety and ameliorate symptoms of major mental illnesses (Trease et al., 1996).[12]

Screening Methods for New Drugs

A scanning procedure designed essentially to distinguish useful drugs from non-useful drugs as comprehensively, rapidly and inexpensively as possible, is what is meant by screening. For screening a series of compounds for a given pharmacological activity, often numerous methods exist. The important factors in the selection of a screening method are the sensitivity

of the assay procedure and the possibility of ranking the compounds that have proved their clinical effectiveness (Turner, 1972).[13,14]

Blind Screening

If a new series of chemical substances becomes available, either through isolation from a natural source or through synthesis, there may be no further information on its pharmacological activity. If they exist, blind screening ought to provide clues to potential activity, atleast and preferably to indicate fields of activity. In addition, it also ought to show pharmacological inertness, if existence of that is present. It is important to note the whole range of qualitative changes produced by a drug and the quantitative relation between them in drug evaluation. Most probably, a drug cannot be properly evaluated until most of the major tests have been performed in a single animal species, under conditions that are similar, and by the intended route of administration to be used clinically. The investigator can obtain a wide range of data from each animal simultaneously and in an integrated form by performing multidimensional procedures. From the data obtained, dose-response relations for different drug actions can be compared in a more meaningful manner, and then extrapolated to their appearance in man (Turner, 1972).[13,14]

MATERIALS AND METHODS

Plant Material

The plant material was collected from kedgaon (pune distrct),all aerial parts was collected and air dried,followed by coarse powder then its was subjected to soxhlet extraction using ethanol: water (90:10) till colourless liquid obtained.the extract dried in rotary drying evaporator and percentage yield was calculated.

Animals

Swiss albino mice weighing 20-25 g and Wistar rats weighing 150-180 g of either sex were used for the study. Animals were procured from the animal house of A.I.S.S.M.S College of Pharmacy and kept in groups of five under standard laboratory condition of temperature (25 ± 2° C) and humidity (55 ± 5 %) under 12:12 light-dark cycle. All the animals had free access to food and water and food was removed 12 hours before experimentation for some of the animal models.

Drugs and Chemicals

Diazepam, scopolamine, piracetam and sodium chloride were procured from Rajesh Chemicals, Pune, India.

Assessment of Neuropharmacological Activity

Acute oral toxicity studies were performed according to OECD guideline 425. All mice and rats used were free of any toxicity as per acceptable range given by OECD guidelines (OECD – 425) upto the dose of 2000 mg/kg. From this data and pilot study reports; two different doses 100 mg/kg and 300 mg/kg were selected for this study.

Elevated Plus Maze (EPM) test

An elevated plus maze consisting of two open arms (35 × 6 cm) and two enclosed arms (35 × 6 × 15 cm) was used. The maze was elevated to the height of 40 cm. Mice were placed individually in the center of the elevated plus maze facing an enclosed arm. The time spent by the mouse during the next 5 min on the open and enclosed arm was recorded. The animals received vehicle or test drug 60 min before and diazepam (1 mg/kg i.p.) 30 min before their placement on the maze. Increased exploratory activity in the open arm was taken as an indication of anxiolytic activity.[15]

Radial Arm Maze Test

The animals for the experiments were preselected by conducting at least one daily training trial. Five groups of animals were made, each group consisting of six mice. In this group I served as normal control, group II as amnesic control, groups III and IV served as treatment and group V as standard.

At the beginning of trial, a food pellet was placed in one receptacle. An overnight fasted mouse was placed in the central hub and allowed to choose the arm freely, to get the food. The trial was considered to be complete when the mouse visited all eight arms.

Three parameters were evaluated here – the re-entries made by the mouse in the same arm, the time taken by the mouse to reach the food pellet (placed in one of the arms throughout the experiment) in the first attempt, and the time taken by the mouse to complete visiting all the eight arms of the maze.

On the 11th day, 60 minutes after the last dose, animals of respective groups were subjected to scopolamine (1 mg/kg i.p.) treatment for inducing amnesia. After 30 minutes each mouse

was placed on central hub & tested again for evaluating the three parameters as mentioned above.[16]

Conditioned Place Preference/Aversion activity in rats using Conditioned Place Preference (CPP) Chamber

The place conditioning procedure consisted of three phases: pre-conditioning, conditioning, and CPP test. All animals were allowed to habituate to the colony room for 1 week upon arrival. Subsequently, each animal was habituated to handling for 3 days before the start of the experiment. Rats were weighed daily and then transported to the testing room in groups of four. Following habituation, animals received a single pre-exposure test in which they were placed in the center choice chamber with the guillotine doors removed to allow access to the entire apparatus for 15 min. The amount of time spent in each chamber was monitored and used to assess unconditioned preferences.

During the following conditioning phase (8 days), rats were assigned to receive drug pairings with one of the two end chambers in a counterbalanced fashion (the 'unbiased' procedure). As well, half of each group began the experiment on the drug-paired side and half on the saline-paired side. The test extract was administered once every other day immediately before the rats were placed into the assigned chamber for 20 min. On alternate days, rats received saline injections (1.0 ml/kg) before being placed in the other chamber. Half of each treatment group received the drug extract on the first, third, fifth, and seventh day; the remaining subjects received the drug extract on the second, fourth, sixth, and eighth days. The center choice chamber was never used during conditioning and was blocked by guillotine doors.

Two days after the last conditioning trial, a test for CPP was given. Animals were placed in the center choice chamber with the guillotine doors removed and allowed free access to the entire apparatus for 15 min. The amount of time spent in each chamber was recorded to assess individual preferences. No injections were given during the CPP test, maintaining the same procedure as that used during the pre-exposure test.[17]

To estimate the concentration of the neurotransmitter dopamine in rat brains by UV Visible Spectroscopy

Brains of adult Wistar rats (5 rats from each group) from Control, Test 1 (100 mg/kg) group and Test 2 (300 mg/kg) group were removed quickly and placed in iced normal saline, and perfused with the same solution to remove blood cells.

Preparation of tissue homogenate

The brains kept in iced normal saline were immediately cut into small pieces and homogenized in phosphate buffer (pH 7.4) individually. The homogenates of the 5 animals from the control group were mixed together to make a single homogenate, and the same procedure was followed for the Test 1 (100 mg/kg) group and the Test 2 (300 mg/kg) group separately. Therefore, three combined homogenates were obtained at this stage – one of the control group animals (n=5), the second of the Test 1 (100 mg/kg) group and third of the Test 2 (300 mg/kg) group. These homogenates were then individually centrifuged at 4000 rpm for 15 minutes at 4°C and the supernatant was removed for estimation.[18]

Determination of brain dopamine level was carried out using UV-visible spectroscopic system [Jasco technologies (V-530 model)]. Serial dilutions of standard dopamine were prepared in distilled water and their absorbances were measured. The detection was performed within the range of 240 nm to 280 nm. A linear standard curve was constructed by plotting absorbance versus the corresponding concentrations, and the linearity equation of the same was obtained.

Similarly, the supernatant obtained from the test centrifugate, for each group was diluted 10 times, and subjected to UV-Visible spectroscopy individually. Obtaining the values of the absorbances of individual groups, these values were inserted in the linearity equation to get the concentration of dopamine in terms of ng/mL, which gave the value of the same in ng/g of brain tissue on further calculations.

Statistical Analysis

Results for all the behavioural tests were expressed as mean ± SEM. Comparison between the groups was made by one way analysis of variance (ANOVA) followed by Dunnett's test.

RESULTS

Anxiolytic Activity Evaluation by Elevated Plus Maze Test

The *Sesbania sesban* (100 mg/kg and 300 mg/kg) and diazepam (1 mg/kg i.p.) showed significant increase in the occupancy in the open arm compared to the control group. The number of entries in the open arm was not significant for the 100 mg/kg dose, but it was highly significant for the 300 mg/kg dose compared to the control for both doses. The animals treated with diazepam showed significant changes for all the parameters observed, and the Test (100 mg/kg) showed a significant decreased preference to the closed arm entries,

but there was a non-significant decrease in the closed arm entries for the 300 mg/kg dose. Similar was the case for mean time spent in the closed arm by the animals, displaying a significant decrease for the 100 mg/kg dose and non-significant decrease for the 300 mg/kg dose in comparison to the control group of animals. However, there was no significant difference shown in the entries at the centre of the maze for both the 100 mg/kg and 300 mg/kg doses. The table below gives a clear picture of the results observed.

Table II: Anxiolytic Activity Evaluation by Elevated Plus Maze Test in Mice.

Open Arm Entries	Time spent in open arm (sec)	Closed Arm Entries control	Time spent in closed arm (sec)	Entries at centre
4.50 ± 1.52	17.17 ± 4.91	15.50 ± 1.73	163.33 ± 6.16	20.00 ± 3.09
Standard [Diazepam (1 mg/ kg i.p.)]				
13.17 ± 1.14 ##	137.67 ± 6.66 ##	7.50 ± 1.12 ##	94.17 ± 9.90 ##	9.17 ± 1.14 ##
Test 1 (100 mg/kg)				
5.50 ± 0.76	95.67 ± 9.04**	8.00 ± 1.29**	119.17 ± 9.33**	14.50 ± 1.38
Test 2 (300 mg/kg)				
11.33 ± 1.14**	150.50 ± 8.59**	13.50 ± 1.09	164.67 ± 6.35	17.50 ± 1.18

Statistical significance test was done by ANOVA followed by Dunnett's test (n=6); Values are mean ± SEM of 6 animals per group; ** □ P < 0.01 vs control (for test groups), ## □ P < 0.01 vs control (for standard group).

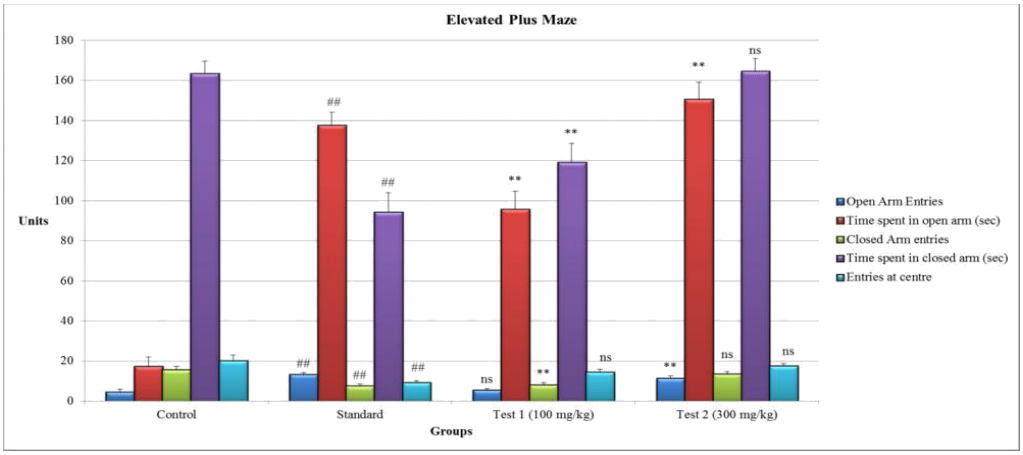

Figure 1: Anxiolytic Activity Evaluation by Elevated Plus Maze Test in Mice (n=6). Five different parameters as shown in the figure were evaluated in a time span of 5 minutes per animal.

Statistical significance test was done by ANOVA followed by Dunnett's test (n=6); Values are mean ± SEM of 6 animals per group; ** □ P < 0.01 vs control (for test groups), ## □ P < 0.01 vs control (for standard group).

Radial Arm Maze Test for Nootropic Activity Evaluation

In the radial arm maze experiment, there were three parameters evaluated – re-entries of an animal into the arms of the maze, the mean time taken by them to reach the food pellet placed in one of the arms throughout the experiment and the mean time taken by the animals to visit all eight arms of the maze once. These three parameters were evaluated daily for 11days, and the results of the 1st, 6th and 11th day taken into consideration for statistical evaluation.

On the first day, the amnesic control group showed an insignificant increase in all the three parameters, whereas the standard and Test (100 mg/kg) groups showed an insignificant decrease in the same, in comparison to the control group. There was an insignificant decrease in the re-entries and mean time taken to visit all eight arms for the Test (300 mg/kg) group, but a significant increase was seen by the same group in the meantime taken by the animals to get to the food pellet in the first attempt, compared to the control group.

The 6th day of evaluation displayed a similar pattern of results, showing significant increase again for the Test (300 mg/kg) group in the mean time taken to reach the food pellet by the animals, in comparison to control group. Another difference seen in the results for this day was a significant decrease in the mean time taken by the animals to reach the food pellet in the first attempt for the standard (piracetam 150 mg/kg, i.p.) group, with respect to the control group.

The 11th day of evaluation of the experiment displayed a significant increase in the mean time taken to reach the food pellet as well as the mean time taken to visit all eight arms by the animals for the amnesic control group (treated with scopolamine 1 mg/kg, i.p., on the 11th day) compared to control group. Significant reduction in the re-entries and the mean time taken to reach the food pellet was also displayed by the standard group of animals. Also, there was a highly significant decrease in the number of re-entries for the Test (100 mg/kg) group, and a similar decrease in the same parameter for the Test (300 mg/kg) group, but with an increase again by a significant value for the mean time taken to reach the food pellet by the animals in comparison to the control group.

Table III: Radial Arm Maze Test for Nootropic Activity Evaluation in Mice.

		Control	
	Re-entries	**Time taken to visit all eight arms pellet (sec)**	**Time taken to visit all eight arms (sec)**
Day 1	4.5 ± 0.76	22.67 ± 3.85	177 ± 8.95
Day 6	4.33 ± 0.67	22.17 ± 2.89	167.5 ± 7.77
Day 11	6.83 ± 1.08	26.67 ± 2.42	163 ± 7.7
Amnesic Control [Scopolamine (1 mg/kg i.p.)]			
	Re-entries	**Time taken to reach food pellet (sec)**	**Time taken to visit all eight arms (sec)**
Day 1	6.83 ± 0.65	29.5 ± 2.33	190.5 ± 5.15
Day 6	5 ± 0.58	19.17 ± 3.9	182.83 ± 4.51
Day 11	7.5 ± 0.43	54.5 ± 1.78 $^{\$\$}$	184.83 ± 3.53 $^{\$}$
Standard [Piracetam (150 mg/ kg i.p.)]			
	Re-entries	**Time taken to reach food pellet (sec)**	**Time taken to visit all eight arms (sec)**
Day 1	6.33 ± 1.2	24.83 ± 3.48	169.5 ± 6.02
Day 6	5 ± 1.13	8.17 ± 0.79 $^{\#\#}$	163.33 ± 6.04
Day 11	2.5 ± 0.56 $^{\#\#}$	18.83 ± 1.19 $^{\#}$	175.67 ± 5.5
		Test 1 (100 mg/kg)	
	Re-entries	**Time taken to reach food pellet (sec)**	**Time taken to visit all eight arms (sec)**
Day 1	6.17 ± 0.79	28.33 ± 2.63	141 ± 5.5
Day 6	4.33 ± 0.71	17.67 ± 1.63	150.17 ± 5.27
Day 11	2.83 ± 0.4**	22.83 ± 1.45	169.33 ± 4.01
		Test 2 (300 mg/kg)	
	Re-entries pellet (sec)	**Time taken to reach food**	**Time taken to visit all eight arms**
Day 1	4.33 ± 0.8	40.17 ± 3.47**	170.5 ± 5.79
Day 6	5.17 ± 1.08	33.83 ± 0.79**	163.83 ± 5.63
Day 11	3 ± 0.37**	34.67 ± 1.94*	167.67 ± 4.68

Statistical significance test was done by ANOVA followed by Dunnett's test (n=6); Values are mean ± SEM of 6 animals per group; $^{\$}$ □ $P < 0.05$ and $^{\$\$}$ □ $P < 0.01$ vs control (for negative control group), $^{\#}$ □ $P < 0.05$ and $^{\#\#}$ □ $P < 0.01$ vs control (for standard group), * □ $P < 0.05$ and ** □ $P < 0.01$ vs control (for test groups)

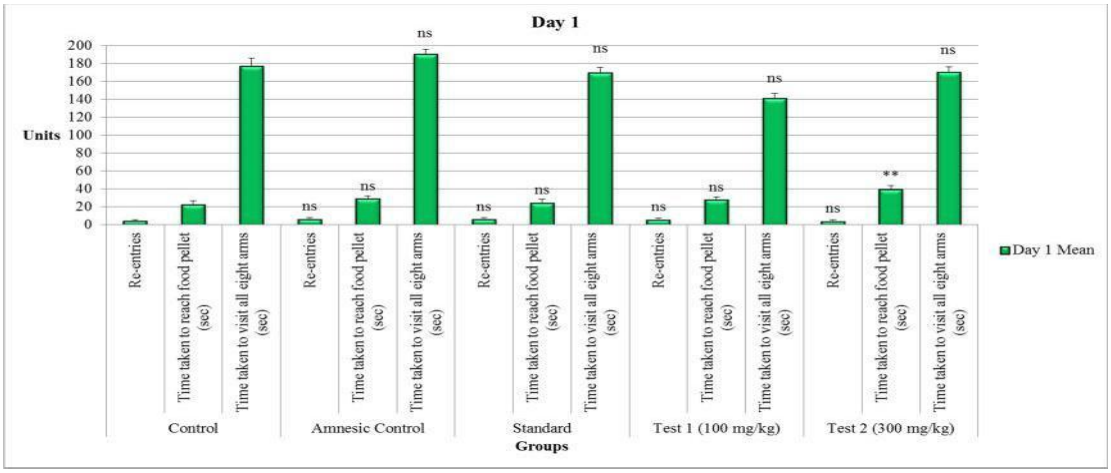

Figure 2: Radial Arm Maze Test for Nootropic Activity Evaluation in Mice [Day 1] (n=6).

Three different parameters as shown in the figure were evaluated for 11 days for each animal. Statistical significance test was done by ANOVA followed by Dunnett's test (n=6); Values are mean ± SEM of 6 animals per group; $ □ P < 0.05 and $$ □ P < 0.01 vs control (for negative control group), # □ P < 0.05 and # □ P < 0.01 vs control (for standard group), * □ P < 0.05 and ** □ P < 0.01 vs control (for test groups).

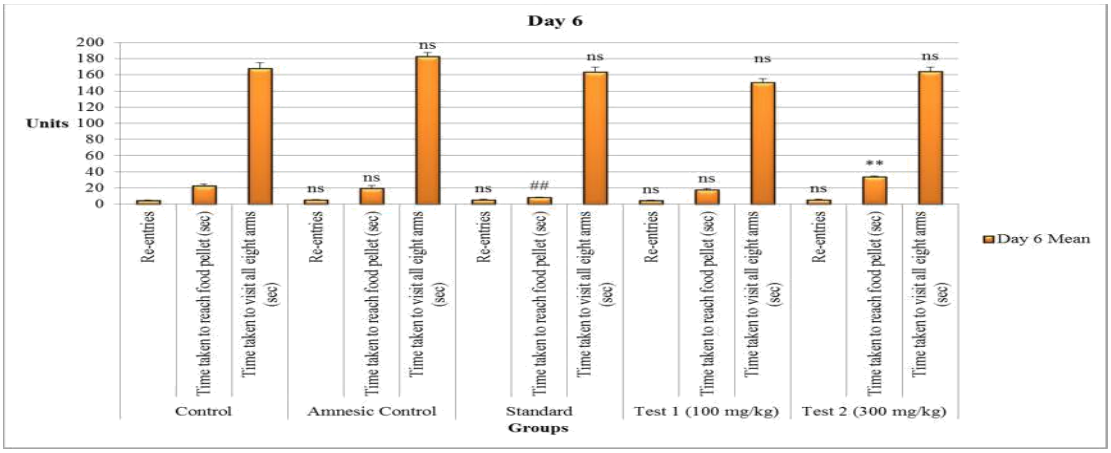

Figure 3: Radial Arm Maze Test for Nootropic Activity Evaluation in Mice [Day 6] (n=6). Three different parameters as shown in the figure were evaluated for 11 days for each animal.

Statistical significance test was done by ANOVA followed by Dunnett's test (n=6); Values are mean ± SEM of 6 animals per group; $ □ P < 0.05 and $$ □ P < 0.01 vs control (for negative control group), # □ P < 0.05 and ## P < 0.01 vs control (for standard group), * □ P < 0.05 and ** □ P < 0.01 vs control (for test groups).

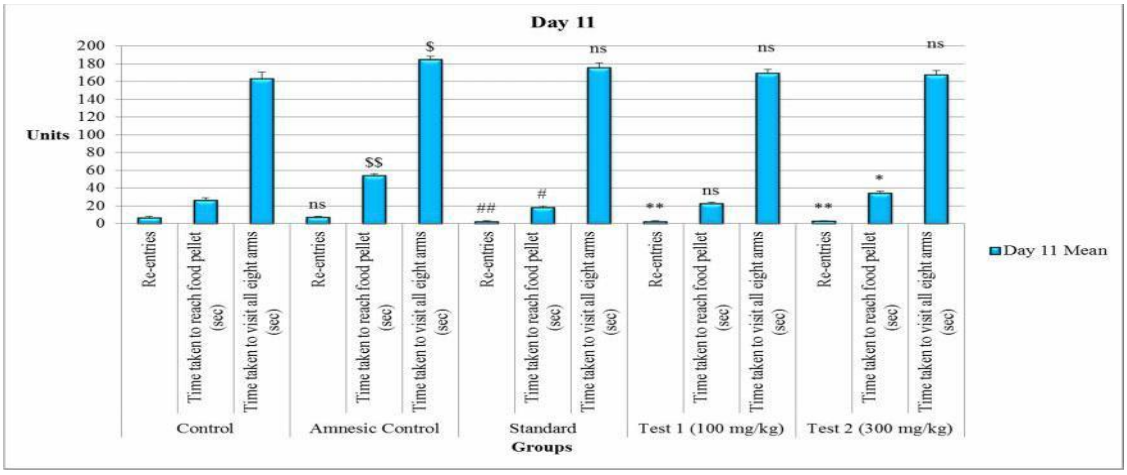

Figure 4: Radial Arm Maze Test for Nootropic Activity Evaluation in Mice [Day 11] (n=6).

Three different parameters as shown in the figure were evaluated for 11 days for each animal.

Statistical significance test was done by ANOVA followed by Dunnett's test (n=6); Values are mean ± SEM of 6 animals per group; $^{\$}$ □ $P < 0.05$ and $^{\$\$}$ □ $P < 0.01$ vs control (for negative control group), $^{\#}$ □ $P < 0.05$ and $^{\#\#}$ $P < 0.01$ vs control (for standard group), * □ $P < 0.05$ and ** □ $P < 0.01$ vs control (for test groups)

Conditioned Place Preference Test

In the Conditioned Place Preference model, the animals were subjected to three phases - Preconditioning, Conditioning and Conditioned Place Preference (CPP) test. The statistical evaluation could be performed for the preconditioning and CPP phases as the animals had the liberty to move freely through the three compartments (zone A, zone B and zone C) of the CPP chamber in these two phases.

The preconditioning phase of the experiment did not show any significant change in the amount of time that the test animals spent in any of the zones in comparison to the control animals, but it showed a similar pattern of behaviour displayed by the animals administered both the Test 1 (100 mg/kg) and Test 2 (300 mg/kg) doses of the *S.sesban*, as animals from all the three groups preferred to stay in the saline-paired chamber (zone A) for most of the time of the preconditioning phase, proving that the environment of that chamber was most comfortable for them.

In the CPP test phase, the animals of the Test 1 (100 mg/kg) group did not show any significant difference in the time spent in the three zones as compared to the control group of animals, but for the Test 2 (300 mg/kg) dose, there was a significant rise in the time spent by the animals in the saline-paired chamber (zone A) and a significant fall in the time spent by them in the neutral chamber (zone B). The time spent by the animals in the drug-paired chamber (zone C) was very less throughout the experiment, in all the phases.

Table IV: Conditioned Place Preference Testing in Wistar rats.

Control			Test 1			Test 2		
Zone A (Saline-paired chamber)	**Zone B (Neutral chamber)**	**Zone C (Drug-paired chamber)**	**Zone A (Saline-paired chamber)**	**Zone B (Neutral chamber)**	**Zone C (Drug-paired chamber)**	**Zone A (Saline-paired chamber)**	**Zone B (Neutral chamber)**	**Zone C (Drug-paired chamber)**
			Preconditioning (seconds)					
821.33 ± 6.67	58.67 ± 5.26	18.33 ± 5.75	826.67 ± 8.60	53.83 ± 6.29	19.50 ± 3.42	843.00 ± 5.49	44.67 ± 4.76	17.00 ± 3.71
			CPP test (seconds)					
826.50 ± 2.60	60.33 ± 3.39	13.17 ± 2.27	840.33 ± 5.11	47.17 ± 4.44	12.50 ± 4.68	850.67± 5.99**	32.83 ± 3.75**	16.50 ± 3.51

Statistical significance test was done by ANOVA followed by Dunnett's test (n=6); Values are mean ± SEM of 6 animals per group; ** □ P < 0.01 vs control (for test groups)

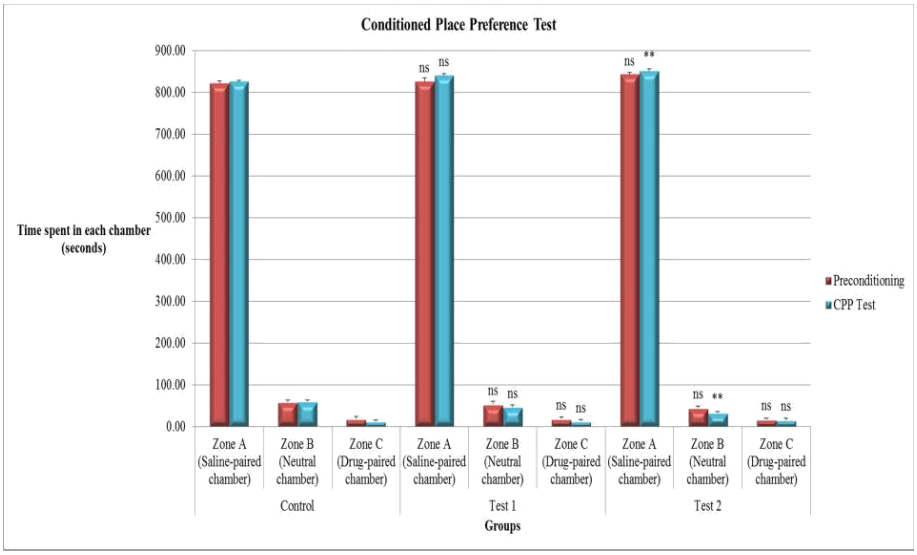

Figure 5: Conditioned Place Preference Testing in Wistar rats (n=6). The results of preconditioning and conditioned place preference testing are represented in the graph.

Statistical significance test was done by ANOVA followed by Dunnett's test (n=6); Values are mean ± SEM of 6 animals per group; ** □ P < 0.01 vs control (for test groups)

Estimation of the concentration of the neurotransmitter dopamine in rat brains by UV-Visible Spectroscopy

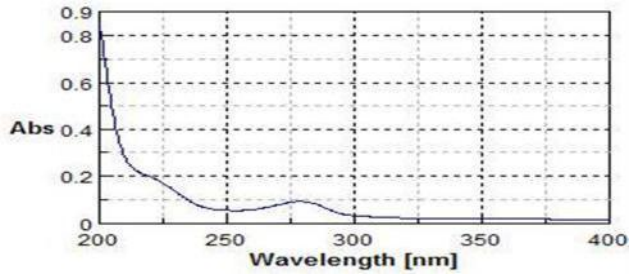

Figure 6: Standard Peak of Dopamine (obtained at 278.9 nm).

Table V: Estimation of dopamine in rat brain (Absorbance values for standard dopamine).

Concentration (ng/mL)	Absorbance
50	0.07
100	0.09
200	0.11
300	0.14
400	0.16
500	0.18

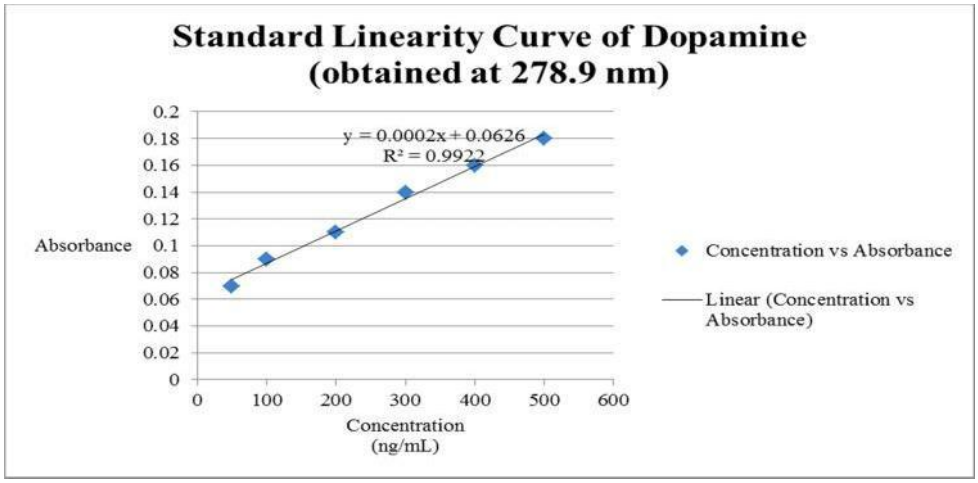

Figure 7: Standard Linearity Curve of Dopamine (obtained at 278.9 nm) Equation of linearity curve of dopamine: y = 0.0002x + 0.0626.

Table VI: Estimation of dopamine in rat brain (Absorbance values for test animals).

y	*M*	*c*	*X*	*x*10 (A)*	*A/5 (B)*	*B/2 (C)*	*y*
Control	0.07031	0.0002	0.0626	38.55	385.50	77.10	38.55
Test 1 (100 mg/kg)	0.07066	0.0002	0.0626	40.30	403.00	80.60	40.30
Test 2 (300 mg/kg)	0.07104	0.0002	0.0626	42.20	422.00	84.40	42.20

where,

y =Absorbance of dopamine

x=Concentration (ng/mL) of dopamine [initially diluted 10 times]□A □ Original concentration of dopamine [for 5 animals per group] B □ Concentration of dopamine per animal [brain weight = 2 grams] C □ Concentration of dopamine in ng/g of brain weight

DISCUSSION

From the overall results of all the animal models as well as the pharmacokinetic studies, we can say that the extract comprising of a combination of various phytochemical constituents like alkaloids, flavonoids, glycosides, polypeptides, steroids, etc. display multiple effects even when used as a combination.

The elevated plus maze model for evaluating the anxiolytic activity also showed significant increases for the open arm entries and the time spent in the open arm of the maze, but with an irregular pattern of results for the closed arm of the maze, showing significant decreases in the closed arm entries and the time spent in the closed arm for the 100 mg/kg dose but no significant decrease for the 300 mg/kg dose. However, the increase in the occupancy by the animal in the open arms of the maze proves that the extract of *S.sesban* possesses considerable anxiolytic properties, due to the synergistic effect of its individual components.

The radial arm maze model for evaluating nootropic activity in mice was also conducted for 11 consecutive days after selection of the animals, and the results of the 1st, 6th and 11th days were reported. There were irregular patterns of nootropic behaviour displayed by the animals, with significant reductions shown only in the re-entries and the time taken to reach the food pellet in the first attempt. However, there was no significant increase or decrease displayed by the animals in the time taken by them to visit all the eight arms of the maze atleast once. Hence, it can be said that the extract shows nootropic activity to some extent, but still more research related to finding out the magnitude of nootrpic activity is essential.

In the conditioned place preference model to determine the addiction potential of the extract, it was observed that the animals spent more than 75-80% of their time in zone A (which is the saline paired chamber), some amount of time in zone B (which is a neutral chamber), and the least amount of time in zone C (which is the drug-paired chamber). This behaviour of the animals was seen in both the pre-conditioning phase as well as during the conditioned place preference test, which proves that the *S.sesban* does not cause any addiction in the animals with respect to the environment associated with the testing conditions. Therefore, the extract

of *S.sesban* proves to be safe and not a substance of abuse on its repeated & long-term administration.

Finally, in the pharmacokinetic parameter of brain dopamine estimation, it is observed that with respect to the dopamine level observed in the animals from the control group, there is only a slight increase in the brain dopamine level in the animals from the test 1 (100 mg/kg) group and the test 2 (300 mg/kg) group, which does not make it clear whether the slight rise in the dopamine level is due to the extract or any other factor/factors. But from the other effects of the extract of *S.sesban* observed above, there is a high probability of the slight elevation of dopamine being due to other factors and not due to the extract itself. This can also mean that the slight increase in nootropic activity observed in the radial arm maze model may be due to the actions of other memory-related neurotransmitters like acetylcholine, glutamate and nitric oxide, but not due to dopamine.

CONCLUSION

Natural products and indigenous drugs provide a wide spectrum of activities since they contain multiple chemical constituents like alkaloids, flavonoids, glycosides, polypeptides, steroids, etc. which together or individually produce favourable therapeutic response. The list of important active ingredients from herbal sources was very exhaustive and the herbs have provided many leads for chemical synthesis of drugs like atropine, scopolamine, codeine, etc. Promising drugs have been discovered over the years which show various activities like anxiolytic, antidepressant, nootropic, sedative, etc.

From the review of literature on plant products used in medicine, one can get an idea of the multiple activities shown by medicinal plants. In the present work, we have carried out the evaluation of neuropharmacological actions of a S.sesban extract as they possess several activities like emollient, aphrodisiac, tonic, antidiarrhoeal, anthelmintic, antimicrobial, antipyretic, etc.

Further, the effects of extract of *S.sesban* were studied in three animal behaviours, such as elevated plus maze, radial arm maze and conditioned place preference test to investigate its possible neuropharmacological activity.

These tests are models for screening central nervous system actions providing information about anxiety, memory and addiction potentials.

The elevated plus maze is considered to be an etiologically valid animal model of anxiety because it uses natural stimuli (fear of a novel open space and fear of balancing on a relatively narrow, raised platform) that can induce anxiety in humans [Dawson and Tricklebank, 1995].[19] An anxiolytic agent increases the frequency of entries into the open arms and increases the time spent in the open arms of the elevated plus maze. In the present study, oral administration of the extract of *S.sesban* under study induced a dose-dependent anxiolytic effect in mice, as it increased the number of entries and the time spent on the open arms.

Nootropic drugs facilitate intellectual performance, learning and memory [Giurgea, 1973].[20] However, the neurological basis of such an action is not known. Although the involvement of the cholinergic system is well established, the role of other neurotransmitters like glutamate and nitric oxide cannot be ignored [Hollander et al., 1986].[21] In the radial arm maze test, the re-entries of animals into the same arms they had entered earlier, the time taken by them to reach the food pellet placed in one of the arms in the first attempt, and the time taken by them to complete entering into all the eight arms of the maze atleast once was recorded for 11 days continuously. The results obtained showed only a slight significance in the re-entries and time taken by the animals to reach the food pellet, but no significance in the time taken to visit all eight arms of the maze. Therefore, it suggested a very slight nootropic activity displayed by the extract, probably due to factors other than dopamine elevation in the brain.

The conditioned place preference test is a model used to assess the addictive properties of various drugs. The animals are placed in a three-chambered rectangular compartment with doors between every adjacent chamber, in which the two chambers at the two ends have a different physical appearance and thereby appear like two different environments to the animals. The animals are dosed with saline and the drug every alternate day for 8-10 days and are alternated between the two end chambers daily, so that all the drug-paired animals always remain in one of the end chambers and the saline-paired animals always remain in the other end chamber. Statistical analysis of the results obtained for this test showed that all the animals preferred the saline-paired chamber over the drug-paired chamber whenever they were given the freedom to move in all three chambers, thereby showing a conditioned place aversion (CPA) to the extract of *S.sesban* under study, stating that the long-term administration of the extract does not cause addiction.

Also, the estimation of brain dopamine levels in rats for this study by UV-Visible spectroscopy showed that administration of the extract of *S.sesban* under consideration caused a very small increase in the dopamine levels in the brain compared to the control, almost negligible, which cannot be necessarily attributed to the extract itself. This negligible increase could be due to other factors elevating brain dopamine levels, and not the extract being administered. There is need for further research on this parameter with more sophisticated techniques in the near future, which will enable the qualitative and quantitative estimation of all neurotransmitters present in the brain.

Therefore, in conclusion, the extract of *S.sesban* was found to possess anxiolytic, partly nootropic and non-addictive properties in a combination, and should be evaluated further for biochemical actions to understand the mechanism of action and other pharmacological and neuropharmacological effects to evaluate its claims and its therapeutic value.

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