ANXIOLYTIC ACTIVITY OF *SEMECARPUS ANACARDIUM (*LINN.) NUT EXTRACT IN MICE.

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Summary

The present study was undertaken to evaluate **anxiolytic** activity of chloroform extract of *Semecarpus anacardium* (Linn.) (CHSA) by using mice. This plant contains steroids, tannins, saponins, flavonides and glycosides etc. Anxiolytic effect of CHSA (100, 200 and 400 mg/kg) was studied and diazepam used as a standard drug by using following animal models, Elevated Plus Maze (EPM), Open-Field Test (OFT) and Light-Dark Transition Test (LDT)**.** On EPM, the diazepam had showed significant anxiolytic activity by increasing open arm entries and time spent in open arm but CHSA was ineffective. In OFT increased total locomotion, central locomotion and decreased number of rearings, immobility time was observed with diazepam and in extract not showed the same effect. In LDT model the anxiolytic activity was observed with diazepam by increasing latency, number of crossings, time spent in light box and decreasing rearings in light box was observed. However it was found that the extract has no anxiolytic activity.

Keywords: *Semecarpus anacardium* (Linn.) Elevated Plus maze, diazepam, anxiolytic activity.

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Introduction

Anxiety is a cardinal symptom of many psychiatric disorders closely allied with appropriate fear and often serving psycho biologically adoptive purposes and is rather infrequently "disease" itself. It is typically associated with the former "psychoneurotic" disorders, hypothesis implicates over activity of adrenergic systems or dysregulation of serotonergic systems in the CNS and symptoms are of anxiety commonly associated with depression[1].

Anxiety affects one-eighth of the total, population worldwide and has become an important area of research in psychopharmacology during this decade. Benzodiazepines (BZDs) are the major class of compounds used in anxiety and they remain the most commonly prescribed treatment for anxiety. However, the realization that BDZs have a narrow safety margin has promoted many researchers to evaluate new compounds in the hope of identifying other anxiolytic drugs with fever unwanted effects[2].

In the present study we selected a plant namely *Semecarpus anacardium* (Linn.) (*S. anacardium*) belonging to the family of Anacardiaceae. It is distributed in the sub-Himalayan tract from the Bias eastwards, ascending in the outer hills up to 3,500 ft., Assam, Khasia hills, Chittagong, Central India and the Western Peninsula. The fruit is acrid in taste, hot, sweetish. In traditional system of medicine it is used as a digestible, aphrodisiac, anthelmintic laxative. It also used treat skin diseases, piles, dysentery, tumors, fevers, loss of appetite, urinary discharges, heals ulcers, and strengthens the teeth, useful in insanity, asthma. The oil is tonic, makes hair black, good for leucoderma, coryza, epilepsy and other nervous diseases. It lessens inflammation, useful in paralysis and superficial pain[3].

Earlier the plant has been studied for its analgesic and anti-inflammatory[4], anti-arthritic[5], antimicrobial[6], antibacterial[7], anthelmintic[8], antimutagenic[9], antidiabetic[10], antitumour[11], antioxident[12], fungistatic[13], hepatocellular crcinoma[14-16], hypocholesterolemic[17], hypolipidemic[18], immunomodulatory[19], and mammary carcinoma[20] activities.

Considering the varied important activities reported in traditional system of medicine with this plant. It was planned to study the effects of nut extract of *Semecarpus anacardium* (Linn.) on CNS mainly for its anxiolytic activity.

Materials and methods

Drugs and Chemicals: Diazepam (Ranbaxy Laboratories Ltd.), PEG (Lobo Cheme PVT. Ltd., Mumbai).

Animals: Albino mice weighing between 18-22 g of either sex were used for in this study. All the animals were procured from Shri Venkateswara Enterprises, Bangalore for experimental purpose. After procuring, all the animals were acclimatized for 7 days and housed in groups of six under standard husbandry condition[21] like room temperature 26 ± 2^0C, relative humidity 45-55% and light/ dark cycle of 12 h.

All the animals were fed with synthetic standard diet (Pranava Agro Industries Ltd., Bangalore.) and water was provided *ad libitum* under strict hygienic conditions. After obtaining permission from Institutional Animal Ethical Committee (IAEC) of T.V.M. College of Pharmacy, Bellary (Karnataka), animal studies were performed as per rules and regulations in accordance to guideline of CPCSEA with R. No. 462/01/CPCSEA, 2001.

Plant material: The nuts of *Semecarpus anacardium* (Linn.) was collected from local market of Bellary (Karnataka State) and authenticated and identified by a Botanist Dr. Govind Raju of A.S.M. College Bellary. The nuts were dried in shadow and slices of nuts were subjected to size reduction by using mixy, to coarse powder.

Preparation of chloroform extract[22, 23]: The air dried nuts were extracted successively with the following solvents of their increasing polarity in a soxhlet extractor. 1) Pet. Ether (60-80%), 2) Chloroform, 3) Alcohol. After alcoholic extraction macerated the mark with chloroform water for 24 h to obtained the aqueous extract. Concentrate the each extract solvent by using flash evaporator to dryness on the water bath in low heat. Weighed the residue obtained with each solvent and determine its % in terms of air dried weight to the nut material (% w/w) to obtained successive solvent extractive values. On the basis of % yield highest percentage of the extract was selected for the study.

Preliminary phytochemical screening[22, 23]: The preliminary phytochemical investigations were carried out with CHSA for qualitative identification of phytochemical constituents. Phytochemical tests were carried out by standard methods**.** All the chemicals and reagents used were of analytical grade.

Determination of acute toxicity (LD_{50})[24]**:** The acute toxicity of CHSA was determined by using albino mice of either sex (18-22 g). The animals were fasted 3 h prior to the experiment, Acute Toxic Class method (OECD guideline No. 423) of CPCSEA was adopted for toxicity studies. Animals were administered with single dose of extract and observed for its mortality during 48 h study period (short term toxicity). Based on short-term toxicity profile of extract the dose for the next animal was determined as per as OECD guideline No.423.

Anxiolytic activity

Elevated plus-maze (Exteroceptive behavior model) [25-27]**:** The plus-maze apparatus comprises of two open arms (16×5 cm) and two closed arms (16×5×12cm) that extend from a common central platform (5×5 cm). The entire maze is elevated to a height of 25 cm above the floor level. Albino mice (18-22 g) of either sex were divided into 05 groups of 06 mice in each was fasted overnight prior to the test but water was supplied *ad libitum*. Group I was maintained as normal control which was given with 3% PEG (10 ml/kg p.o.) only once daily for 7 days, group II received diazepam (2 mg/kg, i.p.) 30 min before the test. Groups III, IV and V were treated with different doses of CHSA (100, 200 and 400 mg/kg, p.o.) respectively once daily for 7 days. On 7th day 60 min after administration of the vehicle or extract and 30 min of standard each mouse was placed in the center of the maze facing one of the open arms. During a 5 min test session, the following parameters were noted.

- Number of entries into open arm
- Number of entries into closed arm
- Time spent in the open arm
- Time spent in the closed arm and
- Total number of entries in open and closed arms

Open-Field Test[28]**:** This method is used to evaluate exploratory activity and emotionality of animal. The open field consisted of a white painted arena measuring 55 cm in diameter with 100 W lamp. The floor of the arena will be divided into several units by black painted lines. The apparatus will be placed in a sound attenuated room, 48 cm above the floor. Albino mice (18-22 g) of either sex were divided into 05 groups of 06 mice in each was fasted overnight prior to the test but water was supplied *ad libitum*. Group I was maintained as normal control which was given with 3% PEG (10 ml/kg p.o.) only once daily for 7 days, group II received

diazepam (2 mg/kg, i.p.) 30 min before the test. Groups III, IV and V were treated with different doses of PEG (100, 200 and 400 mg/kg, p.o.) respectively once daily for 7 days. On 7th day 60 min after administration of the vehicle or extract and 30 min of standard each mouse was placed in the center of open field arena and the following parameters were recorded during a test session of 5 min.

- Total locomotion (number of units entered on the floor)
- Central locomotion
- Rearing frequency (number of times the animal stood on its hind legs)
- Immobility time

Light-Dark Transition test model[29-31]: The light-dark apparatus consists of two-compartment chamber (40×60×20cm/h) comprising of a brightly illuminated area (40×40 cm) and a dark area (40×20 cm) separated by a wall with a round hole (7 cm diameter) will be used. Albino mice (18-22 g) of either sex were divided into 05 groups of 06 mice in each was fasted overnight prior to the test but water was supplied *ad libitum*. Group I was maintained as normal control which was given with 3% PEG (10 ml/kg p.o.) only once daily for 7 days, group II received diazepam (2 mg/kg, i.p.) 30 min before the test. Groups III, IV and V were treated with different doses of CHSA (100, 200 and 400 mg/kg, p.o.) respectively once daily for 7 days. On 7th day 60 min after administration of the vehicle or extract and 30 min of standard each mouse was placed in the center of open field arena and the following parameters were recorded during a test session of 5 min. At the starting of the experiment, the mouse was placed in the illuminated part of the cage. The following parameters were recorded during the test session of 5 min.

- Latency to the first crossing into the dark compartment
- Number of crossings between the light and dark areas
- Total time spent in the illuminated part of the cage

Statistical analysis: The results obtained with various experiments were subjected to statistical analysis by using One-way ANOVA followed by ***Dunnett*** test to assess the significance difference if any among the groups and $P<0.05$ was considered as significant.

Results

Preliminary phytochemical screening: Preliminary phytochemical study was carried out for the presence of various active constituents in the extract. (Table1)

Table 1. Phytochemical screenings of various extracts of *S.anacardium*.

S No.	Phytoconstituents	Successive extraction			
		Pet. Ether extract	Chloroform extract	Alcoholic extract	Aqueous extract
1	Steroids	+	+	–	–
2	Tannins	–	–	+	+
3	Flavanoids	–	–	+	+
4	Saponins	–	–	+	+
5	Alkaloids	–	+	+	–
6	Glycosides	–	–	+	+
7	Carbohydrates	–	–	–	+
8	Starch	–	–	–	+
9	Proteins and amino acids	–	–	+	+
10	Fats and oils	+	+	–	–

Note: '+' Present, '-' Absent

Acute toxicity studies: The extract produced no mortality up to 2000 mg/kg. So 1/5th, 1/10th, and 1/20th of LD_{50} doses were selected for the present study.

Effect of *S.anacardium* on EPM (Exteroceptive behavioral model) in mice: Diazepam has long been reported for its anxiolytic activity in mice with the EPM model. In our study also, a significant anxiolytic effect was recorded with diazepam as increased number of entries in to open and decreased number of entries in to closed arms and with increased time spent in open and central platform but not in closed arms. But in all doses of CHSA i.e. 100, 200 and 400 mg/kg were not exhibited anxiolytic activity as compare to diazepam. (Table 2)

Effect of *S.anacardium* on OFT in mice: Diazepam has long been reported for its anxiolytic activity in mice with the OFT model. In our study also, a significant anxiolytic effect was recorded with diazepam as increased number of total locomotion, central locomotion and decreased number of rearings immobility time. Different doses i.e., 100, 200 and 400 mg/kg of CHSA were subjected for anxiolytic activity using in this model. An insignificant effect anxiolytic activity was observed on total locomotion, central locomotion, rearings and immobility time. (Table 3)

Effect of *S.anacardium* on LDT in mice: These three different doses of CHSA (100, 200 and 400 mg/kg) when administered orally, didn't produced an increase in number of crossings, time spent in light box and decrease in the number of rearings in light compartment. Standard drug diazepam (2 mg/kg) had exhibited significant anxiolytic activity by an increase in number of crossings, time spent in light box and decrease in the number of rearings in light compartment.

Table 2. Effect of *S.anacardium* on EPM in mice.

Group No	Treatment	Dose (Per kg)	No. of entries in 5min session		Time spent in 5min session		Total No. of entries Mean ± SEM
			Open arm Mean ± SEM	Closed arm Mean ± SEM	Open arm Mean ± SEM	Closed arm Mean ± SEM	
Group I	Control (3% PEG)	10 ml p.o.	3..33±0.42	16.5±0.56	26.00±0.36	16.5±0.56	20±0.89
Group II	Diazepam	2mg i.p.	8.66±0.49**	2.66±0.21**	173.00±0.85**	2.66±0.21	11.33±0.66**
Group III	CHSA	100 mg p.o.	4.66±0.33ns	15.83±0.79 ns	29.5±1.28 ns	16.5±0.56 ns	20.5±0.99 ns
Group IV	CHSA	200 mg p.o.	4.16±0.40 ns	17.16±1.01ns	28.83±1.24 ns	16.83±0.94 ns	21.16±1.30 ns
Group V	CHSA	400 mg p.o.	4.00±0.51 ns	16.16±1.30 ns	29.16±1.07 ns	17.5±0.67 ns	20.16±1.40 ns
One-Way ANOVA		F	23.439	51.272	3994.4	99.657	14.322
		DF	29	29	29	29	29

n=6.Significance at P<0.05*, <0.01** and ns-not significant.

Table 3. Effect of *S.anacardium* on OFT in mice.

Groups No	**Treatment**	**Dose (Per kg)**	**Total locomotion Mean ± SEM**	**Central locomotion Mean ± SEM**	**Rearings Mean ± SEM**	**Immobility time Mean ± SEM**
Group I	Control (3% PEG)	10 ml p.o.	101.16±1.276	13.16±0.30	28.5±0.99	19.5±0.42
Group II	Diazepam	2 mg i.p.	210.5±2.202**	46.66±0.42**	7.33±0.49**	7.33±0.42**
Group III	CHSA	100 mg p.o.	102.66±1.606 ns	13.33±0.33 ns	29.16±0.79 ns	20.66±0.76 ns
Group IV	CHSA	200 mg p.o.	103.83±1.62	14.16±0.60	30.33±1.02 ns	20.83±1.13 ns
Group V	CHSA	400 mg p.o.	101±1.63ns	14.83±0.90 ns	30.16±0.94 ns	22.33±1.96 ns
One-Way ANOVA		F	818.32	685.36	130.72	30.820
		DF	29	29	29	29

n=6.Significance at P<0.05*, <0.01** and ns-not significant.

Table 4. Effect of *S. anacardium* on LDT Test in mice.

Groups No	**Treatment**	**Dose (Per kg)**	**Latency Mean ± SEM**	**Number of crossings Mean ± SEM**	**Time spent in L. box in 5min session Mean ± SEM**	**No. of Rearings Mean ± SEM**
Group I	Control (3% PEG)	10 ml p.o.	10.50±0.56	7.50±0.50	97.0±0.44	12.83±0.54
Group II	Diazepam	2 mg i.p.	27.50±0.71**	11.66±0.55**	162.33±2.49**	1.83±0.30**
Group III	CHSA	100 mg p.o.	10.33±0.84ns	7.50±0.34ns	102.17±3.89ns	12.83±0.79ns
Group IV	CHSA	200 mg p.o.	10.50±0.84ns	7.83±0.60ns	95.16±3.64ns	10.16±1.04ns
Group V	CHSA	400 mg p.o	11±0.57ns	7.83±0.47ns	97.50±7.108ns	13.50±0.80ns
One-Way ANOVA		F	110.42	12.74	48.907	42.89
		DF	29	29	29	29

n=6.Significance at P<0.05*, <0.01** and ns-not significant

Discussion

The etiology of most anxiety disorders are not fully understood, but various studies has shown the involvement of GABAergic serotonergic neurotransmission in etiology, expression and treatment of anxiety. The adrenergic and dopaminergic systems have also been shown to a role of in anxiety[32].

Serotonergic hypothesis of fear or anxiety behavior proposes that in stressonergic or threating situations the serotonergic system activity increases where as the reduction of serotonergic systems exerts anxiolytic like effects[33].

The **EPM** test is a well-established animal model for testing anxiolytic as well as nootropic drugs irrespective of parameters observed[34, 35], using diazepam a standard anxiolytic drug. The EPM test is based on a premise where the exposure to an EPM evoked approach-avoidance conflict that was considerably stronger than that evoked by the exposure to an enclosed arm[36]. The decreased aversion to the open arm is the result of anxiolytic effect, expressed by the increase in time spent and total number of entries in to the open arms. The animals treated with CHSA had not showed anxiolytic effect by observing parameters like did not increase the time spent and number of entries in to the open arms with a decrease in total number of entries into the closed arms.

In the **OFT**, the confrontation with the situation induces anxiety behavior in rodents. The anxiety behavior is triggered by two factors, i.e., individual testing as the animal was separated from its social group and agoraphobia, as the arena is very large, relative to the animals breeding or the natural environment. In such situations rodents show thigmotaxic behavior identified by spontaneous preference to the periphery of the apparatus and reduced

ambulation[28]. Anxiolytic treatment decreases this anxiety-induced inhibition of exploratory behavior. However CHSA did not have any significant effect on these parameters such as total locomotion, central locomotion and rearings than control group.

The **LDT** may be useful to predict the anxiolytic like activity of drugs in mice. It has the advantages of being quick and easy to use without food and water deprivation prior training of animals and natural stimuli are used. Transitions have been reported to be an index of activity exploration because of habituation over time and the time spent in each compartment to be a reflection of aversion[36].

In LDT test, the apparatus contains two compartments i.e. light and dark. Animals always try to spend more time in dark compartment because of fear about new environment. In this model, four behavioral events were observed i.e. latency, number of crossings to light compartment, time spent in light box and number of rearings in light box. In this study the CHSA not significantly increased the time spent in light compartment; reduced time spent in dark compartment, number of crossings and decreased the number of rearings in light compartments, indicating that extract ineffective.

In present investigation the extract was ineffective to reduce anxiety, in all these animal models EPM, OFT and LDT test. Finally it is concluded that the chloroform nut extract of *Semecarpus anacardium* (Linn.) has no anxiolytic activity.

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