A STUDY ON CNS EFFECTS OF MILK EXTRACT OF NUTS OF *SEMECARPUS ANACARDIUM. LINN*, (ANACARDIACEAE).

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Summary

The main objective of the proposed work is to evaluate the beneficial effect of nuts of *S. anacardium, Linn.* extracted with milk, on CNS mainly for its locomotor and nootropic activities in different experimental animal models. Dried nuts of *S. anacardium* were extracted using Siddha method of extraction and the extract subjected for preliminary phytochemical tests. LD_{50} studies for the extract were conducted up to 2 g/kg dose by following OECD guidelines No. 425. The locomotor activity studied using photoactometer and the nootropics activity was recorded in both interocepitve and exteroceptive models of amnesia. The milk extract of *S. anacardium* was positively answered for flavonoids, carbohydrates and phenolic compounds. The extract was subjected for LD_{50} value and even upto 2 g/kg has not produced any lethal effect. No significant alteration in locomotor activity was observed with all the doses (75, 150 and 225 mg/kg) of the extract tested but a slight CNS depressant effect was noted with only 150 mg/kg of the extract. The extract was tested on EPM at different dose levels and all the doses (75, 150 225 and 300mg/kg) have shown an increase in inflexion ratios. But statistically significant results were observed with 150 and 225 mg/kg treated groups only. When tested on Passive avoidance paradigm, a significant increase in SDL values were recorded with 75 and 225 mg/kg treated groups but not with 150 mg/kg treated group. Piracetam had significantly reversed the scopolamine-induced amnesia but the extract failed to show a similar effect. In diazepam and electroshock-induced amnesia models both Piracetam and the extract had reversed the amnesia but effect with the extract was found to be statistically insignificant. The milk extract of nuts of *S. anacardium* was found to possess nootropic activity.

Key words: *S. anacardium;* Nut extract; Locomotor activity; Nootropic activity

Introduction

Alzheimer's disease (AD) is a leading cause of dementia in developed countries and primarily affects the elderly persons and currently about 18 million people in the worldwide are suffering with dementia. The prevalence increases with age and may reach nearly 30 to 50% in those with age more than 85 years old. Epidemiological studies on Indian population reveal that dementia is largely a hidden problem and the number is increasing. Especially in rapidly developing and heavily populated regions such as India, China and Latin America.

The Indian system of medicine is replete with medicinal plants claimed to promote learning, memory and intelligence[1]. Plants like *Bacopa monniera*, *Azadirachta indica*, *Withania somnifera*, as well as *Ocimum sanctum* have been investigated for their effect on cognitive functions of the brain[2,3,4,5]. These plants have been grouped under the general class of rejuvenators i.e., drugs that counter the degenerative changes associated with ageing. In recent years, there has been phenomenal rise in the interest of scientific community to explore the pharmacological actions or to confirm the veracity of claims made about herbs in the official books of Ayurveda and Siddha.

Semecarpus anacardium, Linn.[6,7,8,9,10] is a deciduous tree growing on the sub-Himalayan and tropical parts of India, easily recognized by large leaves and the red blaze exuding resin, which blackens on exposure. It is frequently found in drier rather than damp localities. It is commonly known as Bibha, Bhallataka, Marking nut and is well known for its corrosive juice and vesicant nature, is being used in different ways by several groups of physicians. Ayurveda describes it as a potent drug against a variety of ailments and is popularly known as ***'Ardha Vaidya***[11,12].

Semecarpus anacardium Linn, (Anacardiaceae), a plant traditionally used to treat brain diseases, improve memory, as a rejuvenating drug and also used for neurological disorders. The nut extract of *S. anacardium* has been reported for antiarthritic, immunomodulatory, anti-inflammatory and antioxidant activities, and it has been proved that drugs with anti-inflammatory and antioxidant activities are reported to promote memory which prompted us to study the nootropics activity of milk extract prepared with dried nuts.

Methods

Drugs and chemicals:

Piracetam ('Neurocetam syrup', Brown & Burk.), Scopolamine ('Hyoscine' German Remedies, India), Diazepam ('Calmpose' Ranbaxy, India) and Olive oil ('Figaro', Madrid, Spain). Other chemicals used in the study were of analytical grade.

Experimental animals:

Albino mice of either sex weighing between 18-22 gm were used in this study. All the animals were procured from Shri Venkateswara Enterprises, Bangalore for experimental purpose. After procuring, the animals were acclimatized for 7 days and housed in groups of six under standard husbandry condition[13,14] like room temperature 26 ± 2^0C, relative humidity 45-55% and light/ dark cycle of 12 hours. All the animals were fed with synthetic standard diet (Amrut Laboratories Pranava Agro Industries Ltd. Sangli) and water was supplied *ad libitum* under strict hygienic conditions. After obtaining permission from Institutional Animal Ethical Committee (IAEC) of V. L. College of pharmacy Raichur (Karnataka), animal studies were performed as per rules and regulations in accordance to guidelines of CPCSEA with registration number 557/02/C/CPCSEA. Animals were fasted overnight prior to vehicle/standard/extract administration and during the experiment. All experiments were carried out during the light period (8:00 to 16:00 hour).

Preparation of extract:

The extract was prepared according to the method described in the Formulary of Siddha Medicine i.e. by boiling the nuts (200 g) with 500 ml milk. Decanting the decoction, 500 ml of milk was added to the boiling nuts and again boiled for some time. The decoction was recovered and the process was repeated again with the milk (500). All the three portions of milk nut decoction were mixed with ghee (1.5 kg) and boiled till dehydration. Then it was filtered and stored. Olive oil was used as a vehicle for the extract [15].

Preliminary Phytochemical screening:

The preliminary phytochemical investigations were carried out with milk extract of nuts of *S. anacardium* for qualitative identification of phytochemical constituents present with the extract by following standard methods [16-18].

Determination of LD_{50}:

The acute toxicity of milk extract of nuts of *S. anacardium* was determined by using female albino mice (20-30g) those maintained under standard husbandry conditions. The animals were fasted 3 hrs prior to the experiment, up and down procedure (OECD guideline no. 425) of CPCSEA was adopted for toxicity studies [19]. Animals were administered with single dose of extract and observed for its mortality during 48 hours study period (short term) toxicity. Based on short-term profile of drug, the dose of the next animals was determined as per as OECD guideline 425. The LD_{50} of the test extract was calculated using AOT 425 software provided by Environmental protection agency, USA [20,21].

Locomotor activity

Albino mice (18-22 g) of either sex were divided into four groups of six mice in each were fasted overnight prior to the test but water was supplied *ad libitum*. Group I was served as vehicle treated control; Group II, III and IV received milk extract in a dose of 75, 150 and 225 mg/kg respectively. One hour after above treatment, each mouse was placed individually in Photoactometer (INCO, Ambala, India) for a period of 10 min and locomotor activity was measured in terms of scores.

Nootropic activity

A. Elevated plus-maze (Exteroceptive Behavior Model).

Albino mice (18-22 g) of either sex were divided into seven groups of six mice in each were fasted overnight prior to the test but water was supplied *ad libitum*. Group I was maintained as normal control which was given with Olive oil only, Group II with Piracetam (200 mg/kg p.o.) which served as standard, Groups III, IV, V and VI were treated with different doses (75,150, 225 and 300mg/kg) of milk extract of nuts of *S. anacardium* (MENSA) respectively.

Elevated plus-maze served as an exteroceptive behavior model to evaluate learning and memory in mice. The procedure, technique and end point for testing learning and memory are followed as per the parameters described by the investigators working in the area of neuropsychopharmacology [22-25]. The apparatus consisted of two open arms (16 cm ×5 cm) and two enclosed arms (16 cm×5 cm×12 cm). The arms extended from a central platform (5 cm ×5 cm) and the maze is elevated to a height of 25 cm from the floor.

On the first day, 1 hour after the treatment, each mouse was placed at the end of an open arm, facing away from the central platform. TL was recorded i.e. the time taken by mouse with all its four legs to move into any one of the enclosed arms. If the animal did not enter into any one of the enclosed arms within 90 s, it was gently pushed into one of the two enclosed arms and the TL was assigned as 90 s. The mouse was allowed to explore the maze for next 10 s and then returned to its home cage. Retention of this learned-task was examined 24 h after the first day trial. The inflexion ratio was calculated using the formula [26].

Inflexion ratio (IR) = $(L_1 - L_0)/ L_0$, where L_0 is the initial TL (s) on 1st day and L_1 is the TL (s) on the 2nd day.

B. Passive avoidance paradigm

Albino mice (18-22 g) of either sex were divided into five groups of six mice in each were fasted overnight prior to the test but water was supplied *ad libitum*. Group I was maintained as normal control which was given with Olive oil only, Group II with Piracetam (200 mg/kg p.o.) which served as standard, Groups III, IV and V were treated with different doses (75,150 and 225 mg/kg) of milk extract of nuts of *S. anacardium* (MENSA) respectively.

The method described by Papazova et al [27] (1994) is modified as follows. An inverted petridish placed in the centre of the grid floor of a continuous avoidance apparatus (Techno, Lucknow) and the petridish served as the shock-free zone (SFZ).

All groups were treated accordingly as mentioned above and 1 hour after treatment each mouse was placed in the SFZ and upon stepping down from the SFZ was given an electric shock of 20 V through the grid floor. Animals were trained to remain on the SFZ at least for 60 s; mice, which did not meet these criteria in five trials, were rejected. Acquisition i.e. which is the number of trials required to reach the learning criteria. And retention of learning was observed for 10 min at 2 h and 24 h after post-training. The following retention parameters were noted [28]:

- step-down latency (SDL) in seconds,
- step-down error (SDE) as the number of times the animal stepped down from the SFZ and
- the time spent in the shock zone (TSZ) in seconds**.**

C. Scopolamine induced amnesia (Interoceptive Behavior Model)

Albino mice (18-22 g) of either sex divided into four groups of six mice in each were fasted overnight prior to the test but water was supplied *ad libitum*. Group I was maintained as normal control, which was administered with Olive oil only, Groups II, III and IV with Olive oil, Piracetam (200mg/kg p.o.) and MENSA (225mg/kg, p.o.) respectively. 45 min after administration of Olive oil/Piracetam/MENSA groups II, III and IV scopolamine (0.4 mg/kg, i.p.) was administered and 45 min TL (Transfer latency) was recorded on elevated plus maze and retention (memory) of learned task was examined after 24 h. The inflexion ratios were calculated as described earlier.

D. Diazepam-induced amnesia (Interoceptive Behavior Model)

Albino mice (18-22 g) of either sex divided into four groups of six mice in each were fasted overnight prior to the test but water was supplied *ad libitum*. Group I was maintained as normal control, which was given with Olive oil only, Groups II, III and IV were treated with Olive oil, Piracetam (200mg/kg p.o.) and MENSA (225mg/kg, p.o.) respectively. 45 min after administration of Olive oil/Piracetam/MENSA, diazepam (2 mg/kg i.p.) was administered and after 45 min TL (Transfer latency) was recorded on elevated plus maze and retention (memory) of learned task was examined 24 h later. The inflexion ratios were calculated as described earlier.

E. Electroshock-induced amnesia (Interoceptive Behavior Model)

Albino mice (18-22 g) of either sex divided into four groups of six mice in each were fasted overnight prior to the test but water was supplied *ad libitum*. Group I was maintained as normal control, which was given with Olive oil only, Groups II, III and IV were treated with Olive oil, Piracetam (200mg/kg p.o.) and MENSA (225mg/kg, p.o.) respectively. 45 min after administration of Olive oil/Piracetam/MENSA an electroshock of 10 mA capacity was applied for 0.2 sec through ear clip and immediately TL (Transfer latency) was recorded on elevated plus maze. Retention (memory) of learned task was examined 24 h later. The inflexion ratios were calculated as described earlier.

Statistical analysis:

Values are expressed as mean ± SEM. Statistical differences between means were determined by performing one-way ANOVA followed by student's *t*-test. $P < 0.05$ were considered as significant. All the statistical analysis was performed using demo version of Instat® software (Graph pad Inc., Santabarbara, CA)

Results

Phytochemical investigation:-

The milk extract of the nuts of *S. anacardium* (MENSA) was subjected for phytochemical screening and found to contain flavonoids, carbohydrates, and traces of phenolic compounds.

Determination of LD_{50}:

The milk extract of nuts of *S. anacardium* was administered orally to different groups of mice at different dose levels and found that even up to the dose level of 2000 mg/kg body weight did not produce any behavioral symptoms or mortality.

Effect of* MENSA *on Locomotor activity

The effect of MENSA on locomotor activity was tested using photoactometer. When three different doses of MENSA i.e. 75, 150 and 225 mg/kg was administered orally to different groups of mice, it did not exhibited any significant change in locomotor activity when compared to control group. However a slight and insignificant CNS depressant effect was observed with MENSA 150 mg/kg treated group only.

Nootropic activity

Effect of* MENSA *on inflexion ratio in mice (Elevated plus maze model)

Effect of MENSA on inflexion ratios in mice were recorded with elevated plus maze apparatus. Piracetam 200 mg/kg and MENSA with four different dose levels i.e. 75, 150, 225 and 300 mg/kg, treated groups have shown increased inflexion ratios. But statistically significant effect as observed with high doses i.e. 150 and 225 mg/kg, of MENSA treated groups only but not with Piracetam 200mg/kg treated group. However Piracetam has increased the inflexion ratio. (Table 1)

Effect of* MENSA *on Passive avoidance response

Both Piracetam 200 mg/kg and MENSA 225 mg/kg treated groups had not significantly reduced the number of trials required for acquisition in all the groups tested. A significant increase in SDL values were recorded with Piracetam 200 mg /kg and MENSA (225mg) treated group at prefixed time intervals i.e. 2 h and 24 h when compared with control group. MENSA with 75 mg/kg treated group had shown a significant increase in SDL at 24 h only but same effect was not observed with MENSA 150 mg/kg treated group. (Table 2)

Table.1 Effect of MENSA on Locomotor activity.

Group no.	Treatment	Dose (per kg)	Scores
I	Control (vehicle)	10 ml	362.83 ±28.99
II	MENSA p.o.	75 mg	329.83 ±30.74^{ns}
III	MENSA p.o.	150 mg	284.16 ±36.21^{ns}
IV	MENSA p.o.	225 mg	323.50 ±40.01^{ns}
One-way ANOVA	F df P		0.8866 23 -

n=6 in each group. Data is expressed as mean ±SEM
Statistical analysis by one-way ANOVA followed by student's *t*-test.
ns-not significance vs. control group.

Table. 2 Effect of MENSA on inflexion ration in mice (elevated plus maze)

Group no.	Treatment	Dose (per kg)	Inflexion ratio
I	Control (vehicle)	10 ml	0.5934±0.2672
II	Piracetam	200 mg	2.2668±0.2687^{ns}
III	MENSA p.o.	75 mg	1.7719±0.8929^{ns}
IV	MENSA p.o.	150 mg	3.9755±0.8143*
V	MENSA p.o.	225 mg	4.4573±1.7690*
VI	MENSA p.o.	300 mg	1.3123±0.2158^{ns}
One-way ANOVA	F df P		2.905 35 <0.05

n=6 in each group. Data is expressed as mean ±SEM
Statistical analysis by one-way ANOVA followed by student's *t*-test.
Significance at $*P<0.05$ & ns-not significance vs. control group.

Effect of* MENSA *on inflexion ratio in scopolamine induced amnesic model

Scopolamine, an anticholinergic drug with amnesic effect, treated group had shown decrease in inflexion ratio when compared with control group indicating an induction of amnesia. Only Piracetam but not MENSA 225 mg/kg treated group had significantly reversed the scopolamine-induced amnesia. (Table 3)

Table. 3 Effect of MENSA on acquisition and retention performance in passive-avoidance paradigm in mice.

Treatment (per kg p.o.)	Trials required for acquisition (number)	Retention (2 h)			Retention (24 h)		
		SDL (s)	SDE (number)	TSZ (s)	SDL (s)	SDE (number)	TSZ (s)
Control (vehicle) 10 ml	2.83±0.4014	70.50± 13.0350	14.50±4.544	30.50±9.182	355.30±77.192	11.66±2.660	2.33±1.022
Piracetam 200 mg	2.33±0.2108^{ns}	397.00± 97.2930*	4.00±0.894*	9.50±6.850*	555.66±28.040*	4.66±1.406*ss	2.50±1.586^{ns}
MESA 75 mg	2.16±0.1667^{ns}	199.00± 82.8710^{ns}	3.83±1.276*	7.00±2.944*	600.00±00.000**	3.33±0.881**	0.00±0.000^{ns}
MESA 150 mg	3.50±0.5627^{ns}	230.33± 86.5470^{ns}	6.00±1.633^{ns}	4.66±1.994**	449.50±48.001^{ns}	3.50±2.306*	5.16±2.651^{ns}
MESA 225 mg	3.00±0.4472^{ns}	383.00±106.3600*	9.50±2.172^{ns}	3.50±1.708**	574.16±25.833**	1.33±0.714**	0.16±0.166^{ns}
One-way ANOVA F	1.907	2.632	3.321	4.214	5.382	5.125	2.089
One-way ANOVA df	29	29	29	29	29	29	29
One-way ANOVA P	-	<0.05	<0.05	<0.05, <0.01	<0.05, <0.01	<0.05, <0.01	-

n=6 in each group. Data are expressed as mean ±SEM, Statistical analysis by one-way ANOVA followed by student's *t*-test.
Significance at *P<0.05, ** P<0.01 & ns-not significant vs. control.

Effect of MENSA on inflexion ratio in Diazepam induced amnesic model

In diazepam induced amnesia model, a decrease in inflexion ratio was observed when compared to control group. However, only Piracetam but not MENSA 225 mg/kg treated group had exhibited a significant reversal of diazepam induced amnesia. (Table 4)

Table. 4 Effect of MENSA on inflexion ratio in scopolamine induced amnesic model.

Group no.	Treatment	Dose (per kg)	Inflexion ratio
I	Control (vehicle)	10 ml	0.5934±0.2672
II	Vehicle + Scopolamine	10 ml, 0.4 mg	0.2048±0.0654
III	Piracetam p.o.+ Scopolamine i.p.	200 mg, 0.4mg	0.8748±0.0965*
IV	MENSA p.o.+ Scopolamine i.p.	225 mg, 0.4mg	0.2116 ± 0.1126^{ns}
One-way ANOVA	F df P		4.317 23 <0.05

n=6 in each group. Data is expressed as mean ±SEM
Statistical analysis by one-way ANOVA followed by student's *t*-test.
Significance at **P*<0.05 & ns-not significance vs. scopolamine group.

Effect of MENSA on inflexion ratio in Electroshock induced amnesic model

A decrease in inflexion ratio was recorded with electroshock treated group when compared to control. Both Piracetam and MENSA 225 mg/kg treated groups had enhanced inflexion ratios but significant increase in inflexion ratio was observed with Piracetam treated group only. (Tables 5-6)

Table. 5 Effect of MENSA on inflexion ratio in Diazepam induced amnesic model.

Group no.	Treatment	Dose (per kg)	Inflexion ratio
I	Control (vehicle)	10 ml	0.5934±0.2672
II	Vehicle + Diazepam	10 ml, 2 mg	0.0064±0.2176
III	Piracetam p.o. + Diazepam i.p.	200 mg, 2mg	$1.8459\pm0.4126^{*}$
IV	MENSA p.o.+ Diazepam i.p.	225 mg, 2mg	0.9425 ± 0.3388^{ns}
One-way AN OVA	F df P		5.870 23 <0.05

n=6 in each group. Data is expressed as mean ±SEM
Statistical analysis by one-way ANOVA followed by student's *t*-test.
Significance at **P*<0.05 & ns-not significance vs. diazepam group.

Table. 6 Effect of MENSA on inflexion ratio in Electroshock induced amnesic model.

Group no.	Treatment	Dose (per kg)	Inflexion ratio
I	Control (vehicle)	10 ml	0.5934±0.2672
II	Vehicle + Electroshock.	10 ml, 10 mA	-0.1132±0.1297
III	Piracetam p.o. + electroshock.	200 mg, 10 mA	2.7600±0.3932**
IV	MENSA p.o. + electroshock	225 mg, 10 mA	0.2653±0.0486ns
One-way AN OVA	F df P		27.061 23 <0.01

n=6 in each group. Data is expressed as mean ±SEM
Statistical analysis by one-way ANOVA followed by student's *t*-test.
Significance at **P<0.01 & ns-not significance vs. electroshock group.

Discussion

Nootropics represents a new class of psychotropic agents with selective facilitatory effect on integrated functions of the central nervous system, particularly on intellectual performance, learning capacity and memory [29].

Alzheimer's disease is a neurodegenerative disorder associated with a decline in cognitive abilities; patients also frequently have non-cognitive symptoms, such as depression, apathy and psychosis affect day-to-day life. A number of drugs have now been introduced in therapy to ameliorate cognitive deficits [30]. Piracetam, the first representation of a class of nootropic agents, has been shown to improve the condition with memory deficit generally seen in geriatric individuals and further Piracetam administration had improved learning abilities and memory capacities in laboratory animals [31].

In patients with Alzheimer's disease, immunohistochemical studies suggested the existence of chronic inflammation in certain regions of the brain. Since inflammation can cause damage to brain tissue, it was hypothesized that anti-inflammatory drugs might be inhibiting both the onset and the progression of Alzheimer's disease and is supported by the observation that indomethacin (NSAID) halted the progressive memory loss seen in patients with Alzheimer's disease. Moreover, it has also been observed that upon chronic use of anti-inflammatory drugs in elderly patients suffering from Alzheimer's disease showed reduction in symptoms [32]. Indomethacin, a non-steroidal anti-inflammatory drug exhibited a memory protective effect against electroconvulsive shock-induced retrograde amnesia and also reported to act against amyloid deposits in the brain [33].

Though there has been an increase in life span due to improvement in preventive, diagnostic and therapeutic measures for cardiovascular diseases and a variety of cancers still the number of individual afflicted with age-related neurodegenerative disorders like Alzheimer's disease, Parkinson' disease and stroke are increasing.

Though different cell types and brain areas are vulnerable among these, each disorder is developing from activation of a common final cascade of biochemical and cellular events that eventually lead to neuronal dysfunction and death. In this regard, different triggers, like oxidative damage to DNA, over activation of glutamate receptors, and disruption of cellular calcium homeostasis, different genetic and environmental factors, can activate a cascade of intracellular events that induce apoptosis [34]. TNF-α is a pleiotropic cytokine, which plays a critical role in both acute and chronic inflammation [35]. Several inflamogens have the ability of inducing the synthesis of TNF-α. It is linked with formation of a number of small molecular mediators of inflammation TNF-α and thus contributes to the range of mediators that critically control inflammation. TNF-α facilitates inflammatory cell infiltration by promoting the adhesion of neutrophils and lymphocytes to endothelial cells. When TNF-α is specifically blocked, the severity of inflammation is reduced [36]. In neurodegenerative process, the cytokine TNF-α plays a pivotal role within the pathological cascade and its level is elevated in Alzheimer's disease [35]. Earlier *S. anacardium* has been reported for its significant effect in restoring the elevated levels of TNF-α [37] and cellular calcium homeostasis disturbances [38] in diseased conditions.

The crude extracts of *S. anacardium* nuts have been reported to possess antitumour[39], anthelmintic[40] and antiarthritic[41] activities. Though the crude extract was reported to be toxic[39] it is interesting to note that during acute toxicity study the Siddha preparation of *S. anacardium* was nontoxic and did not induce any toxic effect of mortality up to the dose level of 2000 mg[42]. Phytochemical studies with the nut extract revealed the presence of flavonoids [43], phenolic compounds[44] and bhilawanols[45]. An earlier report with Siddha preparation of milk extract the nuts of *S. anacardium* also revealed the presence of flavonoids, phenol and carbohydrates [41]. For centuries, medicinal preparations that contain flavonoids as the principal active constituent have been used by physicians to treat human diseases [46].

The elevated plus maze is used to measure the anxiety state in animals, however transfer latency i.e., the time elapsed between the movement of the animal from an open to an enclosed arm will markedly shorten if the animal has previous experience of entering into open and closed arm and this shortened transfer latency has been shown to be related with memory process and the increase in inflexion ratio indicates nootropic activity. Recent studies of several nootropics and amnestic agents on EPM made this model a widely accepted paradigm to study learning and memory processes in rodents [47]. In EPM, acquisition (learning) can be considered as transfer latency on first day trial and the retention/consolidation (memory) is examined 24 h later. The animals treated with 150mg and 225mg/kg of MENSA showed a significant decrease in transfer latency i.e. increase in inflexion ratio, as compared with the control group, which is an indication for the enhanced cognitive effect of MENSA in rodents.

A protective effect was observed with all the parameters i.e. SDL, SDE and TSZ tested in passive avoidance paradigm. This indicates that *S. anacardium* is effective in consolidating an early retention of memory (immediate recall) and with subsequent improvement.

Animals treated with Piracetam and of MENSA 225 mg/kg showed significant results when tested on passive avoidance paradigm. The impairment of learning and memory induced by scopolamine, an anticholinergic agent, was reflected by prolonged transfer latency from the open arm to the closed arm i.e., decrease inflexion ratio (IR) [48].

MENSA did not reverse the amnesia induced by scopolamine indicates that it is not acting on Ach receptors because it is not able to show action in presence of scopolamine which is a muscranic receptor antagonist.

Diazepam, a GABA mimetic agent induces memory impairment and the subsequent inhibition of GABA-B receptors has been found to facilitate learning and memory [49, 50]. MENSA had offered protective effect in diazepam induced amnesic model that may be responsible for the indirect release of Ach in the brain.

Involvement of chronic inflammation in certain regions of brain and/or free radicals has been implicated in the pathogenesis of Alzheimer's disease. Anti-inflammatory drugs have been found to be effective in enhancing cognitive function in this condition and also shown to maintain memory by offering protective effect against electroshock-induced amnesia. In our study MENSA has exhibited statistically insignificant protective effect against electroshock-induced amnesia. Anti-inflammatory action of *S. anacardium*[37]might be contributing to the observed memory-enhancing activity.

Oxygen free radicals are implicated in the process of ageing and may be responsible for the development of Alzheimer's disease in elderly. Oxygen-free radicals and other products of oxidative metabolism have been shown to be neurotoxic and it was reported that diets with rich antioxidant potency had improved cerebellar physiology and motor learning in aged-rats [51]. Further S. *anacardium* has been reported to possess antioxidant property as well [52]. The protective effect in Alzheimer's disease may attributed for its antioxidant property which results with minimal damage of susceptible brain cells to oxidative stress thereby improving neuronal function the enhances the memory. Further, it has been found that NO is a highly fats soluble free radical, having numerous promiscuous roles. NO synthesis is greatly amplified during inflammation. Several studies have demonstrated that inflammation correlates with the level of NO [53]. In the earlier study *S. ancardium* had shown remarkable reduction in nitrate/nitrite level, which can be attributed to the antioxidant property.

Thus, a combination of anti-inflammatory, antioxidant and neuroprotective role of *S. anacardium* could be leading to the net memory-enhancing effect.

The precise mechanism by which *S. anacardium* elicits its nootropic effect is not known. In vitro studies with *S. anacardium* had shown a potent effect of AchE inhibition and it is shown to neuroprotective especially to the hippocampal regions in stress-induced neurodegeneration [54]. Biochemical as well as electrophysiological evidence exists for interactions between cholinergic, noradrenergic and serotonergic system [55]. Sara (1989) suggested that the behavioral effects of cholinergic degeneration could be alleviated in noradrenergic function [56]. *S. anacardium* may thus indirectly modify Ach concentration, through its influence on the other neurotransmitter systems. Removal of the negative influence (i.e. decreased NE concentration) possibly helped in the manifestation of cholinergic effects resulting in improved cognitive function.

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