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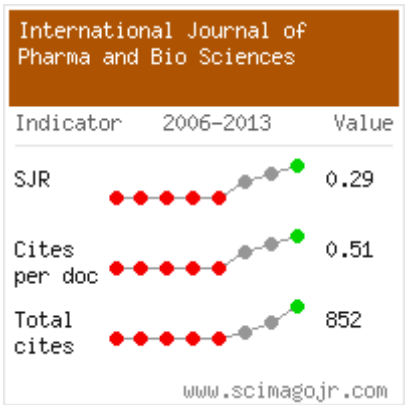

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EVALUATION OF *Sapindus trifoliatus* ON ALZHEIMER'S DISEASE INDUCED BY ALUMINUM CHLORIDE IN RATS

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ABSTRACT

The present study aimed to investigate the possible prophylactic and therapeutic effects of hydro-alcoholic extract of *Sapindus trifoliatus* on AD induced by $AlCl_3$ in rats. In this study thirty adult Wistar albino rats were selected and were divided into 5 groups (six each). Group I, II, III, IV & V served as normal control, negative control, test group and standard control respectively. The rats were given treatment for 30 days and behavioural parameters were determined on 1st, 10th, 20th, &30th day of treatment. After day 30th rats were sacrificed and biochemical parameters (antioxidant levels & Acetylcholinesterase activity) were determined. This study indicated that *Sapindus trifoliatus* when was used for treatment of $AlCl_3$induced AD, it improves the behavioural & biochemical parameters. The histopathological finding in hippocampus of brain tissue neuron appears less or more like normal one when compared to the negative control.

KEYWORDS: Acetylcholine, Acetylcholinesterase, Sapindus trifoliatus, Morris water maze, passive avoidance test, Y-maze.

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1) INTRODUCTION

Alzheimer's disease (AD) is a chronic, progressive, degenerative disease of central nervous system. It is the most common form of dementia[2]. The word dementia describes a set of symptoms that can include memory loss and thinking orientation, comprehension difficulties, problem solving or language.[3] (Dementia literally means loss of memory or thinking) Morphologically, the disease is characterized by brain atrophy and by enlarged cerebral ventricles. From a biological point of view decrease level of choline acetyltransferase and other cholinergic markers. Histologically, AD is characterized by extracellular deposits, called cerebral plaques, the other typical histopathologic hallmark of AD is the neurofibrillary tangles within neurons.[4] The incidence and prevalence of AD increase with increasing age and higher in women. The incidence of AD ranges from 1% at ages 65-70 and approximately 4% over age 85. It is the fourth leading cause of death among 10% of 70 years aged people after myocardial infarction, stroke & cancer.[5] In the United States, the number of new cases per year is expected to triple from approximately 420,000 in 2000 to more than 1.3 million in 2050 and it is the eighth leading cause of death in US.[6] Natural products have been traditionally used in the treatment of several human diseases. The effort to develop natural products as potential therapeutic and advances in extraction and isolation techniques lead to the development of 63% of the natural product derived drugs from 1981-2006 [Newman, David 2007]. Despite the vast availability of medicinal plants, *Sapindus trifoliatus* plant selected in our study and the case for the collection of material its phytochemical constituents and unique importance in this area. *S. trifoliatus* belongs to the family Sapindaceae. Its common name is soapnut (ritha). It is a medium sized deciduous tree growing wild in Nepal, south India and cultivated worldwide. Plant constituents and their activities like flavonoids, saponin, triterpenoids, and anti-inflammatory, antiepileptic are responsible to prevent AD.[7-11]respectively, Literature review suggested that Polyherbal formulation having saponins shown improvement of memory. *S. trifoliatus* is a good source of saponin, flavonoids, triterpenoids and also having anti-inflammatory, antioxidant and antiepileptic activity.[12-17] respectively.

2) MATERIALS AND METHODS

2.1 Materials used

Rivastigmine and Acetylthiocholine iodide was purchased from sun pharma. Mumbai, and Hi-media Mumbai respectively. Fruits of S. trifoliatus was collected from local market of Anantapurmu.

2.2 Preparation of plant extract

The plant was identified and authenticated by Dr. J. Ravindra Reddy, M.Pharm Ph.D., Raghavendra Institute of Pharmaceutical Education and Research, Ananthapuramu and the voucher specimen **(01/15)** was preserved in the department of Pharmacology, RIPER, for further reference. The pericarp of *Sapindus trifoliatus* were separated. The dried material was made powder by using a grinder and sieved. Weighed quantity (500gm) of fresh, finely grounded powder was mixed with hydro alcoholic solvent (Water: Ethanol 1:1) and subjected to maceration with intermittent shaking for seven days. The extract, then obtained was collected by filtration using muslin cloth. Thus obtained filtrate was subjected to solvent evaporation to obtain a solid extract which was weighed and stored in an airtight container.

2.3 Animals

Male Albino Wistar Rats weighing 200-250 g were used for the present study. The animals were collected from Central Animal Facilities, Indian Institute of Science Bangalore. The animals were maintained under controlled conditions of temperature (22 ± 2°C), humidity (50 ± 5%) and 12 hours. light-dark cycles. All the animals were acclimatized for seven days before the study. The animals were housed in sanitized polypropylene cages containing sterile paddy husk as bedding. They had free access to standard pellets as basal diet and water *ad libitum*. All the studies conducted were approved by the Institutional Animal Ethical Committee (IAEC) of Raghavendra Institute of Pharmaceutical Education and Research (RIPER), Ananthapuramu.Approval

No. 878/ac/05/CPCSEA/001/2015 dated 06/01/2015, according to prescribed guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.4 Acute toxicity Studies

An acute toxicity study was carried out using mice (20-25g) as per CPCSEA guidelines. Plant extract was orally administered to different groups of mice at doses of 100 mg, 500 mg, 1000 mg and 2000 mg/kg body weight respectively. Animals were observed for 48h to study the general behaviour of animals, signs of discomfort and nervous manifestations.[15, 18, 19]

2.5 Treatment schedule

Rats were divided into five groups of six each and the groups were as follows

Group	Treatment	Dose
I	Normal Untreated:	-
II	Negative control:	$AlCl_3$ (300 mg/kg-PO)[20]
III	Test group low dose:	$AlCl_3$ (300 mg/kg-PO)+ HAST (200 mg/kg-PO)
IV	Test group high dose:	$AlCl_3$ (300 mg/kg-PO) + HAST (400 mg/kg-PO)
V	Standard control:	$AlCl_3$ (300 mg/kg-PO)+ Rivastigmine (0.3mg/kg-IP)[21]

HAST = Hydro alcoholic extract of Sapindus trifoliatus

2.6 Behavioural stress test

1) Locomotor activity

Levels of activity were measured by determining rat movements using a grid floor activity cage. Each beam interruption was registered as an event on the external counter. The light beam breaks were counted for 5mins.[22]

2) Motor coordination test

The Rota rod apparatus consists of a motor rod with a drum of 7.0 cm diameter. It was adjusted to a speed of 20 revolutions/min during the test session. The latency to fall in a test session of 180 Sec. was taken as a measure of motor coordination, according to the procedure described by Vijitruth et al. [23]

3) Elevated plus-maze (EPM) test for spatial memory in rats

This test is based on the natural aversion of rodents to the open arms and high spaces. The animals prefer the enclosed arm and try to reach it as quickly as possible because they experience fear in the open arms of the maze. Itoh*et al*., in 1990, demonstrated that the time taken by an animal to move from an open arm to an enclosed arm is significantly reduced if the animal had previous experience of entering the enclosed arm. The EPM, consisting of two open arms (35 × 6 cm) and two enclosed arms (35 × 6 × 15 cm), was elevated to a height of 50 cm. Acquisition of memory was assessed by placing the animals individually at the end of either of the open arms, facing away from the central platform. The time taken by each animal to move from the open arm to either of the closed arms was recorded. This duration of time was called transfer latency (TL). If the animal did not enter into any of the enclosed arms within 600 seconds, it was gently pushed into any of the enclosed arms and the TL was assigned as 600 seconds. Later, the animal was allowed to explore the plus-maze for 5 minutes and then placed back in the home cage. [24]

4) Morris water maze test in Rats

The Morris Water Maze is a circular pool filled with water. Rats are trained to find an escape platform hidden just below the surface of the opaque water (Morris, 1984). The hidden platform version of the Morris Water Maze is a test of spatial memory which is sensitive to hippocampal damage, while the visible platform version of the Morris Water Maze is a non-hippocampal task, which is disrupted by dorsal striatum lesions. During acquisition and reversal training the Swim latency (time to find and mount the escape platform in seconds) and swim distance (distance to find out the hidden plate in cm) were measured. [22, 23, 25]

5) Passive avoidance test

Pole climbing apparatus chamber is used for a passive avoidance response where the pole is replaced by a wooden platform fixed on electrified grid floor. When rats stepped off the platform, they receive a continuous foot shock from grid floor. The normal reaction of rat was to jump back to the wooden platform. After about 4-5 trials the animals acquired the

passive avoidance response and they refrained from stepping down. The criterion was reached when the animal remained on the platform for at least 60 seconds. The animals were then subjected to the passive avoidance test, and parameters were noted as step down latency, step down error and time spent in shock zone. [26]

6) Y-maze test

Animals were introduced from the base of the Y-maze and allowed to choose one of the goal arms abutting the other end of the stem. The trial was carried out twice in quick succession. At the second trial, the rodent tended to choose the arm not visited before, reflecting a memory of the first choice. This is called 'spontaneous alternation'. This tendency was reinforced by starving the animal for 24 h before the test and rewarding it with a preferred food item if it alternates. Both spontaneous and rewarded alternations are very sensitive to dysfunction of the hippocampus; however, other brain structures are also involved and in this test time taken to reach previsited arm was recorded. [27]

2.7 Oxidative parameters in brain tissue homogenate]

1) Catalase (CAT)

Two ml of tissue homogenate diluted with 1 ml of $\mathbf{H_2O_2}$ and takes the absorbance at 240 nm for 3 min. with 30 Sec interval. (Add H_2O_2 just before taking O.D)**[Aebi-1984]**

2) Reduced glutathione (GSH)

The supernatant homogenate was precipitated with 20% trichloro acetic acid (TCA) and centrifuged. 0.25 ml supernatant was taken for GSH estimation using freshly prepared DTNB solution (2 ml) and volume up to 3 ml with phosphate buffer (pH 8). The intensity of the yellow colour formed was read at 412 nm against blank for each sample without reagent was run. The GSH content was calculated by using $\varepsilon = 13.6 \times 10^3\ M^{-1}\ cm^{-1}$ expressed as nmol/g of wet tissue.[28]

3) Lipid peroxidation (LPO)

Lipid peroxidation was evaluated as an index of oxidative damage and was assessed by measuring thiobarbituric acid reactive substances (TBARS) - Malandialdehyde (MDA) in the brain. MDA is an end product of the lipid peroxidation process. MDA level is commonly known as a marker of oxidative stress. Briefly, 500 µL supernatant of tissue homogenate, 500 µL of TBA reagent and 1.5 ml of 15% trichloroacetic acid were combined in a 10 ml screw-cap Pyrex centrifuge tube, mixed, and heated for 45 min in boiling water. After cooling in an ice bath, 3 ml of n-butanol was added, mixed and centrifuged, and the chromogen extracted. The absorbance of the pink coloured organic phase was determined spectro-photometrically at 512 nm against a blank. The amount of lipid peroxidation was determined by using the formula $\varepsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$ and expressed as nmol of MDA per gm of wet tissue.[29]

4) Superoxide dismutase (SOD)

0.5ml of tissue homogenate was diluted with 0.5 ml distilled water, to this add all chilled reagents, 0.25 ml ethanol, 0.5 ml of chloroform and shaken for 1 min and centrifuged at 2000 RPM for 20 min. supernatant enzymatic activity was determined to it 0.05ml of carbonate buffer (0.05 M pH 10.2) and 0.5 ml EDTA (0.49 M) was added. The reaction was initiated by the addition of 0.4 ml epinephrine and the change in optical density/mm was measured at 480 nm. SOD was expressed as U/mg wet tissue. [30]

2.8 Acetylcholinesterase (AchE) activity

Acetylcholinesterase has a very short half-life so direct estimation of acetylcholine is a little difficult in brain homogenates. There are some approaches to evaluate cholinergic function indirectly. Estimation of acetylcholinesterase activity provides a valuable and a relatively easy assessment of cholinergic function. Estimation of acetylcholinesterase activity is popularly done by Ellman's method named after George Ellman, who developed this method in 1961. The esterase activity was measured by providing an artificial substrate, acetylthiocholine. Because of cleavage of the acetylcholine by acetylcholinesterase is allowed to react with the –SH group of reagent DTNB, which is reduced to thionitrobenzoic acid, a yellow coloured anion with an absorption maxima at 420 nm by UV-spectrophotometer. A 0.4 ml homogenate was added to a cuvette containing 2.6 ml of phosphate buffer (pH 8, 0.1 M). 100 µl of

DTNB reagent were added to the photocell. The absorbance was measured at 420 nm, when this had stopped increasing, the photometer silt was opened so that the absorbance was set to zero. Determine the acetylcholinesterase activity at 25°c, over 1 minute in a photocell after addition of 20 µl of acetylthiocholine iodide as a substrate.[31-33]

2.9 Histopathology

The hippocampus was separated and immediately fixed with 10% formalin. Thereafter, the specimens were embedded in paraffin, sectioned at 5 µm and stained with hematoxyline and eosin.

Table 1
Effect of HAST on Loco motor activity (Actophotometer)

S.No.	Group	Locomotor activity (No. of counts/5min)			
		1st Day	10th Day	20th Day	30th Day
1	Normal	405.7±3.062	410.2±4.888	412.5±3.096	416.5±2.861
2	Negative control	402.3±0.8433[a]	324.2±6.1120[b]	306.3±4.2400[b]	308.7±3.3830[b]
3	Low dose of HAST	403.3±1.585[a]	382.2±6.145[c]	406.0±2.113[c]	380.0±5.231[c]
4	High dose of HAST	408.3±4.216[a]	410.3±4.551[c]	407.8±2.833[c]	408.0±4.066[c]
5	Standard control	403.5±1.384[a]	426.0±8.347[c]	408.0±2.206[c]	407.3±2.418[c]

Each Value represents the mean±S.E.M (n=6). One-way ANOVA followed by Tukey'sposthoctest.[a] indicates non significance values [b] indicates P<0.001 when compared to Normal control [c] indicates p<0.001 when compared to negative control

Table 2
Effect of HAST on motor coordination (Rotarod)

S.No.	ıup	Fall off time (in sec.)			
		1st Day	10th Day	20th Day	30th Day
01	Normal	15.83±0.4773[a]	15.17±1.078	20.00± 1.826	17.00±1.506
02	Negative control	12.59±0.6540[a]	7.500±0.4282[b]	6.167±0.8724[b]	8.000±0.9309[b]
03	Low dose HAST	14.17±1.108[a]	12.67±0.6146[c]	12.67±0.954[d]	13.17±1.195[d]
04	High dose HAST	14.17±0.4773[a]	14.67±0.7149[c]	14.50±1.025[e]	14.83±1.108[e]
05	Standard control	14.33±0.8433[a]	15.33±0.8819[c]	15.00±1.826[e]	16.00±1.862[e]

Each Value represents the mean±S.E.M (n=6). One-way ANOVA followed by Tukey'sposthoc test. [a]indicates non-significantvalues[b] indicates P<0.001 when compared to Normal control[c, d, e] indicates p<0.001, p<0.05 and p<0.01 respectively when compared to negative control

Table 3
Effect of HAST on special memory test

S.No.	Group	Transfer latency (in sec.)			
		1st Day	10th Day	20th Day	30th Day
01	Normal	35.67±0.5578	38.17± 1.424	34.83±1.222	40.33±1.838
02	Negative control	33.17±0.792[a]	51.33±1.856[b]	53.33±2.092[b]	55.67±1.874[b]
03	Low dose HAST	35.00±0.856[a]	41.33±2.011[c]	43.00±1.095[c]	43.83±1.600[d]
04	High dose HAST	34.67±1.256[a]	35.17±0.833[d]	40.00±1.826[d]	40.00±1.317[d]
05	Standard control	37.57±1.976[a]	33.00±1.506[d]	39.00±2.129[d]	44.50±1.285[d]

Each Value represents the mean±S.E.M (n=6). One-way ANOVA followed by Tukey'sposthoctest.[a]indicates non-significantvalues[b] indicates P<0.001 when compared to Normal control[c& d] indicates p<0.01 and p< 0.001 when compared to negative control

Table 4
Effect of HAST on Morris water maze test (Swim latency (time in sec)

S. No.	Group	Swim latency (time in sec)			
		1st Day	10th Day	20th Day	30th Day
01	Normal	21.50±1.607	22.17±1.376	23.00±1.826	22.33±1.333
02	Negative control	19.33±1.667 [a]	35.00±1.653[b]	39.17±2.386[b]	37.83±1.493[b]
03	Low dose HAST	18.67±0.6667 [a]	22.00±1.155[c]	22.17±1.138[c]	21.50±0.8466[c]
04	High dose HAST	22.33±1.476 [a]	16.00±1.155[c]	21.33±1.138[c]	18.67±0.4944[c]
05	Standard control	21.83±0.9458 [a]	17.33±0.8028[c]	21.67±1.282[c]	16.33±0.9888[c]

Each Value represents the mean±S.E.M (n=6). One-way ANOVA followed by Tukey'sposthoc test. [a]indicates non-significant values [b& c] indicates P<0.001 and p< 0.001respectively when compared to Normal control.

Table 5
Effect of HAST on Morris water maze test (Swim distance (cm.)

S.No.	Group	Swim distance (in cm)			
		1st Day	10th Day	20th Day	30th Day
01	Normal	15.00±1.826	18.00±2.191	17.83±2.120	16.33±1.308
02	Negative control	17.17±1.167[a]	31.33±1.498[b]	33.83±1.327[b]	37.67±2.044[b]
03	Low dose HAST	16.67±1.308 [a]	22.50±1.258[c]	24.33±1.498[c]	25.33±1.308[d]
04	High dose HAST	16.83±1.195 [a]	20.67±0.4944[d]	24.17±1.167[c]	24.17±1.108[d]
05	Standard control	18.17±1.641[a]	20.00±1.653 [d]	23.67±1.229 [d]	22.17±1.249 [d]

Each Value represents the mean±S.E.M (n=6). One-way ANOVA followed by Tukey'sposthoctest. [a] indicates non-significant values [b] indicates P<0.001 when compared to Normal control[c& d] indicates p<0.01 and p< 0.001 respectively when compared to negative control.

Table 6
Effect of HAST on Passive avoidance test

Group	TRFA (no.)	Retention											
		Day 1st			Day 10th			Day 20th			Day 30th		
		SDL (sec)	SDE (no.)	TSZ (sec)	SDL (sec)	SDE (no.)	TSZ (sec)	SDL (sec)	SDE (no.)	TSZ (sec)	SDL (sec)	SDE (no.)	TSZ (sec)
Normal	5	46.2± 2.394	1.0± 0.408	13.75± 2.394	45.50± 2.062	0.75± 0.25	15.50± 2.102	45.00± 2.041	0.75± 0.25	16.00± 2.614	43.75± 2.394	1.000± 0.4082	13.75± 1.250
Negative control	5	50.0± 9.350[a]	1.0± 0.408 [a]	16.25± 2.394 [a]	20.00± 4.564[b]	3.0± 0.40[e]	30.00± 2.041[b]	15.00± 2.041[b]	3.25± 0.47[e]	35.25± 1.702[b]	20.00± 2.041[b]	3.750± 0.2500[b]	32.50± 3.227[b]
Low dose HAST	4	46.2± 3.030[a]	0.75± 0.250 [a]	13.75± 3.038 [a]	35.00± 2.041[c]	1.0± 0.40[d]	17.75± 1.109[d]	36.25± 2.394[f]	1.25± 0.25[d]	17.50± 3.227[d]	31.25± 1.250[d]	1.250± 0.2500[d]	20.00± 2.887[d]
High dose HAST	5	48.7± 4.270 [a]	1.0± 0.408 [a]	11.75± 3.838 [a]	40.00± 3.742[d]	0.75± 0.25[d]	14.00± 1.958[f]	37.50± 3.227 [f]	1.00± 0.40 [d]	15.25± 3.544[f]	35.00± 2.041[f]	1.250± 0.4787[d]	15.50± 1.658[f]
Standard control	4	48.7± 4.270 [a]	1.0± 0.408 [a]	11.75± 3.838 [a]	39.00± 3.674 [d]	0.50± 0.28[f]	12.75± 1.109[f]	43.25± 1.031 [f]	0.50± 0.28[f]	12.00± 1.414[f]	40.00± 2.041[f]	1.000± 0.4082[f]	13.75± 1.493[f]

TRFA= Trials required for acquisition SDL= step down latency, SDE= step down error, TSZ= Time spent in shock zone Each Value represents the mean±S.E.M (n=6). One-way ANOVA followed by Tukey'sposthoctest[a] indicates non-significant values [b& e] indicates P<0.001 and p<0.01 respectively when compared to Normal control[c, d &f]indicates p< 0.05, p<0.01 and p<0.001 respetivelywhen compared to negative control.

Table 7
Effect of HAST on Y maze test

S. No.	Group	Time to reach pre-visited arm (sec.)	
		Before treatment	After treatment
01	Normal	27.00±2.569	27.00±1.880
02	Negative control	25.40±3.356	45.00±1.438
03	Low dose HAST	25.20±1.158	36.00±1.342
04	High dose HAST	24.00±1.517	33.83±1.222
05	Standard control	24.00±1.049	32.83±1.558

Values are expressed in mean ± S.E.M

Table 8
Effect of HAST on oxidative parameters in brain tissue homogenate

S.No	Group	SOD (U/mg wet tissue)	CAT (µmol H_2O_2 decomposed/mg wet tissue)	GSH (nmol GSH/mg wet tissue)	LPO (nmol MDA/mg wet tissue)
01	Normal	30.33 ± 0.3333	343.2 ± 2.088	55.67 ±0.8433	63.50 ± 1.285
02	Negative control	17.17 ± 0.7032[a]	105.8 ±1.167 [a]	47.83 ± 0.8724 [a]	86.17 ± 2.227 [a]
03	Low dose	22.33 ± 1.563[b]	240.7 ±6.146 [c]	52.17 ± 0.4773[b]	51.17 ± 1.493 [c]
04	High dose	26.50 ± 0.7188[c]	280.2 ±6.332 [c]	54.67 ± 0.8433 [c]	56.67 ± 1.174 [c]
05	Standard control	25.00 ± 0.5164[c]	309.2 ±3.103 [c]	54.50 ± 0.9220 [c]	61.17 ± 0.4773[c]

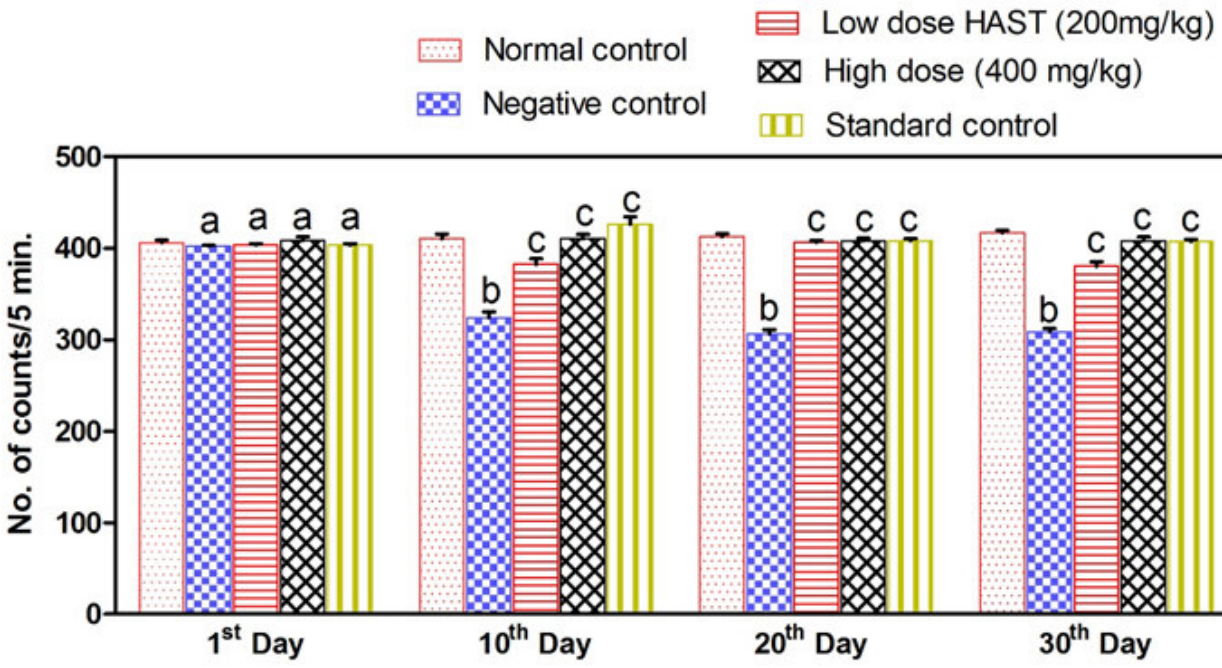
Each Value represents the mean±S.E.M (n=6). One-way ANOVA followed by Tukey'sposthoctest[a] indicates P<0.001 when compared to Normal control[b&c] indicates p<0.01 and p<0.001 when compared to Negative control.

Table 9
Effect of HAST on Acetylcholinesterase activity

S.No.	Group	AchE activity (µ mol/min/mg wet tissue)
01	Normal	15.50 ± 1.057
02	Negative control	27.00 ± 1.211[a]
03	Low dose	20.83 ± 0.3073[b]
04	High dose	19.33 ± 1.202[c]
05	Standard control	16.67 ± 0.7601[c]

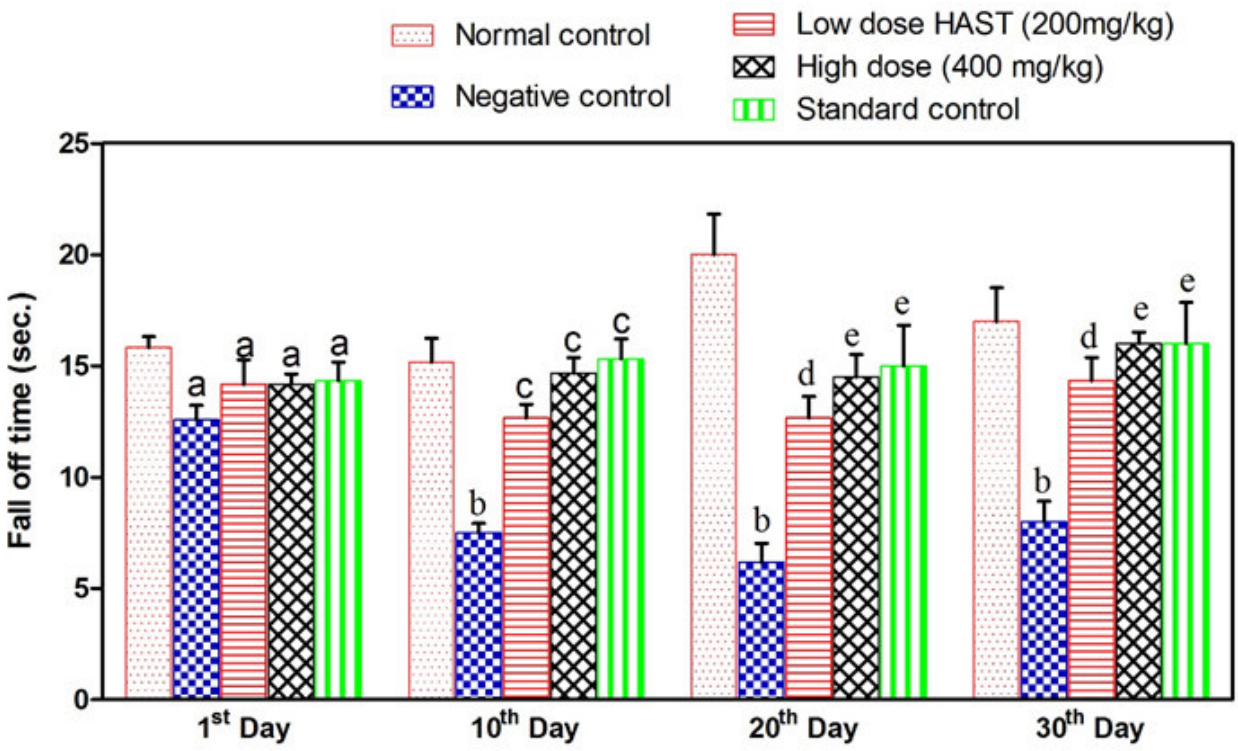
Each value represents the mean±S.E.M (n=6). One-way ANOVA followed by Tukey'sposthoc test [a] indicates P<0.001 when compared to Normal control, [b& c] indicates p<0.01and p< 0.001 respectively when compared to Negative control.

Figure 1
Effect of HAST on Loco motor activity (Actophotometer)



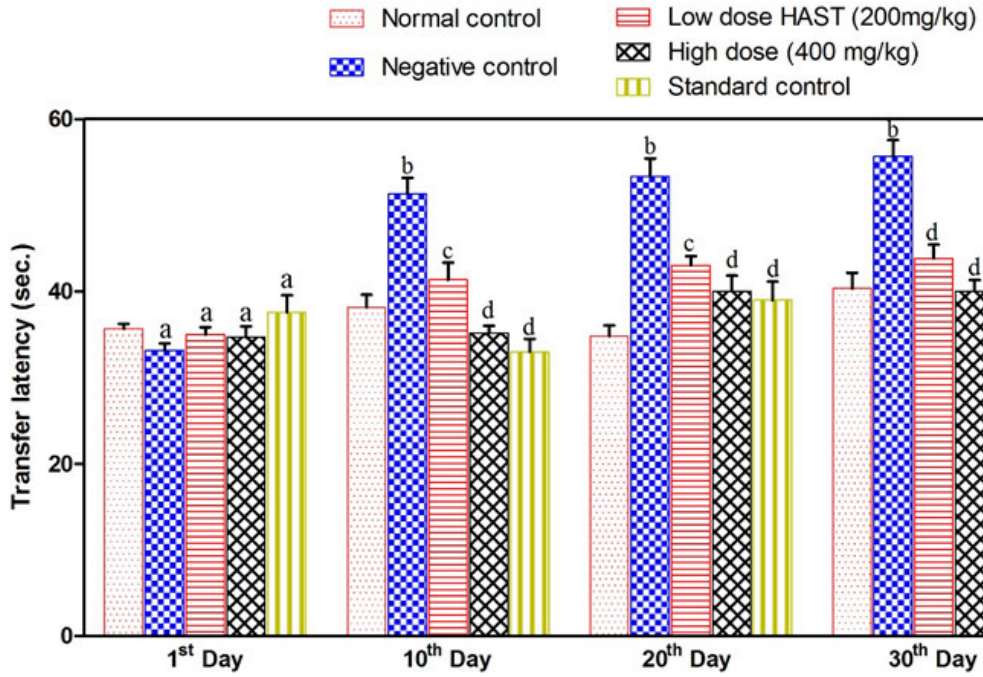
Each bar represents the mean±S.E.M (n=6). One-way ANOVA followed by Tukey'sposthoc test. [a] indicates non-significant values[b] indicates P<0.001 when compared to Normal control [c] indicates p<0.001 when compared to negative control

Figure 2
Effect of HAST on motor coordination (Rotarod)



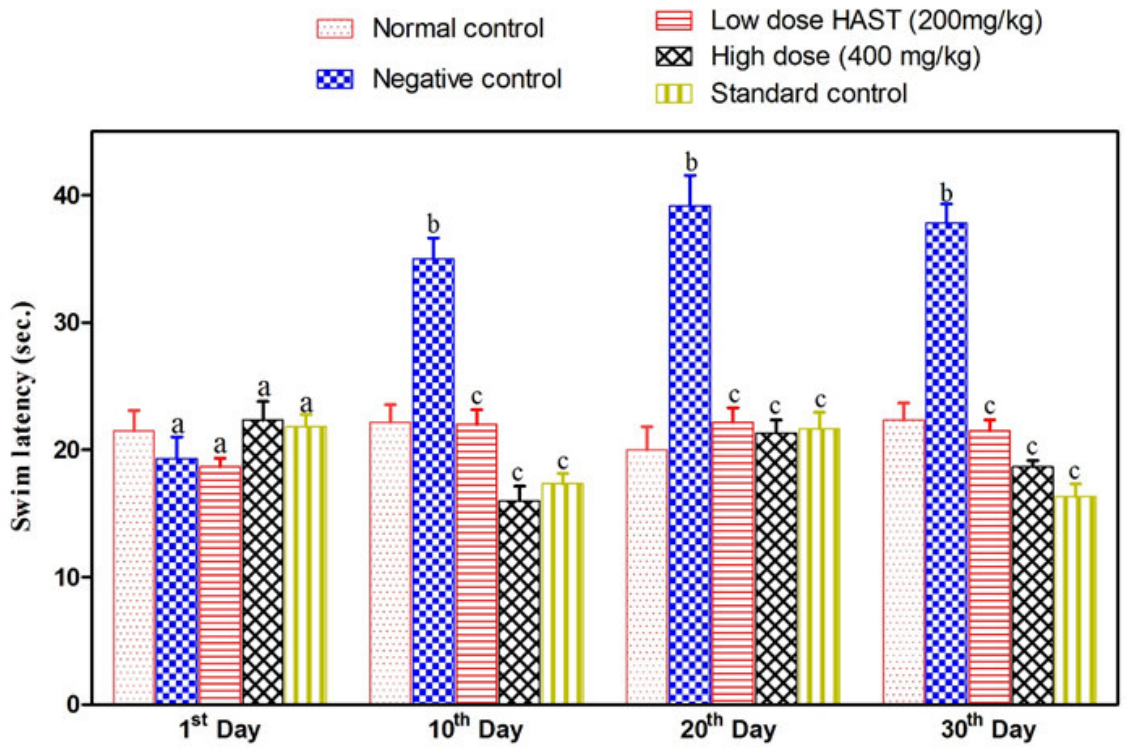
Each bar represents the mean±S.E.M (n=6). One-way ANOVA followed by Tukey'sposthoc test. [a]indicates non-significantvalues[b] indicates P<0.001 when compared to Normal control[c, d & e] indicates p<0.001, p<0.05 and p<0.01 respectively when compared to negative control.

Figure 3
Effect of HAST on special memory test



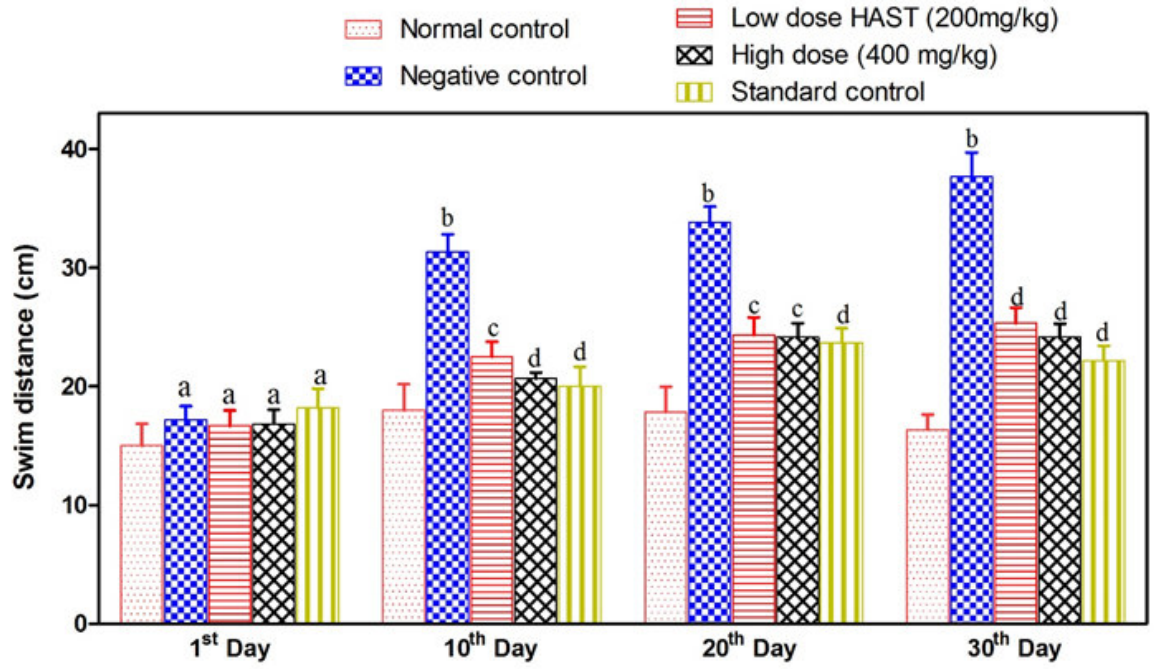
Each bar represents the mean±S.E.M (n=6). One-way ANOVA followed by Tukey'sposthoc test. [a]indicates non-significant values,[b] indicates P<0.001 when compared to Normal control [c& d] indicates p<0.01 and p<0.001 respectively when compared to negative control.

Figure 4
Effect of HAST on Morris water maze test (swim latency)



Each bar represents the mean±S.E.M (n=6). One-way ANOVA followed by Tukey'sposthoc test. [a] indicates non-significant values,[b] indicates P<0.001 when compared to Normal control [c]indicates p< 0.001 when compared to negative control

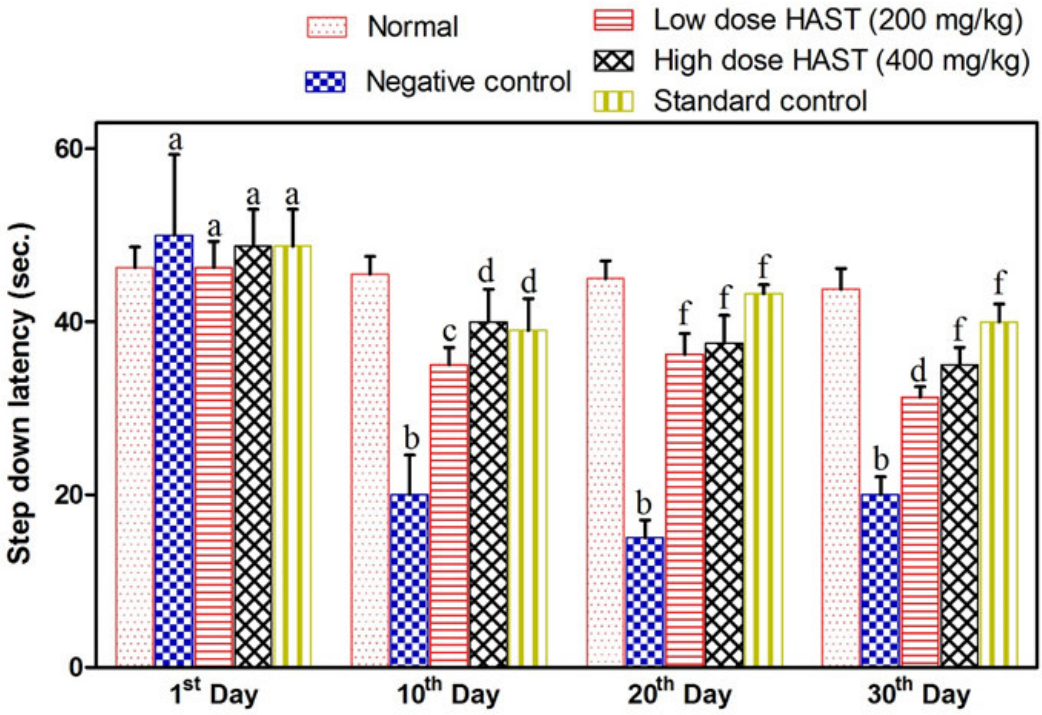
Figure 5
Effect of HAST on Morris water maze test (Swim distance (cm.)



Each bar represents the mean±S.E.M (n=6). One-way ANOVA followed by Tukey'sposthoctest[a]indicates non-significant values [b]indicates P<0.001 when compared to Normal control[c& d] indicates p<0.01 and p<0.001 respectively when compared to negative control

Figure 6

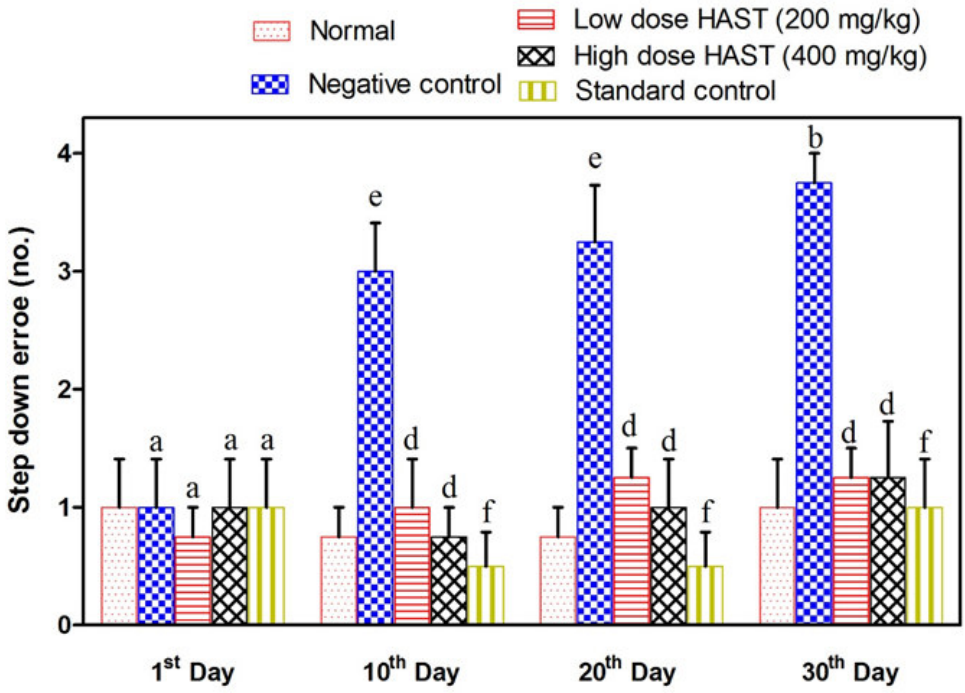
Effect of HAST on Step down latency of passive avoidance test



Each Value represents the mean±S.E.M (n=6). One-way ANOVA followed by Tukey'sposthoctest[a] indicates non-significant values [b]indicates P<0.001 when compared to normal control[c, d &f]indicates p< 0.05, p<0.01 and p<0.001 respetivelywhen compared to negative control

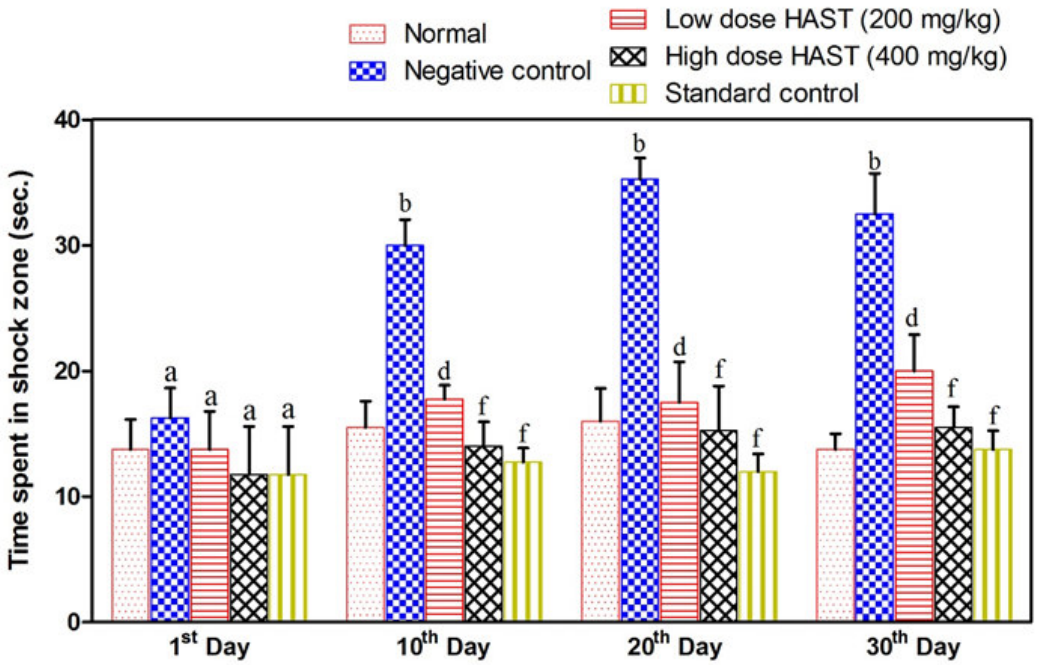
Figure7

Effect of HAST on Step down error of passive avoidance test



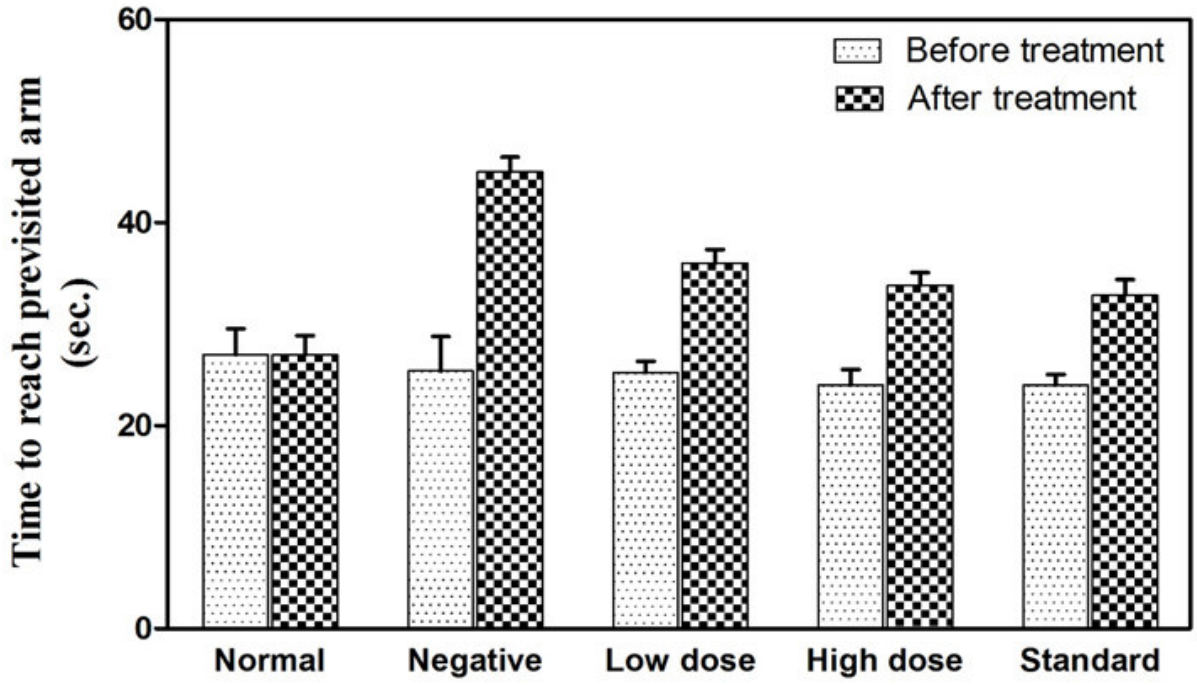
Each Value represents the mean±S.E.M (n=6). One-way ANOVA followed by Tukey'sposthoctest[a] indicates non-significant values [b& e] indicates P<0.001 and p<0.01 respectively when compared to Normal control[d&f]indicates p<0.01 and p<0.001 respectively when compared to negative control.

Figure 8
Effect of HAST on time spent in shock zone of passive avoidance test



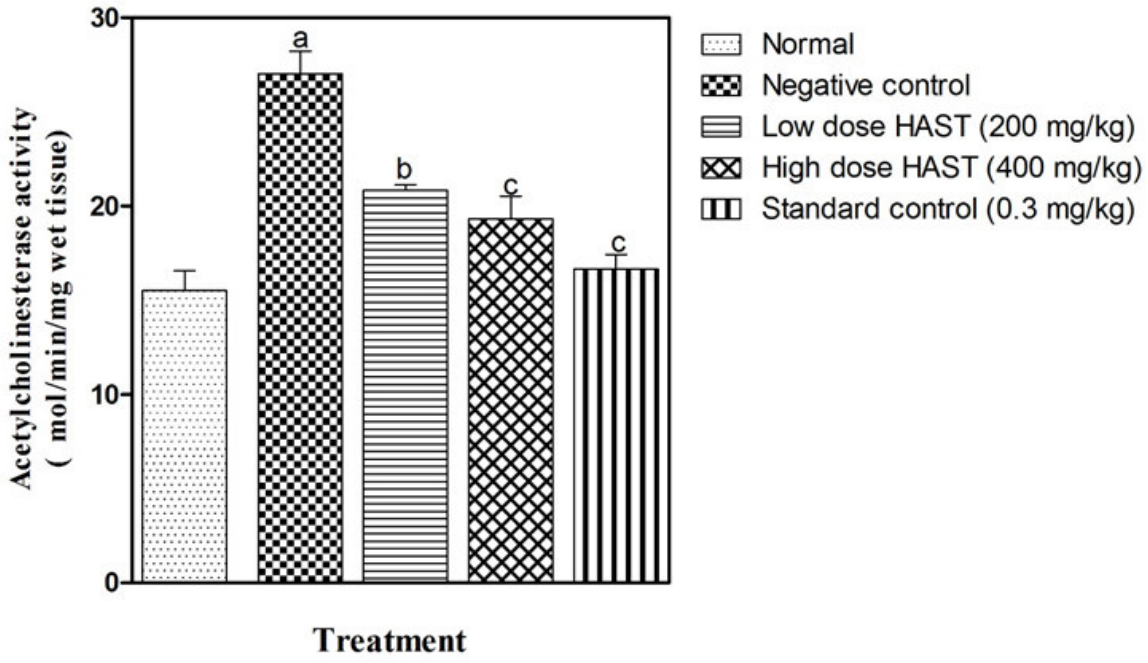
Each Value represents the mean±S.E.M (n=6). One-way ANOVA followed by Tukey'sposthoctest[a] indicates non-significant values [b]indicates P<0.001when compared to Normal control[d&f]indicates p<0.01 and p<0.001 respectively when compared to negative control.

Figure 9
Effect of HAST on Y maze test



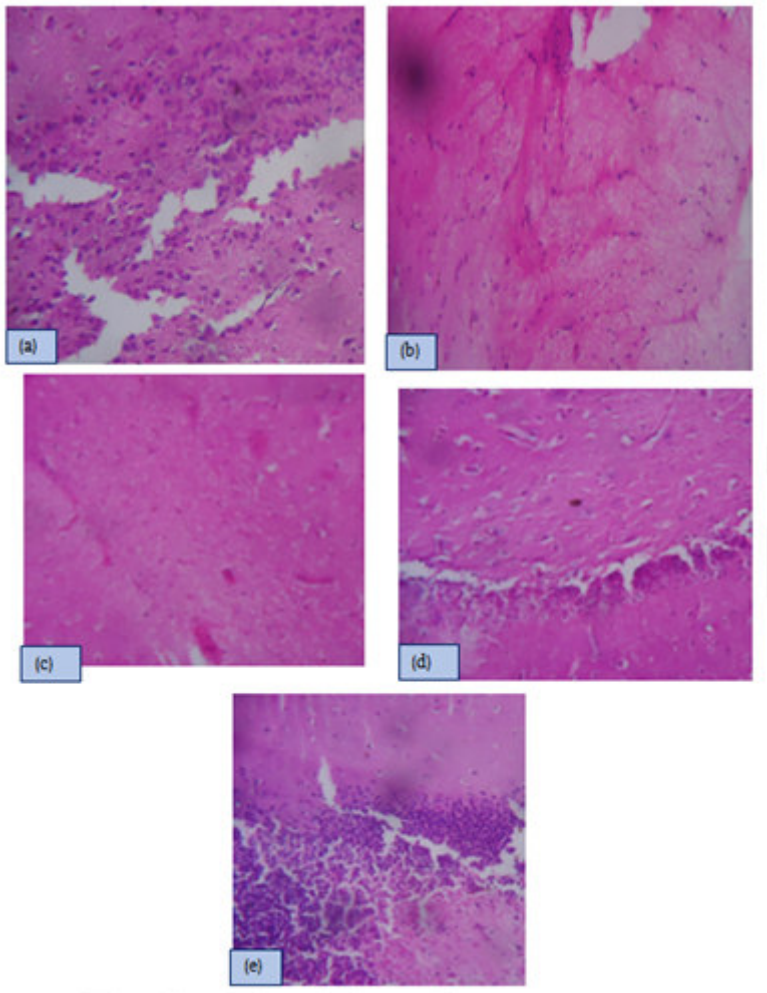
Values are expressed in mean ± S.E.M. (N=6)

Figure 10
Effect of HAST on Acetylcholinesterase activity



Each bar represents the mean±S.E.M (n=6). One-way ANOVA followed by Tukey'sposthoctest[a] indicates P<0.001 when compared to Normal control[b & c] indicates p<0.01and p< 0.001 respectively when compared to Negative control.

Figure 11
Light microscopic examination of hippocampus of brain.



3) RESULTS

3.1 Determination of acute toxicity

The mice treat with Sapindus trifoliatus at a dose of 2000 mg/kg body weight, P.O. exhibited normal behaviour without any sign of passivity, stereotype and vocalization. Their motor activity and secretory sign were also normal and no sign of depression and no any mortality up to the dose of 2000 mg/kg of body weight. So 200 and 400 mg/kg dose was selected for our study as low and high doses.

3.2 Locomotor activity

The results in Table 1 and Figure 1 showed a significant decrease in activity of rats treated with $AlCl_3$ (negative control group) for 30 days as compared with normal group. While rats treated with HAST and rivastigmine exhibited a significant increase of rat activity in combination with $AlCl_3$ when compared with the negative control group.

3.3 Motor coordination test

The results in Table 2 and Figure 2 showed a significant decrease in fall off time of rats treated with $AlCl_3$ (negative control group) for 30 days as compared with normal group. While rats treated with HAST and rivastigmine exhibited a significant increase of rats fall off time in combination with $AlCl_3$ when compared with the negative control group.

3.4 Elevated plus-maze (EPM) test for spatial memory in rats

The results in Table 3 and Figure 3 showed a significant increase in transfer latency of rats treated with $AlCl_3$ (negative control group) for 30 days as compared with normal group. While rats treated with HAST and rivastigmine exhibited a significant decrease of rats transfer latency in combination with $AlCl_3$ when compared with the negative control group.

3.5 Morris water maze test in Rats

The results in Table 4,5 and Figure 4,5 showed a significant increase in swim latency and swim a distance respectively of rats treated with $AlCl_3$ (negative control group) for 30 days as compared with normal group. While rats treated with HAST and rivastigmine exhibited a significant decrease of swim latency and swim a distance respectively in combination with $AlCl_3$ when compared with the negative control group.

3.6 Passive avoidance test

The results in Table 6 and Figure 6,7,8 showed a significant decrease in step down latency, significant increase in step down error and time spent in the shock zone respectively of rats treated with $AlCl_3$ (negative control group) for 30 days as compared with normal group. While rats treated with HAST and rivastigmine exhibited a significant increase in step down latency, significant decrease in step down error and time spent in the shock zone respectively in combination with $AlCl_3$ when compared with the negative control group.

3.7 Y-maze test

The results in Table 7 and Figure 9 showed a significant increase in time to reach previsited arm of rats treated with $AlCl_3$ (negative control group). While rats treated with HAST and rivastigmine exhibited a significant decrease in time to reach previsited arm in combination with $AlCl_3$ when compared to before treatment and after 30 days treatment.

3.8 Oxidative parameter

The results in Table 8 showed a significant decrease in SOD, catalase, GSH and a significant increase in LPO of rat brain homogenate treated with $AlCl_3$ (negative control group) for 30 days as compared with normal group. While rats treated with HAST and rivastigmine exhibited a significant increase SOD, catalase, GSH and significant decrease in LPO of rats brain homogenate in combination with $AlCl_3$ when compared with negative control group.

3.9 Acetylcholinesterase (AchE) activity

The results in Table 9 and Figure 10 showed a significant increase in Acetylcholinesterase activity of rat brain homogenate treated with $AlCl_3$ (negative control group) for 30 days as compared with normal group. While rats treated with HAST and rivastigmine exhibited a significant decrease in Acetylcholinesterase activity of rat brain homogenate in combination with $AlCl_3$ when compared with the negative control group.

3.10 Histopathology of hippocampus

Light microscopic examination (H&E × 100) shown in figure 11 (a) The image of the brain section of a normal control rat showing normal histological structure of the hippocampus. (b) The image of the brain section of a negative control rat showing plaques in hippocampus. (c) The image of the brain section that treated with 200 mg/kg of HAST of 30 days followed by $AlCl_3$ showing neuron that appear more or less like a normal one. (d) The image of the brain section that treated with 400 mg/kg of HAST of 30 days followed by $AlCl_3$ showing neuron that appear like a normal one. (e) The image of the brain section that treated with 0.3 mg/kg of HAST of 30 days followed by $AlCl_3$ showing neuron that appear like a normal one.

4) DISCUSSION

AD is a chronic, progressive, degenerative disease of central nervous system. It is the most common form of dementia. The word dementia describes a set of symptoms that can include memory loss and thinking orientation, comprehension difficulties, problem solving or language.[3] Neurodegeneration in the hippocampus and neocortex are associated with the special memory impairment.[34] While no drug has been shown to completely protect neurons, agents that inhibit the degradation of acetylcholine within the synapse are the mainstay of treatment of AD. Herbal plant products play an important role in disease management without side effects. The present study has outlined the effectiveness of HAST as a therapeutic agent for AD in experimental animal models of AD. Aluminum is present in water as municipal water supplies treated with both aluminum chloride and aluminum sulphate. As well as in food as cans used for beverages, coals and fruit drinks, beside aluminum containers increase in the amount of toxic metal in food. Also on medication as antidiarrheal, eye drops, vaccines and intravenous solution and in air born aluminum come from industrial sources or increase the frequency of use of antiperspirants.[35] It is well established that aluminium a neurotoxic agent that induces the production of free radicles in the brain. Accumulation of free radicals may cause degenerative events of aging such as AD. In the present study rats treated with the $AlCl_3$ to develop AD. The exact pathogenesis of AD by $AlCl_3$ is still unclear. Deficiency of Ach is critical in the genesis of the symptoms of AD [36], inflammation of the brain also plays a key role in AD pathogenesis.[37] Plant antioxidants cooperate with the body enzymes to protect the brain from free radical damage. *Sapindus trifoliatus* is a plant which is having a good antioxidant activity and high amount of flavonoids content. In the present study, rats treated with $AlCl_3$ showed a decrease in levels of activity in the activity cages and in the duration of rotation on the Rotarod as well as an increase in the length of time taken by rats to reach the previsited arm in Y-maze test, rats also showed increase the transfer latency in elevated plus maze, not climb the pole in pole climb response apparatus, increase the swim latency and swim distance in Morris water maze test, decrease the step down latency, increase the step down error and time spent in shock zone in passive avoidance test. The AD rats also showed a significant an increase in AchE activity. Histopathology of brain tissues revealed the presence of amyloid plaques in the hippocampus. AD-induced rats showed significant decrease in SOD, catalase and GSH. And significant increase in MDA. This indicated that the mechanism by which $AlCl_3$ induced AD involves induction of oxidative stress. In this investigation we studied the protective and therapeutic effects of HAST (200 & 400 mg/kg body weight) and of rivastigmine (as a reference drug) to determine their effects on the results of behavioural stress activities and on brain levels of AchE in $AlCl_3$ induced AD rats. Rivastigmine was used as standardized drugs as it is the only proven pharmacological therapy for the symptomatic treatment of AD. Treatment of AD rats with rivastigmine as a protective or therapeutic agent lead to an improvement in the oxidative stress, decrease the levels of AchE, all the behavioural parameters and histopathology of hippocampus when compared to $AlCl_3$ treated group. The results of the present study reveal that the protective and the therapeutic group of AD induced rats treated with HAST (200 or 400 mg/kg) exhibited a significant improvements in the AD induced in rats, as improvement of behavioural parameters, oxidative parameter, histopathology of hippocampus and significant decrease in the

AchE level in the brain when compared to $AlCl_3$ treated group.

5) CONCLUSION

From the behavioural, biochemical and histopathological parameters the present investigation suggest that treatment of hydro-alcoholic extract of Sapindus trifoliatus can protect the $AlCl_3$ induced Alzheimer's disease (AD) in rats. This beneficial effect might be partly due to suppression of oxidative stress and formation of plaques in the hippocampus area of the brain. Overall the title plant could be promising therapeutic option for the management of AD. Further study is required to approach the new insight for the possible mechanism of action.

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