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Pharmacological investigation of *Achras sapota* against scopolamine induce amnesia and cognitive impairment in laboratory animals

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ABSTRACT

The present study was undertaken to investigate the effect of *Achras sapota* (*A. sapota*) fruits in scopolamine induced amnesia & cognitive impairment in mice. *A. sapota* commonly known as Chiku belong to Sapotaceae family. Memory impairment was induced in Swiss albino mice by a single injection of scopolamine (1 mg/kg, i.p). Animals (Swiss albino mice) were divided into five separate groups of six animals each. Positive control group received CMC (carboxy methyl cellulose) as vehicle, negative control group received scopolamine along with vehicle, standard group received Donepezil (5 mg/kg, p.o) with scopolamine. Ethanolic extract of *A. sapota* (EEAS, 200 mg & 400 mg/kg, p.o) was administered to group Test 1 and Test 2 respectively along with scopolamine. Elevated plus maze (EPM), modified passive avoidance test, Morris water maze (MWM) models and locomotor activity were employed as exteroceptive behaviour models to assess learning and memory activity. Thereafter lipid peroxidation, reduced glutathione and catalase level were estimated in homogenized brain of mice. The extract showed the presence of different chemical constituents like flavonoids, tannins, glycosides and alkaloids. The pre-treatment of mice with EEAS (200 mg/kg & 400 mg/kg) significantly reduced the scopolamine induced increase in EL time in MWM, whereas in EPM administration of extract produces significant decrease in TL. In Modified passive avoidance test significant increase in SDL, was shown by the animals. In locomotor activity, treatment of EEAS did not alter normal locomotor activity whereas lipid peroxidation was significantly decreased, catalase & reduced glutathione levels were significantly increased in animals of test 1 & test 2 when compared to negative control group. Hence it would be worthwhile to explore the potential of this plant in management of cognitive impairment and other memory disorders

1. Introduction

Learning is one of the most characteristic attributes of humans and higher animals. It is defined as the process of acquiring new information or skills, while the subsequent retention of that information is called memory [1]. Memory is a crucial function of the brain, enabling individuals to record sensory stimuli and information, retain it over short or long periods, and recall it when needed, which is vital for survival [2]. Once memories are stored in the brain, they become part of the brain's processing mechanism and involve multiple neuronal pathways and neurotransmitters when recalled in the future [3,4]. The cholinergic system plays a significant role in learning and memory, with the neurotransmitter acetylcholine being important for recognition functions such as memory [5,6]. Acetylcholine is synthesized in certain neurons by the enzyme choline acetyltransferase from choline and acetyl-CoA [7]. Acetylcholinesterase (AChE) is an enzyme that breaks down acetylcholine into choline and acetate. A loss of cholinergic neurons and reduced choline acetyltransferase activity in the cerebral cortex and hippocampus are consistent with findings in Alzheimer's disease [8,9]. For the neurobiologist, clinical management of dementia remains a nightmare [10]. The cornerstone of AD treatment to date has been cholinesterase inhibitors such as donepezil, rivastigmine, galantamine,

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etc. The rationale behind developing programs aimed at treating Alzheimer's symptoms is the administration of AchE inhibitors, which increase the availability of acetylcholine (Ach) at cholinergic synapses [4]. Anti-inflammatory drugs, antioxidants, nonsteroids, and some neuroprotective agents have also been used, albeit with varying degrees of success. Alzheimer's disease-related confusion, or dementia, is treated with donepezil. While it doesn't treat Alzheimer's, it might help with functioning, memory, and consciousness. This drug, an enzyme blocker, functions by reestablishing the proper ratio of organic compounds (neurotransmitters) in the brain. Scopolamine is used to stop motion sickness and post-operative anesthesia-related nausea and vomiting. Although scopolamine-induced models are utilized to investigate novel compounds in the realm of learning, scopolamine is also used to treat some intestinal or stomach issues, muscle spasms, and Parkinson-like diseases [10]. Drugs and natural therapies have been prescribed for centuries to help people's memories. Ayurveda, the Indian System of Medicine, possesses an abundance of memory-boosting medications that are widely used today owing to their demonstrated efficaciousness. The brain-affecting herbs are known as nootropic herbs, and the separate ingredients in them are known as smart medications. Herbs that improve memory also improve blood circulation in the brain.

Locally, *Achras sapota* (*A. sapota*) (Sapotaceae) is referred to as "chiku," a therapeutic herb. Numerous investigations have demonstrated the high antioxidant potential, analgesic, mosquitocidal, hypoglycemic, and other properties of *A. sapota.* Fruit part has traditionally mentioned to cure brain diseases. A. sapota possessed various pharmacological actions such as anti-inflammatory, anti-pyretic activity, antioxidant potential, analgesic activity, mosquitocidal activity, anticancer activity, hypoglycemic activity, antitumor activity, acute and sub-chronic toxicity study, *in vitro* antibacterial activity, *in vitro* anti-arthritic activity, antibacterial activity [11–15]. To the best of our knowledge, no research has been done on its anti- amnesic properties; instead, this study examined the effects of ethanolic extract of *Achras sapota* fruit at varying doses on memory and learning in response to scopolamine-induced amnesia in lab animals.

2. Materials and methods

2.1. Plant material

The *A. sapota* fruit were collected from Lucknow, India during the month of November 2015. The plant material was authenticated by NISCAIR (The National Institute of Science Communication and Information Resources), New Delhi, India and voucher specimens were deposited for future reference. (Reference no NISCAIR/RHMD/Consult/2015/2908/101).

2.2. Preparation of plant extracts

The peels of the fruits were removed from the pulp part and pulp were shade dried and then crushed to make a very small piece. First, it was treated 'with petroleum ether to remove the impurities. The dried pulp powder of sapota fruit was extracted by the process of maceration by using 70 % ethanol as a solvent. After 72 hrs. the extract was filtered with Whatman No.1 filter paper and dried. Extract was further stored for all further studies [11].

2.3. Phytochemical screening

The ethanolic extract of *A. sapota* (EEAS) was qualitatively tested for the presence of various chemical constituents like alkaloids, flavonoids, glycosides, tannins, saponins etc. by using standard methods. The percentage yield was found to be 11.4 % W/V [16].

2.4. Animals

Swiss albino mice weighing 18–25 g of either sex were used for the study after the approval of the Institutional Animal ethics committee. (BBDNIIT/IAEC/05/16/04). Mice used for the study was obtained from the animal house of Babu Banarasi Das Northern India institute of Technology, Lucknow, U.P, India**.** Animals were house in clean polypropylene cage containing husk to keep them dry throughout the experiments. The bedding material of the cages was changed every day. The animals were housed under standard laboratory conditions such as room temperature at 22±3°C, humidity at 30–70 %, and with natural day and night cycle. They were kept on standard pallet diet and water *ad libitum*.

2.5. Drugs and chemicals

Scopolamine hydrobromide (Sigma Aldrich, USA), 5,5-dithio-bis2-nitrobenzoic acid, (Ellman's reagent), trichloroacetic acid, thiobarbituric acid (TBA) were purchased from Sigma–Aldrich (Bangalore, India) Dithiobis-nitrobezoic acid (SD Fine Chem Limited), Donepezil (as a gift sample from Vasudha Pharma, Hyderabad), All other chemicals and reagents used for the study were of analytical grade.

2.6. Pharmacological models

2.6.1. Grouping and treatment protocol

Elevated plus maze (EPM), modified passive avoidance test, Morris water maze (MWM) models and locomotor activity were employed as exteroceptive behaviour models to assess learning and memory activity. Animals were randomly divided into five groups of six animals each. The groupings for pharmacological screening models were as follows:

Group 1: Positive control (PC) mice received only vehicle.

Group 2: Negative control (NC) mice received scopolamine (1 mg/kg, i.p.) [17].

Group 3: Standard drug (STD) Donepezil (5 mg/kg, i.p.) and scopolamine (1 mg/kg, i.p.)

Group 4: Lower dose of ethanolic extract of *A. sapota* (EEAS, 200 mg/kg, p.o) and Scopolamine (1 mg/kg, i.p.).

Group 5: Higher dose of ethanolic extract of *A. sapota* (EEAS, 400 mg/kg, p.o) and scopolamine (1 mg/kg, i.p.).

2.6.2. Elevated plus maze

The exteroceptive behavioural model used to assess mice's long-term memory was the elevated plus-maze. Two open (16 ×5) and two closed (16 ×5 x 20) arms made up the maze. Every treatment was administered for 14 days. After 60 minutes of medication delivery on the fourteenth day, all groups—aside from group 1—were given an intraperitoneal injection of scopolamine (1 mg/kg). Following a 30-minute scopolamine injection, each animal was positioned at the end of an open arm, with its back to the centre. The duration of time it took the animal to transition from an open to a closed arm, i.e. There was a documented transfer latency (TL) during the acquisition trial. The amount of time (measured in seconds) that the animals need to transition from the exposed arm to a covered arm with all four legs was called TL. The mice were placed into one of the closed arms for 15 seconds to investigate it if they were unable to reach the closed arm within the 180-second time limit. In this case, the TL was recorded as 180 seconds. The process was repeated after 24 hours of exposure, and on the day of retention, TL was noted as the memory parameter [18–21].

2.6.3. Morris water maze

The maze consisted of a white circular pool that was 100 cm in circumference and 50 cm in height. The water inside had a featureless inner face and was 20 ± 1°c, with a depth of 30 cm. The animals received the therapy for a period of 14 days. An hour following all treatments on day 14, scopolamine was administered. From the tenth to

the thirteenth day, the animals were taught for the maze task four times a day, with a five-minute interval between each attempt. The mice were given a 120-second initial learning phase and 180-second scopolamine-induced amnesia trial before failing to locate the platform and spending 30 seconds on it. On day 14th, water made opaque by adding milk, and animals was tested 3 times with each of the three trials being performed between 30- and 45-min following scopolamine injection. Escape latency, no. of crossing and residence time was evaluated as parameters for testing of spatial learning [22].

2.6.4. Modified passive avoidance test

Long-term memory was examined using the passive avoidance apparatus, which is based on negative reinforcement. The device was a rectangular box measuring 27 × 27 × 27 cm3, with three wood walls and one Plexiglas wall. The apparatus included a wooden platform measuring 10 × 7 × 1.7 cm3 in the center of the grid floor, which was equipped with three mm stainless steel rods spaced eight mm apart. The grid floor was subjected to an electric shock. Throughout the trial, a 15 W bulb was used to light the box. The animals received all treatments for 14 days, and on the fourteenth day, scopolamine was injected into the animals following a 60-minute medication delivery period. Each mouse received a 30-minute dose of scopolamine before being placed on a wooden platform in the middle of the grid floor. Step-down latency (SDL) was recorded when the mouse stepped down and placed its paw on the grid floor. Foot shock (50 Hz; 1.5 mA; 1 s) was then applied. The amount of time it takes the mice to step down and plant all four paws on the grid floor is known as the SDL. We used mice with SDL who were between the ages of two and fifteen for both the acquisition and retention tasks. The training session ended and the acquisition job was completed 90 minutes later. Animals in the acquisition test were taken out of the shock-free zone if they did not step down after a certain amount of time. During the acquisition test, animals were removed from the shock-free zone if they did not step down for a period of 60 sec. Retention was tested after 24 h in a similar manner, except with an upper cut-off time of 180 sec [23,24].

2.6.5. Locomotor activity

Actophotometer, which use photoelectric cells connected in circuit to a counter, were used to study locomotor activity. When an animal blocks the light beam landing on a photo cell, a count is recorded. For five minutes, each animal was left alone in the activity cage, and the animals' behaviour was observed. The number of photo cells was recorded, and the change in locomotor activity was computed [24,25].

2.7. Biochemical estimation of markers of oxidative stress

2.7.1. Measurement of lipid peroxidation (LPO)

The brain was homogenized in 50 mM phosphate buffer and centrifuged at 15,375 × g for 20 minutes at 4 ºC to measure the level of malondialdehyde (MAD). The brain was then deproteinized using 40 % trichloroacetic acid (TCA) and 5 M hydrochloric acid, and 2 % (w/v) thiobarbituric acid was added in 0.5 M sodium hydroxide. After 15 minutes of heating the reaction mixture in a water bath at 90℃, it was centrifuged for 10 minutes at 12,000 × g. Using spectrophotometry, the pink chromate that developed was detected at 532 nm [26].

2.7.2. Catalase activity

A 10 % homogenate brain was created by mixing 1.95 mL of ice-cold, phosphate-buffered saline (0.05 M, pH 7) with 1 mL of hydrogen peroxide (0.019 M). The mixture was then centrifuged for 15 minutes at 10,000 rpm, −4º C, and the resulting supernatant was used to monitor changes in absorbance at 240 nm [27].

2.7.3. Glutathione levels

The Ellman (1959) procedure was used to assess the GSH level as non-protein thiols. After centrifuging the homogenate for two minutes at 15,000 g in cooled trichloroacetic acid 10 %, the supernatant was incubated with DTNB in a 1 M phosphate buffer at a pH of 7.0. At 412 nm, absorbances were measured. GSH levels were determined using a standard reduced glutathione curve [28].

2.8. Statistical analysis

Statistical analysis was performed by one-way analysis of variance (ANOVA) with Tukey post test. Values are expressed as mean ± SEM.

3. Results

3.1. Preliminary phytochemical screening

Preliminary phytochemical testing was performed to find out different phytochemical constituents present in *A. sapota*. The observation showed the presence of alkaloids, glycosides, tannins, flavonoids and saponins (Table 1).

Positive sign indicates the presence of various phytoconstituents with respective to concentration.

3.2. Acute toxicity study

In research on acute toxicity, mice treated with EEAS did not die. Since no significant behavioural effects were seen even at this higher dose of 4000 mg/kg, the study employed doses of 200 mg/kg and 400 mg/kg.

3.3. Elevated plus maze

The effects of the vehicle, control negative, EEAS (200 and 400 mg/kg), and donepezil (5 mg/kg) were assessed. When compared to positive control mice, the scopolamine (1 mg/kg) control group's TL values significantly increased on both the acquisition and retention days, suggesting a learning and memory impairment. It was determined that the outcomes shown in Table 2 were statistically significant. Day 11 showed a significant drop in the TL's EEAS (400 mg/kg) as compared to the scopolamine control group. When comparing the donepezil (5 mg/kg, p.o.) group to the scopolamine control group, the highest reduction in TL was seen.

3.4. Morris water maze

Scopolamine increased the delay to find the concealed platform in the Morris Water Maze test, and the preceding 400 mg/kg dose of EEAS significantly reduced the scopolamine-induced amnesia. The greatest reduction (Table 3) was observed with donepezil (5 mg/kg p.o.), suggesting that this medication improved learning and memory in the retention experiment.

3.5. Modified passive avoidance paradigm

In an acquisition and retention test, scopolamine (1 mg/kg i.p.) reduced step down latency, suggesting a memory deficit. When *A. saporata* was given orally for 14 days at doses of 200 mg/kg and

Table 1
Phytochemical analysis of EEAS.

S. No	Test	Present (+)/ Absent (-)
1.	Alkaloids (Dragendorff's test, Mayer's test, Wagner's test)	+
2.	Flavonoids (Lead acetate test, Shindo test)	+
3.	Glycosides (Modified Borntrager's test)	+
4.	Tannins (Ferric chloride test)	+
5.	Saponins (Foam formation test)	+

Table 2
Effect of EEAS on transfer latency in scopolamine induced amnesia in mice.

Groups	Treatments	Transfer Latency	
		Acquisition Day	Retention day
Positive Control (CMC)	0.5 % w/v	20.02±0.42	13.86±0.58
Negative Control (Scopolamine)	1 mg/kg	35.30±0.46	40.22±0.25
Standard (Donepezil + Scopolamine)	5 mg/kg + 1 mg/kg	22.92±0.30	14.46 ±0.25***
EEAS+ Scopolamine	200 mg/kg + 1 mg/kg	32.39±0.32	36.40±2.33
EEAS+ Scopolamine	400 mg/kg + 1 mg/kg	30.60±0.65	31.29 ±2.50**

Results expressed as Mean ± SEM (n=6) and **P< 0.001, ***P< 0.0001 as Compared with negative control group by One Way ANOVA followed by Tukey Test.

Table 3
Effect of EEAS on Escape Latency in scopolamine induced amnesia in mice.

Groups	Treatments	Acquisition latency (sec) Before scopolamine		Retention latency (sec) After Scopolamine
		Day 1	Day 3	Day 4
Positive Control (CMC)	0.5 % w/v	24.13 ±0.27	20.01 ±0.99	19.72±0.34
Negative Control (Scopolamine)	1 mg/kg	38.08 ±0.19	25.20 ±1.54	43.10±2.40
Standard (Donepezil + Scopolamine)	5 mg/kg + 1 mg/kg	23.35 ±0.20	18.44 ±0.83	20.60±0.29***
EEAS+ Scopolamine	200 mg/kg + 1 mg/kg	37.38 ±0.46	21.82 ±1.31	41.00±1.35
EEAS+ Scopolamine	400 mg/kg + 1 mg/kg	37.43 ±0.34	23.34 ±0.53	36.63±0.58*

Results expressed as Mean ± SEM (n=6) and *P< 0.05, ***P<0.0001 as Compared with negative control group by One Way ANOVA followed by Tukey Test.

400 mg/kg, there was a significant increase in step down latency (Table 4) and the scopolamine-induced amnesia was reversed. The mice in the donepezil (5 mg/kg p.o.) treatment group exhibited considerable improvements in memory and corrected scopolamine-induced amnesia.

3.6. Locomotor activity

Comparing the EEAS-administered 200 and 400 mg/kg p.o. to the control rats that received only the vehicle, no discernible decrease in locomotor activity was seen. (Table 5). Furthermore, neither the donepezil-treated group nor the scopolamine control group was able to significantly alter locomotor activity in comparison to the control. This suggests that the mice developed learning difficulties as a result of the scopolamine treatment, rather than any effect on their overall locomotor activities. Figs. 1–7

Table 4
Effect of EEAS on step down latency in scopolamine induced amnesia in mice.

Groups	Treatments	Step Down Latency	
		Step Down Latency (Day 1)	Step Down Latency (Day 2)
Positive Control (CMC)	0.5 % w/v	7.00±0.37	12.68±0.29
Negative Control (Scopolamine)	1 mg/kg	7.96±0.55	5.56±0.18
Standard (Donepezil + Scopolamine)	5 mg/kg + 1 mg/kg	8.16±0.27	10.28±0.74***
EEAS+ Scopolamine	200 mg/kg + 1 mg/kg	4.37±0.16	5.62±0.30
EEAS+ Scopolamine	400 mg/kg + 1 mg/kg	5.12±0.21	7.67±0.54

Results expressed as Mean ± SEM (n=6) ***P<0.0001 as Compared with negative control group by One Way ANOVA followed by Tukey Test.

Table 5
Effect of EEAS on Locomotor activity in scopolamine induced amnesia in mice.

Groups	Treatments	Locomotor activity (Counts/ 5 minutes)
Positive Control (CMC)	0.5 % w/v	112.50±6.95
Negative Control (Scopolamine)	1 mg/kg	111.67±6.77
Standard (Donepezil + Scopolamine)	5 mg/kg + 1 mg/kg	113.17±6.89
EEAS+ Scopolamine	200 mg/kg + 1 mg/kg	103±3.03
EEAS+ Scopolamine	400 mg/kg + 1 mg/kg	106.17±3.25

For all groups n=6, all the data for locomoter activity are represented as mean ± standard error of the (SEM), and were statically analyzed by One Way ANOVA followed by Tukey Test.

3.7. Biochemical parameters

Oral EEAS administration at a dose of 200 mg/kg did not substantially lower the level of lipid peroxidation; however, at a dose of 400 mg/kg, the level of lipid peroxidation was impressively (**P<0.001) lower than in the corresponding control negative group. In comparison to their respective negative control group, the oral administration of EEAS at a dose of 200 mg/kg did not significantly raise the level of catalase. However, at a dose of 400 mg/kg, the level of catalase increased remarkably (**P<0.001). When compared to Scopolamine treatment, donepezil significantly increases (p<0.001) (0.38±0.01) catalase activity (Table 6).

4. Discussion

Alzheimer's disease (AD) is a brain condition that worsens over time [29]. The development of neurofibrillary tangles in the cortical regions of the brain and the medial temporal lobe, as well as senile plaques, are the hallmarks of this neurodegenerative condition that is linked to a reduction in cognitive function [30]. It is a prevalent kind of dementia in the elderly that can have catastrophic effects on the person with the diagnosis, their family and caregivers, and society as a whole. It is a leading cause of death and disability, and it is thought to have an annual impact of more than $100 billion on health care expenses, including direct and indirect medical and social service costs.

The typical onset of AD is a gradual loss of memory that eventually

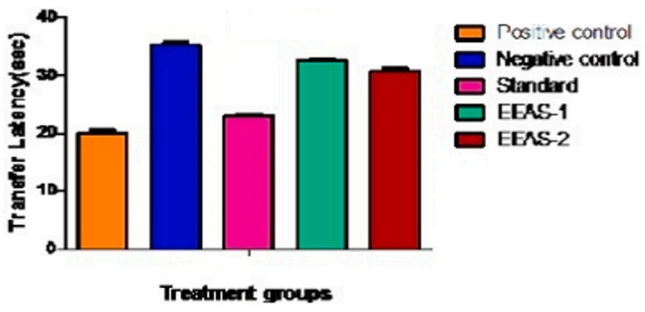

Fig. 1. Effect of EEAS on Transfer Latency in scopolamine induced amnesia in mice on Day 1.

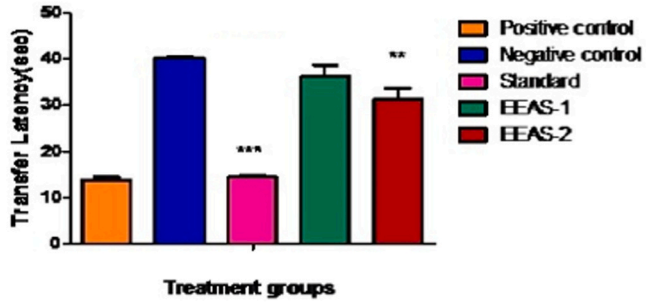

Fig. 2. Effect of EEAS on Transfer Latency in scopolamine induced amnesia in mice on Day 2.

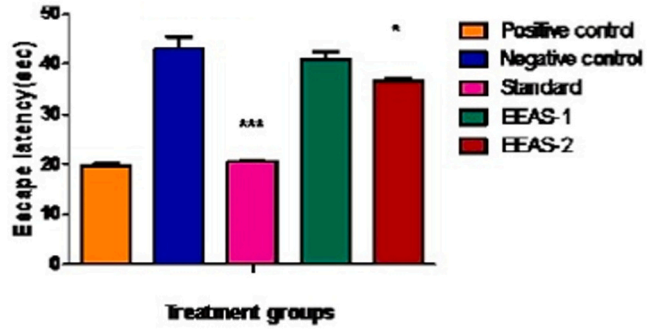

Fig. 3. Effect of EEAS on escape latency in scopolamine induced amnesia in mice.

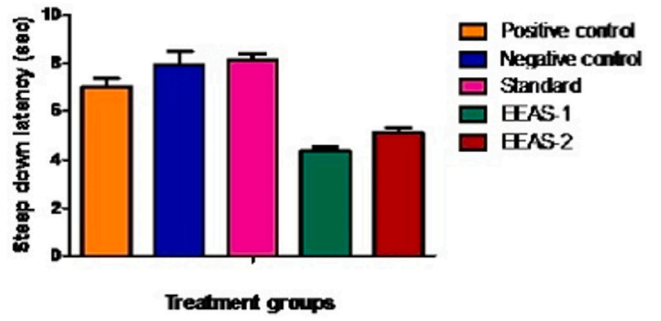

Fig. 4. Effect of EEAS on Steep down latency in scopolamine induced amnesia In mice on Day 1.

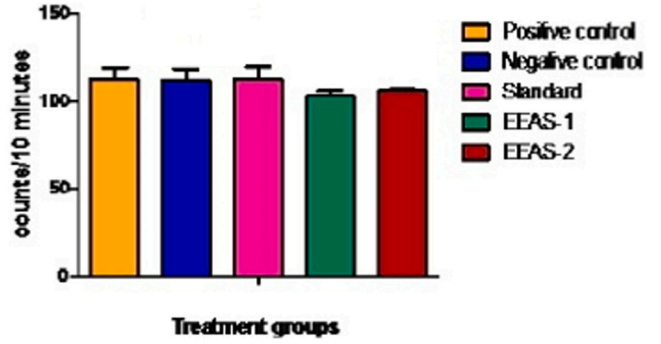

Fig. 5. Effect of EEAS on Locomotor activity in scopolamine induced amnesia in mice.

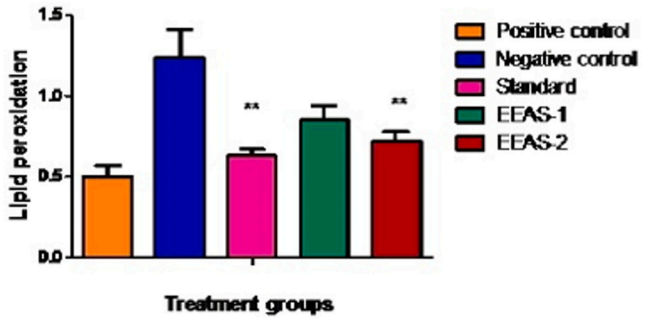

Fig. 6. Effect of EEAS on Lipid peroxidation in scopolamine induced amnesia in mice.

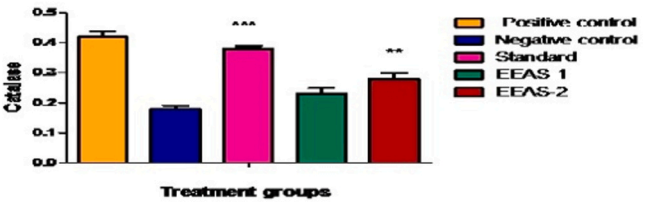

Fig. 7. Effect of EEAS on Catalase in scopolamine induced amnesia in mice.

Table 6
Effect of EEAS on lipid peroxidation and catalase in scopolamine induced amnesia in mice.

Groups	Treatments	Lipid Peroxidation (µmole /mg protein)	Catalase (µmole of H_2O_2 decomposed/ mL n/mg protein)	GSH (µmol of GSH/mg/ Tissue)
Positive Control (CMC)	0.5 % w/v	0.50±0.07	0.42±0.02	0.51 ±0.03
Negative Control (Scopolamine)	1 mg/kg	1.24±0.17	0.18±0.01	0.43 ±0.04
Standard (Donepezil + Scopolamine)	5 mg/kg + 1 mg/kg	0.63±0.04**	0.38±0.01***	0.66 ±0.05***
EEAS+ Scopolamine	200 mg/kg + 1 mg/kg	0.85±0.09	0.23±0.02	0.52 ±0.03
EEAS+ Scopolamine	400 mg/kg + 1 mg/kg	0.70±0.03**	0.28±0.02**	0.57 ±0.04**

Result expressed as Mean ± SEM (n=6) and **P<0.001, ***P<0.0001 as Compared with negative control group by One Way ANOVA followed by Tukey Test.

impairs language, computations, visuospatial perception, and executive functions. In AD, behavioral and psychological signs are also common. Acetylcholinesterase (AChE) inhibitors and muscarinic or nicotinic receptor ligands have been the cornerstones of treatment for AD-related cognitive decline. medications that can cause unfavorable side effects like cramping in the muscles, nausea, vomiting, diarrhea, drowsiness, and bradycardia [30]. There is still no effective counteragent offered by the allopathic medical system, despite the seriousness and widespread occurrence of this illness [24].

Since ancient times, natural items like herbal remedies have been utilized as an alternate form of treatment for neurodegenerative illnesses like AD. The connection between natural goods and AD is clear. As a result, the current study adopts a mouse model of scopolamine-induced amnesia to investigate the memory-enhancing properties of the EEAS.

The phytoconstituents found in the plant, primarily alkaloids, flavonoids, and tannins (gallic acid), may be the cause of its potential anti-amnesic properties.

Gallic acid is a naturally occurring phenol antioxidant. Studies have shown that using phytoconstituents as pharmacological therapy to treat oxidative stress-related illnesses and scavenge free radicals is both clinically effective and less hazardous than current drug therapies. Based on our findings, it's possible that EEAS's anti-amnesic properties stem from its antioxidant properties. In terms of biochemical parameters, EEAS has demonstrated dose-dependent effects; in all animals, a 400 mg/kg dose of EEAS produced more notable outcomes than a 200 mg/kg dose, suggesting more efficacies in the recovery from scopolamine-induced memory deficits [31].

The current study shows that scopolamine-induced amnesia can benefit from the use of ethanolic extract of A. sapota (EEAS). The Elevated Plus Maze (EPM), Modified Passive Avoidance Test (MPA), Morris Water Maze (MWM), and locomotor activity—behaviour tests that are commonly used to evaluate animal learning and memory paradigms—all demonstrated significant ant amnesic action in the EEAS. We also looked into how EEAS affected reduced glutathione, catalase, and lipid peroxidation.

In a pharmaceutical paradigm, scopolamine, a non-selective muscarinic cholinergic antagonist, is employed to produce amnesia [32]. Because scopolamine interferes with acetylcholine in the brain, it induces oxidative stress and impairs cognitive function. AChE and MDA levels in the brain and hippocampus are markedly elevated by scopolamine [33]. An essential function of hippocampal cholinergic neurotransmission is to underpin memory and learning. Nonselective muscarinic antagonist scopolamine inhibits cholinergic signalling without altering acetylcholine levels, resulting in memory deficits resembling those associated with aging-related senile central nervous system failure.

The enzyme that catalyses the hydrolysis of acetylcholine, which ends cholinergic transmission, is called acetyl cholinesterase. As of right now, it's thought that this enzyme's activity might have an impact on the fundamental mechanisms of AD [4]. We administered scopolamine (1 mg/kg, i.p.) to the mice and saw that it increased TL in the elevated plus maze, EL in the modified passive avoidance test, and decreased SDL in the modified passive avoidance task. These results demonstrated that our paradigm is a useful tool for assessing learning and memory in mice.

A well-known medication for treating Alzheimer's type dementia brought on by scopolamine is donepezil [34]. Acetyl cholinesterase's active core is more strongly bound by donepezil [35]. It improves cholinergic neurotransmission by delaying the breakdown of acetylcholine delivered into synaptic cleft [36]. When compared to the negative control group, the outcomes of EPM, MWM, and MPA demonstrated considerable effectiveness in the parameters that were observed [36].

A classic model for assessing anxiety-like behaviours in mice and rats is the elevated-plus maze [34]. However, transfer latency—that is, the amount of time it takes for an animal to move from an open to an enclosed arm—was significantly reduced if the animal had previously entered both open and closed arms. This reduced transfer latency has been linked to memory functions. This paradigm has gained widespread acceptance as a means of studying rodent learning and memory processes, thanks to recent investigations on the effects of various nootropics and amnestic drugs on EPM.In EPM, retention/consolidation (memory) is assessed 24 hours after the initial day of trials, and acquisition (learning) can be viewed as transfer delay [3]. The current study demonstrates that EEAS have memory-enhancing activity since they help people with scopolamine-induced cognitive impairment retain their spatial recall. In comparison to their respective negative control group, the oral administration of EEAS at a dose of 200 mg/kg did not substantially increase TL. However, at a dose of 400 mg/kg, TL increased remarkably (**$P<0.001$). When donepezil is used instead of Scopolamine, there is a considerable rise in TL.

An effective method for estimating standard learning and memory is the passive avoidance test. Then, both short-term and long-term memory are assessed using this test [28]. When compared to the negative control group, mice in the current study treated with EEAS (200 mg/kg and 400 mg/kg po.) showed increases in SDL on the fourteenth day, indicating a significant improvement in memory. Donepezil dramatically improves SDL in comparison to the negative group.

The MWM model included in this study is one of the most generally recognized models for evaluating learning and memory, and it is used to evaluate the capacity for hippocampal-dependent spatial learning [37]. The working and reference memory processes could be analyzed simultaneously thanks to the paradigm we employed in the Morris water maze test. Daily reductions in escape latency are indicative of learning related to long-term memory or reference.

Long-term memory impairment was noted in the group receiving scopolamine treatment. While EEAS at dose (400 mg/kg, p.o.) significantly reduced the escape latency time that had received the scopolamine, EEAS at dose (200 mg/kg) did not significantly shorten the escape latency, indicating that EEAS alleviated the memory impairment. The majority of CNS medications affect how people and animals move. The locomotor activity mean scores for each mouse in the current investigation were comparatively constant and did not significantly differ between groups. When the test medications were compared to the negative control group, there was no discernible difference in the amount of locomotor activity [38].

There is a growing body of evidence in the literature that indicates oxidative stress as a crucial factor for several disorders including dementia, furthermore data indicates that oxidative stress is one of the earliest events in pathogenesis of memory impairment [39]. Oxidative stress is thought to be important in the progression of AD and is temporally linked to the development of plaques and NFTs [34]. The lipid peroxidation is well known elements to play an essential role in oxidative stress balance [38]. In experimental models of Alzheimer's disease, there is evidence of increased oxidation of lipids, proteins, and deoxyribonucleic acid; there are also changes in mitochondrial activity and a potential involvement of amyloid beta and its precursor protein in oxidative processes. An important measure for lipid peroxidation in the frontal cortex and hippocampal regions is scopolamine-induced thiobarbituric acid reactive substance activity. Because, as observed in Alzheimer's disease patients, oxidative stress plays a major role in the disruption of calcium homeostasis and the ensuing apoptosis [4]. Considerable data suggests that scopolamine causes oxidative stress in rats, which impairs cognitive function [38].

Depletion of GSH level in the brain, which is frequently regarded as the cell's first line of defence by this endogenous antioxidant against oxidative stress, may exacerbate lipid peroxidation. There is evidence that the glutathione system plays a major role in mediating the neural defence against H_2O_2, which is the most damaging chemical to the brain. Tri-peptide GSH is an endogenous antioxidant that varies in quantity in all animal cells and is a highly reliable marker of oxidative stress [32]. One sign of lipid peroxidation is MDA. When compared to positive control mice (0.50 ± 0.07), the injection of scopolamine in this investigation resulted in an increase in MDA levels (1.21 ± 0.17). Donepezil significantly reduced ($p<0.001$) (0.03 ± 0.04) the MDA level in mice given Scopolamine.

When EEAS (200 mg/kg, p.o.) was provided to mice instead of Scopolamine, the MDA level did not alter significantly ($P>0.05$). When EEAS was given to mice at a dose of 400 mg/kg, p.o., the MDA level was significantly lower (0.70 ± 0.03) than in the negative control group. The main enzyme that aerobic cells use to fend off the harmful effects of superoxide radicles is catalase [40]·H_2O_2 radicals can be detoxified by catalase. The release of H_2O_2 stimulates the synthesis of a wide range of additional oxidant species, which plays a significant role in oxidative stress and the pathogenesis of AD [32]. The current work demonstrates that pretreatment with EEAS (400 mg/kg, p.o.) significantly restored catalase activities after scopolamine therapy reduced brain catalase

activity. Catalase activity improved in both the conventional and EEAS treated groups [4].

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5. Conclusion

EEAS extract has been shown to improve memory across various experimental models, such as the Modified Passive Avoidance Test (MPAT), the Elevated Plus Maze (EPM), and the Modified Verbal Avoidance Test (MWM). In comparison to a negative control, EEAS displayed antioxidant properties at different doses by inhibiting lipid peroxidation, enhancing the levels of endogenous antioxidant enzymes, and reducing glutathione and catalase levels. These effects are likely due to the presence of phytoconstituents. While the exact mechanism of EEAS is not yet fully understood, further research into its potential for treating memory disorders and cognitive impairments would be valuable. Additionally, EEAS may offer benefits as an antioxidant therapy for patients with neurodegenerative conditions and elevated brain oxidative stress.

Ethics approval and consent to participate

Approval for the Swiss albino mice (Laboratory experimental animals) will only be done by IAEC. Swiss albino male mice were used after the complete approval of Institutional Animal Ethics Committee (IAEC).

Human and animal rights

No humans were used in this study.

Consent for publication

Not applicable.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

Data will be made available on request.

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