

Research Paper: Evaluation of nootropic activity of standardized *Epipremnum aureum* extract against scopolamine-induced amnesia in experimental animals



Sreemoy Kanti das¹, G. S Chakraborty², Tulika Chakrabarti³, Prasun Chakrabarti⁴, Mohammad Javad Gholamzadeh^{5*}, Mohammad Nami^{6,7,8,9}

1. Lecturer, Faculty of Pharmacy, Lincoln University College, Selangor, Malaysia

2. Principal and Professor, Parul Institute of Pharmacy & Research, Gujarat, India

3. Department of Chemistry, Sir Padampat Singhania University, Udaipur 313601, Rajasthan, India

4. Provost and Institute Endowed Distinguished Senior Chair Professor, Techno India NJR Institute of Technology, Udaipur 313003, Rajasthan, India

5. Students' Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran

6. Department of Neuroscience, School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran

7. Neuroscience laboratory-NSL (Brain, Cognition, and Behavior), School of Advanced Medical Sciences and Technologies, Shiraz University of Medical Sciences, Shiraz, Iran

8. Neuroscience Center, Instituto de Investigaciones Científicas y Servicios de Alta Tecnología (INDICASAT AIP), City of Knowledge, Panama

9. Dana Brain Health Institute, Iranian Neuroscience Society, Fars Chapter, Shiraz, Iran

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ABSTRACT

Introduction: Various plant species of genus *Epipremnum* have already been reported to have different types of pharmacological activities. However, another plant of the same genus *Epipremnum aureum* has not been scientifically exposed to a significant extent to date. Although it contains many bioactives, it has only been studied for antidepressant activity. The present study aims to evaluate the nootropic potential of standardized extract of *Epipremnum aureum* against scopolamine-induced amnesia in experimental animals.

Method: The nootropic potential of *Epipremnum aureum* was evaluated using an elevated plus maze and Morris water maze apparatus. A dose of 400mg/kg and 600mg/kg was used to access the nootropic activity. Scopolamine (0.4 mg/kg) was used to induce amnesia in mice. Additionally, the anti-acetylcholinesterase activity of the extract was evaluated by measuring the level of acetylcholinesterase in the mice brain.

Result: *Epipremnum aureum* was found to increase memory and reverse the amnesic action of scopolamine in a dose-dependent manner. In elevated plus maze and Morris water maze, *Epipremnum aureum* decreased the transfer latency as compared to the control group. Further biochemical investigation revealed an increased level of acetylcholine and decreased level of TBARS resulting in reversing the effect of scopolamine in amnesic mice.

Conclusion: *Epipremnum aureum* showed positive results in reversing the amnesia action of scopolamine which may be the probable mechanism for its memory retention activity. Based on the experimental outcome, the present study provides a piece of scientific evidence for the nootropic potential of *Epipremnum aureum* in experimental animals.

* Corresponding Author:

Mohammad Javad gholamzadeh

Address: Students' Research Committee, Shiraz University of Medical Sciences, Shiraz, Iran.

Tel: +989174829217

E-mail: mohammadjavadgholamzadeh@gmail.com

1. Background

Memory is the process to encode, accumulate, and recall information and past experiences. The loss of memory leads to dementia, and Alzheimer's disease (AD) is the most prevalent type of dementia, which accounts for 60-80% of cases worldwide [1]. AD is a primary neurodegenerative disease with characteristic neuropathological and neurochemical features. Dementia, an age-related mental problem is a characteristic symptom of Alzheimer's disease. The cholinergic hypothesis claims that a decrease in cognitive function in dementia is predominantly related to a decrease in cholinergic neurotransmission. The cholinergic muscarinic antagonist like scopolamine is the most widely used drug to induce amnesia in experimental animals. Nootropic drugs like Piracetam and cholinesterase inhibitors are clinically used to improve learning and memory abilities, mood, and behavior in those neurodegenerative diseases [2]. But the resulting side-effects associated with these synthetic drugs have made their utility limited [3]. Therefore, it is worthwhile to explore the utility of herbal medicines in the treatment of various neuropharmacological disorders associated with cognitive dysfunction as they are considered to be safe and economical.

Epipremnum aureum is known by many names but most common is "Money Plant." It is a large root-climber that belongs to the botanical family of Araceae. The plant generally stands at a height of between 5 m and 9 m and has a total spread of 1.5–2.5 m. This plant is widely known in Malaysia and Singapore and has a reputation as a traditional anticancer preparation as well as a remedy for skin diseases. A decoction of the fresh leaves with meat or eggs or as tea was reported to be a common practice among the locals. Leaves of *E. aureum* show great potential to reduce stress [4]. Stress is an important factor in the pathogenesis of cognitive dysfunction. Various plant species of genus *Epipremnum* have already been reported to have different types of pharmacological activities. *Epipremnum pinnatum* showed antimicrobial and anticancer activity. However, another plant of the same genus *E. aureum* has not been scientifically exposed to a significant extent to date. The leaves of an analogous species viz. *E. pinnatum* are ingested to heal chest pain. The aqueous leaf extract of *E.*

pinnatum finds use in the treatment of gonorrhoea, malaria, and diabetes, to lessen toothache, joint problems, and problems of the bone. In the present study, an attempt has been made to investigate the nootropic activity using a standardized extract of *E. aureum* given the reported anti-stress activity of this plant.

2. Method

A. Collection and authentication of plant

The fresh whole plant of *E. aureum* was collected from Kepong district, Malaysia. The official identification of the plant was carried out at Forest Research Institute Malaysia. The voucher specimen (No. SBID: 001/15) was prepared and deposited in the Faculty of Pharmacy, Lincoln University College, Malaysia for imminent reference.

B. Extraction of Plant material

E. aureum leaves were collected and chopped into small pieces and dried under shade for 2 weeks. The dried leaves were grounded to powder using a mechanical grinder. The coarse powder of leaves was obtained by passing through sieve no 20. Extraction of *E. aureum* was carried out in batches with 500g of powdered material in each batch. A continuous hot percolation process using Soxhlet apparatus was used for the extraction of the actives from crude leaves of *E. aureum*. After completion of extraction, the resulting extract was concentrated using a rotary evaporator and stored in desiccators until further use [5].

C. Determination of yield and physical characterization of extract

The dried residue was weighed and its physical characters such as color and consistency were investigated. Yield percent was determined by the following formula:

Extraction yield (%) = (weight of the freeze-dried extract x 100) / (weight of the original sample)

D. Thin-Layer Chromatography

Thin-layer chromatography (TLC) was used for the detection of phytochemicals in extracts, monitoring

the progress of column chromatography, and testing the homogeneity of the isolates [6].

E. Preparation of chromatographic plates

The chromatographic plates (15 × 8 × 0.4cm) were cleaned thoroughly and dried in a hot air oven at 105°C. A uniform suspension of silica gel G was prepared by dispersing one part of the adsorbent in 2.5 parts of distilled water using glass mortar and pestle. The suspension was applied to plates as films care was taken to eliminate air bubbles in the slurry. The plates were allowed to dry at room temperature and then activated at 105°C for one hour in a hot air oven. The plates were taken out and cooled to room temperature before use.

F. Detection of compounds

Standard solutions of caffeic acid, rosmarinic acid, and ferulic acid were prepared by dissolving 10mg of the individual substances in 1mL of distilled water. Substances were identified using UV detection at 254nm. Visualization was carried out by spraying with a solution of iron III chloride (2% in methanol) and aluminum chloride (1% in ethanol). Individual blue spots indicated the presence of caffeic acid, rosmarinic acid, and ferulic acid [7]. The R_f value of the sample was calculated and compared with the R_f value of the standard compounds.

R_f value = distance traveled by the solute/distance traveled by the solvent

G. Experimental animals

Swiss albino mice (18–25g) were selected for the study. Animals were kept in the animal house at 26 ± 2°C and 44–56% relative humidity, light and dark cycles of 12h respectively for 1 week before and during the experiments. Animals were provided with a standard rodent pellet diet, and the food was withdrawn 24 h before the experiment though the water was allowed ad libitum. All studies were performed under the guidelines for the care and use of laboratory animals, as adopted and promulgated by the Institutional Animal Ethics Committee, Lincoln University College, Malaysia.

H. Acute toxicity studies

The determination of lethal dose (LD_{50}) was performed according to OECD guideline 423. The plant extract at a dose of 2000mg/kg did not produce any observable toxic effects during the entire duration of the study and all animals survived 14 days of observation [8].

I. Animal

The experimental investigation was carried out using Swiss albino mice of either sex, weighing between 25 to 30g having 6 animals in each group.

J. Extraceptive behavioral model using the elevated plus maze

The elevated plus-maze consisting of two open arms (16 × 5cm) and two closed arms (16 × 5 × 12cm) was used [9]. The maze was elevated to the height of 25cm. Mice were placed individually at the end of an open arm facing away from the central platform and the time taken to move from the end of the open arm to either of the closed arm (Transfer latency, TL) was recorded. Where the animal did not enter into one of the enclosed arms within 90 seconds, it was gently pushed into one of the two enclosed arms, and the TL was assigned as 90 seconds. The mice were allowed to explore the maze for another 10 seconds and then returned to their home cage. After 90 minutes of administration on the 10th day, TL was recorded. Retention of the learned task was examined after 24 hours after the first-day trial.

K. Experimental design

Eight groups of animals were made, each group consisting of six mice:

- Group I: Received 1% CMC in normal saline (1ml) p.o saline water once daily for 10 days
- Group II: Received 0.4 mg/kg of Scopolamine
- Group III: Received 100 mg/kg of piracetam orally once daily for 10 days
- Group IV: Received 100mg/kg of piracetam extract orally once daily for 10 days. TL was recorded on completion of 90min of post-administration of scopolamine (0.4 mg/kg) intraperitoneally and the same procedure was repeated for recording, after

24 hours i.e. on the 11th day.

- Group V: Received 400mg/kg of ethanolic extract orally once daily for 10 days
- Group VI: Received 400mg/kg of ethanolic extract orally once daily for 10 days. TL was recorded on completion of 90 min of post-administration of scopolamine (0.4 mg/kg) intraperitoneally and the same procedure was repeated for recording, after 24 hours i.e. on the 11th day
- Group VII: Received 600mg/kg of ethanolic extract orally once daily for 10 days
- Group VIII: Received 600mg/kg of ethanolic extract orally once daily for 10 days. TL was recorded on completion of 90 min of post-administration of scopolamine (0.4 mg/kg) intraperitoneally and the same procedure was repeated for recording, after 24 hours i.e. on the 11th day.

L. Morris Water Maze design

This model was followed according to the method as described by Smith et al. [10]. Each animal was subjected to seven consecutive trials on each day with a gap of 5 minutes. The animals were gently placed in the water of the pool between quadrants, facing the wall of the pool with drop location changing for each trial, and allowed 120 seconds to locate the submerged platform. Then, it was allowed to stay on the platform for another 20 seconds. If it failed to find the platform within 120 seconds, it was guided gently onto the platform and allowed to remain there for 20 seconds. Escape latency time (ELT) to locate the hidden platform in the water maze was noted as an index of acquisition or learning. Animals were subjected to seven acquisition trials daily for seven consecutive days. On the eighth day, the platform was removed and each rat was allowed to explore in the pool for 120 seconds. The average time spent in all four quadrants was noted. The mean time spent by the animal in the target quadrant searching for the hidden platform was noted as an index of retrieval (memory).

For the acquisition trial, each rat was subjected to four trials each day. A rest period of 5 minutes was allowed between trials. Four trials per day were repeated for four consecutive days. Starting position on each day to conduct four acquisition trials was changed as described below and Q4 was

maintained as the target quadrant in all acquisition trials.

Day 1	Q1	Q2	Q3	Q4
Day2	Q2	Q3	Q4	Q1
Day3	Q3	Q4	Q1	Q2
Day4	Q4	Q1	Q2	Q3

Mean escape latency time (ELT) calculated for each day during acquisition trials day 5 ELT was used as an index of acquisition.

For the retrieval trial, on the eighth day, the platform was removed. The mouse was placed in the water maze and allowed to explore for 120 seconds. Each mouse was subjected to four such trials and each trial was started from a different quadrant. The average time spent in all three quadrants i.e. Q1, Q2, and Q3 was recorded and the time spent in the target quadrant i.e. Q4 in search of the missing platform provided an index of retrieval. Care was taken that relative location of the water maze with respect to other objects in the laboratory and prominent visual clues were not disturbed during the total duration of the study. All the trials were in a semi-soundproof laboratory.

N. Brain Acetylcholinesterase Activity (AChE)

Inhibition of AChE was assessed by a modified colorimetric method as described by Perry et al. [11]. The AChE activity was determined in a reaction mixture containing 200 µL of a solution of AChE (0.415 U/mL in 0.1 M phosphate buffer, pH 8.0), 100 µL of a solution of 5,5'-dithio-bis(2-nitrobenzoic) acid (3.3 mM in 0.1 M phosphate-buffered solution, pH 7.0) containing NaHCO₃ (6 mM), extract dilutions (0 to 100 µL), and 500 µL of phosphate buffer, pH 8.0. After incubation for 20 min at 25°C, acetylthiocholine iodide (100 µL of 0.05 mM solution) was added as the substrate, and AChE activity was determined with an ultraviolet spectrophotometer from the absorbance changes at 412 nm for 3.0 min at 25°C. The AChE inhibitory activities were expressed as percentage inhibition.

O. Estimation of Thiobarbituric Acid Reactive Substances (TBARS)

The quantitative measurement of thiobarbituric acid reactive substances (TBARS), an index of lipid peroxidation in the brain was performed according

to the method described by Ohkawa et al. [12]. 0.2 ml of supernatant of homogenate was pipetted out in a test tube, followed by the addition of 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 30% acetic acid (pH 3.5), 1.5 ml of 0.8% of thiobarbituric acid, and the volume was made up to 4 ml with distilled water. The test tubes were incubated for 1 hour at 95°C, then cooled and added 1 ml of distilled water followed by the addition of 5 ml of n-butanol-pyridine mixture (15:1 v/v). The tubes were centrifuged at 4000 g for 10 minutes. The absorbance of the developed pink color was measured spectrophotometrically (UV 2600 Shimadzu) at 532 nm. A standard calibration curve was prepared using 1-10 nM of 1, 1, 3, 3-tetra methoxy propane. The TBARS value was expressed as nanomoles per mg of protein.

3. Results

The qualitative determination of constituents by TLC analysis showed the presence of three different phenolic compounds caffeic acid, ferulic acid, and rosmarinic acid in the investigated extracts. The ethanolic extract showed the presence of caffeic acid, rosmarinic acid, and ferulic acid whereas the acetone and chloroform extract have shown the presence of rosmarinic acid only. Moreover, the extract also showed the presence of alkaloids and flavonoids in different solvent systems.

The Preliminary detection of various phenolic compounds against reference compounds is given in table 1.

Table 1. Preliminary detection of various phenolic compounds against reference compounds

Extract	Compounds	R _f value of sample	R _f value of reference compound
Ethanol	Caffeic acid	0.99, 0.75, 0.90	0.90
	Rosmarinic acid	0.83 , 0.78, 0.78	0.83
	Ferulic acid	0.91, 0.95 , 0.96	0.95

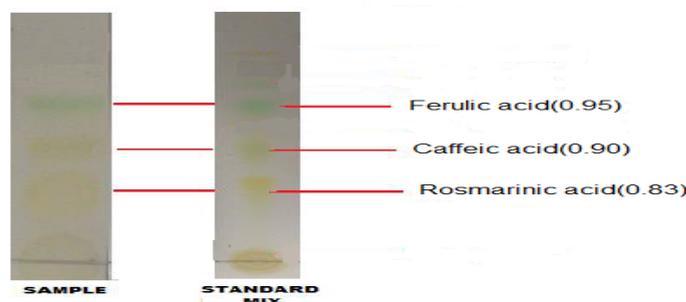


Figure 1. TLC chromatogram of Standard Mix and Extract

Table 2. Effect of *E. aureum* extract on the transfer latency in Elevated plus maze apparatus

Group	Treatment	TL on 10 th day	TL after 24 hrs
Group I	1% CMC in normal saline (1 mL) p.o	19.74±1.24	18.12±1.11
Group II	Scopolamine (0.4 mg/kg i.p)	39.24±1.12	38.24±1.12
Group III	100 mg/kg p.o of piracetam	9.24±1.12	7.12±1.27***
Group IV	100 mg/kg p.o of piracetam + scopolamine	11.74±1.24	10.12±1.97***
Group V	400mg/kg of extract extract p.o	17.79±1.57	16.12±1.08
Group VI	400mg/kg of extract extract + scopolamine	16.74±1.27	16.12±1.52
Group VII	600 mg/kg of extract	16.98±1.11	15.58±1.03***
Group VIII	600mg/of extract + scopolamine 1 g/kg	17.87±1.85	16.12±1.32***

Values are mean ± SEM expressed of each group. Data analysis was performed using one-way ANOVA followed by Bonferroni multiple comparison test against the respective control. Significance at *P < 0.05, **P < 0.01, and ***P < 0.001 was considered statistically significant, ns: not significant. SEM: Standard error of mean.

Table 3. Effect of ethanol extract of *E. aureum* on spatial memory in Morris water maze task in mice

Group	Treatment	Escape latency		
		Day 1	Day 4	Day 5
Group I	1% CMC in normal saline (1 mL) p.o	57.54±1.12	47.54±1.11	37.54±1.18
Group II	Scopolamine (0.4 mg/kg, i.p.)	89.84±1.22	85.33±1.25	81.51±1.42
Group III	Piracetam (100 mg/kg, i.p.) + Scopolamine (0.4 mg/kg, i.p.)	51.24±1.35	31.21±1.21	21.14±1.24***
Group IV	Piracetam (100 mg/kg, i.p.)	49.14±1.13	22.54±1.12	15.54±1.12**
Group V	Ethanol extract (400 mg/kg, p.o.)	54.84±1.32	49.24±1.34	43.54±1.20*
Group VI	Ethanol extract (400 mg/kg, p.o.) + Scopolamine (0.4 mg/kg, i.p.)	55.02±1.29	52.51±1.25	48.38±1.19*
Group VII	Ethanol extract (600 mg/kg, p.o.)	54.12±1.39	48.11±1.15	43.38±1.19***
Group VIII	Ethanol extract (600 mg/kg, p.o.) + Scopolamine (0.4 mg/kg, i.p.)	51.52±1.22	47.31±1.32	40.38±1.17***

Values are mean ± SEM expressed of each group. Data analysis was performed using one-way ANOVA followed by Bonferroni multiple comparison test against the respective control. Significance at *P < 0.05, **P < 0.01, and ***P < 0.001 was considered statistically significant, ns: not significant. SEM: Standard error of mean

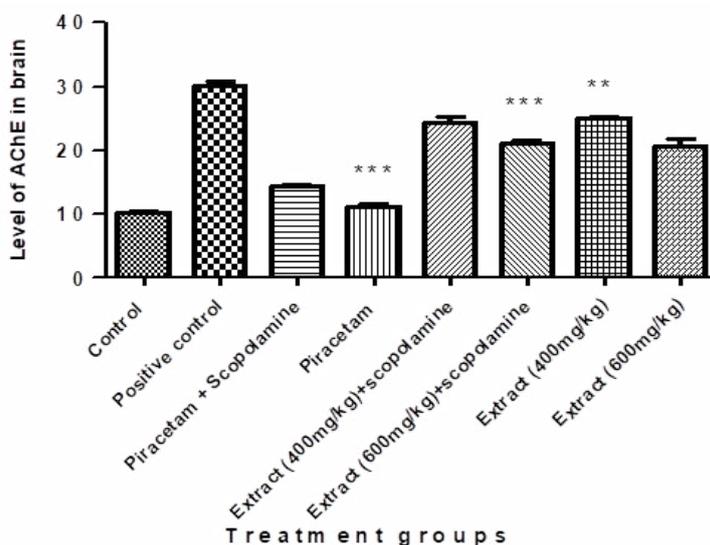


Figure 2. Effect of *E. aureum* extract on anti-acetylcholinesterase (AChE) activity

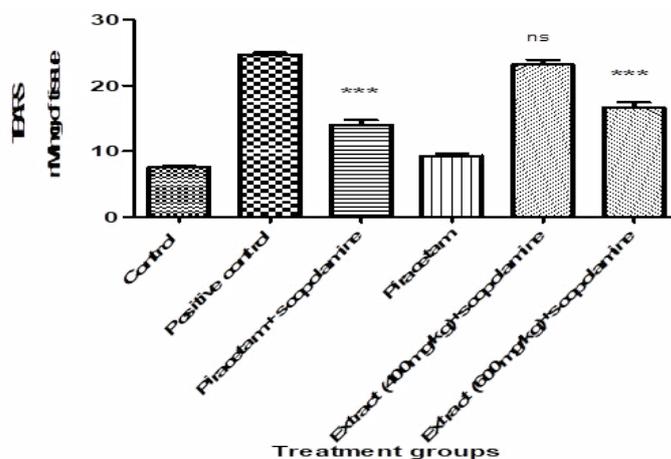


Figure 3. Effect of ethanol extract of *E. aureum* on brain levels of Thiobarbituric Acid Reactive Substances (TBARS)

Values are mean ± SEM expressed of each group. Data analysis was performed using one-way ANOVA followed by Bonferroni multiple comparison test against the respective control. Significance at *P < 0.05, **P < 0.01, and ***P < 0.001 was considered statistically significant, ns: not significant. SEM: Standard error of mean.

4. Discussion

Consolidation is the process of conversion of registered short-term information to a long-term memory trace, which involves physicochemical changes in neuronal networks. The stored information is made accessible by the process known as retrieval. Behavioral, pharmacological, and neurobiological studies have provided evidence for a cholinergic involvement in learning and memory [13]. The cholinergic hypothesis claims that the decline in cognitive function in dementia is predominantly related to a decrease in cholinergic neurotransmission [14]. The cholinergic muscarinic antagonist scopolamine is the drug most widely used to induce amnesia in experimental animals [15]. Nature provides a new opportunity to regain one's full mental capacity. Several herbs have been extensively studied and reported to have memory-enhancing properties.

The elevated plus maze was 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 was markedly shortened for the animals that had previously experienced entering open and closed arms. This shortened transfer latency is related to memory processes. In the elevated plus maze, acquisition (learning) was considered as transfer latency on the first-day trials and the retention/consolidation (memory) was examined 24 hours later. The animals treated with *E. aureum* extracts showed a significant decrease in transfer latency as compared to the control group. Piracetam at a dose of 100mg/kg significantly decreased the escape latency of scopolamine-induced amnesic animals.

The water maze task has been used extensively to study the neurobiological mechanisms that underlie spatial learning and memory, age-associated changes in spatial navigation, and the ability of nootropic agents to influence specific cognitive processes. Since scopolamine-induced amnesia is due to blockade of the cholinergic neurotransmission, it provides a rationale for the use of this drug to model the cognitive deficits that are observed in aging and dementia. In Morris water maze, a decrease in escape latency during training and increase in time spent in the target quadrant during retrieval indicated improvement of learning and memory,

respectively; and vice versa. The decrease in escape latency on day 8 was a positive indication for memory retention activity. Further, in the estimation of the brain's acetylcholine level, it was found that the level of acetylcholine was decreased after the administration of scopolamine. Post administration of fraction at a dose of 600mg/kg increased the brain's acetylcholine level. Moreover, scopolamine administration increases the level of TBARS which causes memory deficit in animals. The nootropic potential of *E. aureum* was due to the increase in the level of acetylcholine, which plays an important role in memory function and retention.

The present study, therefore, demonstrates the probable Anti-Amnesic Activity of *Epipremnum aureum* in Scopolamine Induced Amnesia in mice mechanism by which *E. aureum* enhanced the anti-amnesic activity by increasing the performance of learning and memory. It had been suggested that the varying degrees of behavioral impairments are associated with aging and age-associated neurodegenerative diseases [16]. The qualitative determination of constituents by TLC analysis showed the presence of three different phenolic compounds caffeic acid, ferulic acid, and rosmarinic acid in the investigated extract. Pharmacology studies have also shown that caffeic acid exerts a protective effect against hydrogen peroxide-induced oxidative damage in the brain [17]. Ferulic acid has been proposed as a potential treatment for many disorders including Alzheimer's disease [18]. So it can be concluded that the nootropic potential of *E. aureum* may be attributed to the presence of phenolic acid and flavonoids [19].

5. Conclusion

The probable mechanism behind the present observations may be the increased level of acetylcholine following the administration of *Epipremnum aureum* which was decreased after scopolamine administration producing an amnesic effect. Furthermore, the level of TBARS was decreased in the *E. aureum*-treated animals indicating its neuroprotective effect against free radical-mediated oxidative damage of neurons resulting in amnesia. Further studies are necessary for the isolation and characterization of the active compound(s) from the active extract of *Epipremnum aureum*.

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