Original Article

# Cognitive Enhancing And Antioxidant Activity Of Ethyl Acetate Soluble Fraction Of The Methanol Extract Of Pisonia Alba Leaves In Scopolamine-Induced Amnesia

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## Abstract

Evaluation of Pisonia alba's cognitive-improving and antioxidant activity was the goal of the current investigation. In mice, scopolamine (1 mg/kg, i.p.) administration decreased learning and memory, which is related to altered brain oxidative status. To evaluate cognitively enhancing activity, the object recognition test (ORT) and passive avoidance test (PAT) were used. The methanol extract of P. alba was administered to the animals at doses of 25, 50, and 100 mg/kg, p.o. The methanol extract of P. alba's ethyl acetate soluble fraction (EASF) reduced amnesia brought on by scopolamine and age. The older and scopolamine group in ORT showed a considerably lower discrimination index (DI). EASF pretreatment greatly raised the DI. Mice given scopolamine had considerably lower step-down latencies in PAT (SDL). EASF therapy resulted in a notable rise in SDL in all animal groups, including young, old, and scopolamine-treated animals. Scopolamine therapy enhanced lipid peroxidation and lowered levels of superoxide dismutase (SOD) and glutathione reductase, according to the biochemical study of the brain (GSH). By drastically reducing LPO and reversing the decline in brain SOD and GSH levels, extract was administered. P. alba treatment enhanced memory in amnesic mice and reduced the oxidative stress brought on by scopolamine. Increased cellular antioxidants may be the cause of P. alba's protective mechanism. The findings of the current investigation indicated that P. alba may be useful in the treatment of cognitive disorders because it may have a protective effect against scopolamine-and age-induced forgetfulness.

**Keywords:** Cognitive enhancing, Pisonia alba, lipid peroxidation, oxidative stress, passive avoidance test, scopolamine

## INTRODUCTION

Alzheimer's disease (AD) is a neurological condition that primarily affects older people and causes dementia and memory loss. [1] A dysfunctional beta-amyloid (A) protein metabolism, anomalies in glutaminergic, adrenergic, serotonergic, and dopaminergic neurotransmission, and the probable involvement of inflammatory and oxidative

pathways are all part of the complicated pathophysiology of AD. [2] Acetylcholinesterase (AChE) inhibitors, which raise acetylcholine (ACh) availability at cholinergic synapses, and nootropic drugs like piracetam are being used to treat AD. The resultant negative effects of these drugs, however, have restricted their use. Additional non-cholinergic AD treatments are urgently needed because cholinesterase inhibitors only provide limited benefits. In the fight against AD, multipotent agents are predicted to perform better than their counterparts who aim at a single target. [2] Memory impairment in scopolamine-induced animal models is linked to increased brain oxidative stress levels. [3] The forebrain cholinergic system may be affected by oxidative stress, according to compelling evidence. The use of medications having antioxidant properties may help to maintain brain function. Enzymes that fight free radicals aid in reducing oxidative stress. [4] In the part of the brain affected by Alzheimer's disease, antioxidant enzymes show diminished activity. Furthermore, the improvement of cognitive and/or psychomotor functions has been linked to the decrease in intracellular oxidised protein under these circumstances. Finding therapeutic drugs that could lessen oxidative damage and encourage a functional recovery in degenerative illnesses has thus been a focus of research.

Members of the Nyctaginaceae family include Pisonia umbellifera, Pisonia alba, and Pisonia alba spanoghe. It can be found on many of the Seychelles Islands that have undergone habitat restoration, and it is an important part of the habitat with a high level of biodiversity and a complex food web. The Seychelles warbler, a unique land bird that was saved from extinction through meticulous habitat management and transfer, was discovered to frequently nest on pisonia trees, demonstrating the importance of considering the entire island ecology. Other natural tree species cannot simply take the place of the Pisonia. The leaves can be eaten. Vegetables are made from the young leaves. In addition to being fed to animals, leaves are commonly utilised as rheumatism and arthritis treatments. The leaves are cooked and eaten for arthritis, they are a carminative and an antidote for snake bites, and they are used as an anti-diabetic in traditional Indian medicine. The indigenous people also utilise the leaves as cattle feed. However, there is no evidence that it possesses anti-anxiety properties. The purpose of this investigation was to examine the analgesic and anti-inflammatory effects of different fractions of Pisonia alba root extract. [5-15] Although P. alba has been shown to have antioxidant properties in a number of models, there are no significant investigative findings that address its ability to improve cognition. The goal of the study was to examine P. alba's ability to improve memory and function in old mice and mice with amnesia caused by scopolamine.

## MATERIALS AND METHODS

### Plant material

The roots were collected in the month of November, 2021 from local area of Chennai, India and authenticated. A voucher specimen of the plant has been deposited at Botanical Survey of India. The plant was sun-dried and ground into a coarse powder. The Soxhlet extractor used 1 kg of the powdered plant material to defattify it with petroleum ether at 60–80 °C. For an additional 72 h, methanol was used to extract the defatted marc. Filtering and concentration of the extract were done at low pressure. 6.2% w/w was found to be the yield of the methanol extract of P. alba roots. To get the ethyl acetate soluble (EASF, 1.7 w/w) & ethyl acetate insoluble fractions (EAISF, 1.3 w/w), the methanol extract of P. alba was also thoroughly extracted with ethyl acetate. The ethyl acetate soluble fraction was dissolved in distilled water and suspended in Tween 80 (0.2% v/v) (p.o.).

### Animals

The study used Swiss albino mice of either sex (young, 8 weeks old, 18-20 g, and older, 32 weeks old, 35-40 g). Animals were kept in polypropylene cages under conventional laboratory environmental conditions, including a temperature of 25 2 °C, a 12-h light/dark cycle, and a relative humidity of 50 5%, with unlimited access to food and water. Prior to the test, animals were acclimated to the laboratory environment. There were five (n = 5) animals in each group. The light period was used for all of the trials (08:00-16:00 h).

## Drugs and chemicals

Donepezil hydrochloride (Glenmark Pharmaceutical, Mumbai), Piracetam hydrochloride (UCB India Pvt. Ltd. Vapi, Gujarat), and Scopolamine hydrobromide (Sigma Aldrich, USA). The medium was distilled water.

## Phytochemical screening of P. alba

Following the previously described protocols, the EASF of the methanol extract of P. alba roots was phytochemically screened for the presence of flavonoids, glycosides, saponins, alkaloids, and sterols. [16,17]

## Treatment schedule

Mice of all ages (young and old) were placed into 16 groups of five each. Different groups of young and old mice received different doses of EASF (25, 50, and 100 mg/kg, p.o.). Scopolamine was used to exclusively cause amnesia in young mice. Groups I and II are Control (young and old, 0.2% v/v Tween 80, p.o. ); Groups III and IV are Standard (young and old, piracetam 200 mg/kg, i.p. ); Group V is Scopolamine (1 mg/kg, i.p. ); Groups VI, VII, & VIII are EASF (young, 25, 50, and 100 mg/kg, p.o. ); and Group I Groups IX, X, XI-EASF (25, 50 and 100 mg/kg, p.o. in aged respectively); Groups XII, XIII, XIV-EASF (25, 50 and 100 mg/kg, p.o. + Scopolamine in young respectively); Group XV-Piracetam (200 mg/kg, i.p. + Scopolamine in young); Group XVI-Donepezil (1 mg/kg, i.p. + Scopolamine in young).

## Object recognition test

Animal memory performance is evaluated using the object recognition test (ORT), a behavioural test that is frequently utilised. The ORT bases memory performance on animals' innate need to investigate new objects. The device is a 70 by 60 by 30 cm open box made of plywood painted white with a nicely furnished floor. A 60 W bulb positioned 50 cm above the box illuminates the device. The two different shapes of the 8 cm plywood pieces that need to be distinguished are constructed in black and white. Mice were given a habituation session the day before the test, during which they were free to explore the box for two minutes. During the habituation trial, there was nothing put inside the box. On test day, two identical objects were placed in two opposing corners of the box for the first trial (T 1), and it was timed how long it took each mouse to explore each object for 20 seconds. Exploration was defined as aiming the nose towards an object from a distance of less than 2 cm and/or touching it with the nose or forepaw. The behaviours of turning around or sitting on the object were not regarded as exploratory. A familiar object from the first trial was replaced with a new object for the second trial (T 2, 90 minutes after T 1), and mice were left in the box for 5 minutes. The discriminating index (DI) was determined after the time (s) spent exploring the familiar (F) and new (N) objects were separately recorded. DI = N-F/ N + F, where N denotes investigation of a novel object and F denotes exploration of a known object. [18, 19]

Young mice received EASF (25, 50, and 100 mg/kg, p.o.) or piracetam (200 mg/kg, i.p.) or donepezil (1 mg/kg, i.p.) or vehicle, and the first trial was administered 45 minutes following the injection of scopolamine. The first trial was given to aged mice 45 minutes after EASF (25, 50, & 100 mg/kg, p.o.) treatment.

## Passive avoidance paradigm/test

The long-term memory was tested using passive avoidance behaviour based on negative reinforcement. The device was a box (27×27×27 cm) with three wood walls and one Plexiglass wall, a grid floor made of stainless steel rods spaced 3 mm apart and a wooden platform (10×7×1.7 cm) in the middle. Throughout the testing period, a 15 W bulb

was used to illuminate the box. The grid floor received a 20 V AC electric shock. Two sessions with comparable content were used for training. On the wooden platform positioned in the middle of the grid floor, each mouse was carefully placed. The mouse was shocked for 15 seconds after stepping down and placing all four paws on the grid floor, and the step-down latency (SDL) was measured. SDL was defined as the amount of time needed for the mouse to step all the way down from the wood platform to the grid floor. For the second session and the retention test, animals that displayed SDL in the range of (2–15 s) during the first test were used. After the initial test, the second session was conducted for 90 minutes. The animals received an electric shock for 15 seconds if they stood still for less than 60 seconds. In the second experiment, animals were taken out of the shock-free area if they didn't step down within 60 seconds. Similar retention tests were conducted after 24 h, with the exception that the grid floor did not receive any electric shocks. Once more, each mouse was put on the platform, and the SDL was recorded with a 300-second upper cutoff. For 8 days, oral doses of EASF (25, 50, and 100 mg/kg, po), piracetam (200 mg/kg, i.p. ), donepezil (1 mg/kg, i.p.), or vehicle were given. On the eighth day, 45 minutes after the last dosage was provided, and again after 24 h, or on the ninth day, SDL was noted. In the group that received scopolamine,

Scopolamine (1 mg/kg) was administered intraperitoneally (i.p.) 45 min after EASF (25, 50, and 100 mg/kg), piracetam, donepezil, or vehicle. SDL was measured after 45 minutes, or on the eighth day, and after 24 h, or on the ninth day. The animals were killed by cervical dislocation nine days after the SDL measurement, and antioxidant measures including lipid peroxidation (LPO), superoxide dismutase activity (SOD), and glutathione reductase (GSH) levels in the brain were assessed. [20]

## Dissection and homogenization

The mice of groups I, V, XII, XIII, XIV, XV, and XVI were killed by cervical dislocation at the conclusion of the trial, and their brains were removed. They were then weighed after receiving a thorough rinsing with ice-chilled 0.9% NaCl. In 0.1 M phosphate buffer, a homogenate of tissues at 10% (w/v) was created (pH 7.4). The homogenate was centrifuged (Remi-C-30, Remi Industries Ltd, Mumbai, India) at 12000 g for 60 min at 4 $^{o}$C to extract the post nuclear fraction. For the following tests, a spectrophotometer model Shimadzu-160A was employed. [21]

## Biochemical analysis

## Lipid peroxidation assay

The Wills method was used to measure LPO in the brain quantitatively in 1966. Thiobarbituric acid was used to measure the amount of malondialdehyde (MDA) produced in the process at 532 nm. Using the molar extension coefficient of the chromophore (1.56 10 5 M-1 cm-1), the results were represented as nanomole of MDA per milligramme of protein. [22]

## Superoxide dismutase activity

By inhibiting the reduction of nitroblue tetrazolium chloride (NBT), which was detected at 560 nm spectrophotometrically, superoxide dismutase activity (SOD) was evaluated in accordance with Kono's method from 1978. In a nutshell, the reaction was started by adding hydroxylamine hydrochloride to a reaction mixture that contained NBT and brain homogenate's post-nuclear fraction. Inhibition was used to express the results. [23]

## Estimation of reduced glutathione

According to Ellman's approach, reduced glutathione (GSH) in the brain was estimated in 1959. With 0.75 ml of 4% sulfosalicylic acid, a homogenate sample volume of 0.1 ml was precipitated. In 0.1 M phosphate buffer at pH 8.0, the test mixture contained 4.5 ml of DTNB and 0.5 ml of supernatant. At 412 nm, the yellow tint that had emerged was readily detected. In terms of nanomoles of GSH per milligramme of protein, the results were presented. [24]

### Protein estimation

Bovine serum albumin was used as the reference standard for the Lowery et al. (1951) method, and the protein content was calculated as g protein/mg of tissue. [25]

### Statistical analysis

The statistical data analysis was carried out using one-way analysis of variance (ANOVA), followed by Dunnett's test, and the results are shown as mean S.E.M. Statistical significance was defined as a probability level less than 0.05.

## RESULTS

### Phytochemical screening

The EASF of P. alba underwent phytochemical screening, which identified the presence of glycosides, flavonoids, tannins, and saponins.

### Object recognition test

In the elderly and scopolamine groups, the discrimination index was considerably ($P < 0.05$; $P < 0.01$) lower than in the control group, demonstrating memory impairment in the object recognition test. In the elderly and scopolamine-treated group, pretreatment with EASF of P. alba (25, 50, and 100 mg/kg) significantly enhanced the DI, showing improvement in short-term memory and reversal of scopolamine-induced amnesia. In the elderly and scopolamine-treated group, piracetam and donepezil likewise significantly enhanced short-term memory (P 0.01) as shown in Table 1.

**Table 1:** Effect of EASF of P. alba on discrimination index in young and aged mice in object recognition test

| Treatment | Discrimination Index |
|---|---|
| Control (Young) | 0.0428±0.01 |
| Control (Aged) | 0.0433±0.02$^{\$}$ |
| Piracetam (200mg/kg, i.p., in young) | 0.282±0.02** |
| Piracetam (200mg/kg, i.p., in aged) | 0.104±0.09$^{@}$ |
| Scopolamine (1 mg/kg, i.p., in young) | -0.152±0.04** |
| EASF of P. alba (25 mg/kg , p.o. in young) | 0.0394±0.01 |
| EASF of P. alba (50 mg/kg , p.o. in young) | 0.081±0.02 |
| EASF of P. alba (100 mg/kg , p.o. in young) | 0.0552±0.02 |
| EASF of P. alba (25 mg/kg , p.o. in aged) | 0.0317±0.01 |
| EASF of P. alba (50 mg/kg , p.o. in aged) | 0.0684±0.02 |
| EASF of P. alba (100 mg/kg , p.o. in aged) | 0.0393±0.01$^{@}$ |
| EASF of P. alba + Scopolamine (25 mg/kg , p.o. + 1 mg/kg, i.p., in young) | -0.0296±0.30 |
| EASF of P. alba + Scopolamine (50 mg/kg , p.o. + 1 mg/kg, i.p., in young) | -0.0196±0.028$^{a}$ |

| | |
|---|---|
| EASF of P. alba + Scopolamine (100 mg/kg , p.o. + 1 mg/kg, i.p., in young) | 0.0702±0.020[b] |
| Piracetam + Scopolamine (200mg/kg, i.p. + 1 mg/kg, i.p., in young) | 0.08324±0.060[b] |
| Donepezil + Scopolamine (1 mg/kg, i.p. +1 mg/kg, i.p., in young) | 0.0954±0.029[b] |

Values are expressed as mean ± SEM (n=5); *P < 0.05; **P < 0.01 vs. Control group (in young group); [$]P < 0.05; [@]P < 0.01 vs. Control (aged control group); [a]P < 0.05; [b]P < 0.01 vs. Scopolamine treated group; (One-way ANOVA followed by Dunnett's test).

## Passive avoidance paradigm/test

Animals' long-term memory was indicated by their SDL on the second day (the ninth day of drug treatment). As compared to the control group, the SDL was considerably lower in the older and scopolamine-treated group (P < 0.01). Young and old mice were given EASF of P. alba (50 and 100 mg/kg) for 8 days, and the SDL increased in comparison to the corresponding control groups (P < 0.05; P < 0.01). Scopolamine-and aging-induced amnesia-induced memory deficits were corrected by administering P. alba EASF for 8 days. In the young, elderly, and scopolamine-treated groups, piracetam and donepezil also improved memory (P < 0.01) as summarized in Table 2.

**Table 2:** Effect of EASF of P. alba on transfer latencies of young and aged mice in passive avoidance test

| Treatment | Step down latency (s) |
|---|---|
| Control (Young) | 150.4±8.8 |
| Control (Aged) | 69.7±10.2[**] |
| Piracetam (200mg/kg, i.p., in young) | 249.2±12.5** |
| Piracetam (200mg/kg, i.p., in aged) | 227.5±4.4[@] |
| Scopolamine (1 mg/kg, i.p., in young) | 13.8±2.2** |
| EASF of P. alba (25 mg/kg , p.o. in young) | 196.2±16.2 |
| EASF of P. alba (50 mg/kg , p.o. in young) | 211.3±12.2* |
| EASF of P. alba (100 mg/kg , p.o. in young) | 226.1±10.2** |
| EASF of P. alba (25 mg/kg , p.o. in aged) | 161.8±22.2[$] |
| EASF of P. alba (50 mg/kg , p.o. in aged) | 196.8±15.4[@] |
| EASF of P. alba (100 mg/kg , p.o. in aged) | 216.4±30.3[@] |
| EASF of P. alba + Scopolamine (25 mg/kg , p.o. + 1 mg/kg, i.p., in young) | 68.3±4.6[b] |
| EASF of P. alba + Scopolamine (50 mg/kg , p.o. + 1 mg/kg, i.p., in young) | 81.6±1.0[b] |
| EASF of P. alba + Scopolamine (100 mg/kg , p.o. + 1 mg/kg, i.p., in young) | 97.4±4.0[b] |
| Piracetam + Scopolamine (200mg/kg, i.p. + 1 mg/kg, i.p. in young) | 206.1±4.6[b] |
| Donepezil + Scopolamine (1 mg/kg, i.p. +1 mg/kg, i.p. in young) | 255.2±4.1[b] |

Values are expressed as mean ± SEM (n=5); *P < 0.05; **P < 0.01 vs. Control group (in young group); [$]P < 0.05; [@]P < 0.01 vs. Control (aged control group); [a]P < 0.05; [b]P < 0.01 vs. Scopolamine treated group; (One-way ANOVA followed by Dunnett's test).

## Biochemical effect

## Lipid peroxidation assay

The level of MDA was examined nine days after SLD measurement. The administration of EASF of P. alba (25, 50, and 100 mg/kg) considerably (P < 0.01) decreased the level of MDA as compared to the scopolamine group, whereas the level of MDA was significantly (P < 0.01) raised in the scopolamine group as compared to the control group. In

the piracetam and donepezil group, the MDA level was similarly significantly reduced ($P < 0.01$) as mentioned in Table 3.

**Table 3:** Effect of EASF on antioxidant parameters in scopolamine-induced oxidative stress

| Treatment | LPO (nmoles of MDA/mg protein | GSH (µ moles of GSH/ mg protein | SOD (% inhibition of control) |
|---|---|---|---|
| Control (Young) | 124.6±6.2 | 13.91±0.2 | 93.44±0.9 |
| Scopolamine (1 mg/kg, i.p., in young) | 347.6±2.1** | 8.86±0.4** | 83.37±1.7** |
| Donepezil + Scopolamine (1 mg/kg, i.p. +1 mg/kg, i.p., in young) | 108.21±1.0[b] | 12.81±5.6[a] | 92.34±0.4[b] |
| Piracetam + Scopolamine (200mg/kg, i.p. + 1 mg/kg, i.p., in young) | 112.11±2.1[b] | 13.61±2.2[a] | 94.30±0.2[b] |
| EASF of P. alba + Scopolamine (25 mg/kg , p.o. + 1 mg/kg, i.p., in young) | 264.50±18.3[b] | 10.02±0.2 | 88.24±2.1 |
| EASF of P. alba + Scopolamine (50 mg/kg , p.o. + 1 mg/kg, i.p., in young) | 199.71±6.1[b] | 10.61±0.2 | 90.68±1.59[a] |
| EASF of P. alba + Scopolamine (100 mg/kg , p.o. + 1 mg/kg, i.p., in young) | 161.8±12.3[b] | 12.14±0.1[a] | 91.1±1.42[b] |

Values are expressed as mean ± SEM (n=5); *$P < 0.05$; **$P < 0.01$ vs. Control group (in young group); [$]$P < 0.05$; [@]$P < 0.01$ vs. Control (aged control group); [a]$P < 0.05$; [b]$P < 0.01$ vs. Scopolamine treated group; (One-way ANOVA followed by Dunnett's test).

## Effect on brain SOD level

As compared to the control group, the scopolamine group had significantly lower levels of the protective antioxidant enzyme SOD ($P < 0.01$). Pretreatment with P. alba EASF (100 mg/kg) led to an increase in SOD ($P < 0.01$) in comparison to the group treated with scopolamine. The piracetam and donepezil group similarly experienced a statistically significant rise in SOD levels ($P < 0.05$) and shown in Table 3.

## Effect on brain GSH level

Scopolamine causes neurotoxicity in mice, as seen by the considerably lower levels of GSH ($P < 0.01$) in the scopolamine-treated group compared to the control group. In contrast, when EASF of P. alba (50 and 100 mg/kg) was administered, the GSH level was discovered to be considerably higher ($P < 0.05$; $P < 0.01$) than in the scopolamine-treated group. Additionally considerably raising GSH levels were piracetam and donepezil (Table 3).

## DISCUSSION

In the current investigation, EASF of P. alba (25, 50, and 100 mg/kg) significantly enhanced mice's learning and memory in the interoceptive behavioural models used. Through ORT and PAT, a distinction between working memory and reference memory can be made simultaneously. Non-selective muscarinic antagonist scopolamine inhibits cholinergic signalling and causes memory deficits that are comparable to those seen in senile CNS dysfunction brought

on by ageing. Reference (long term) and working (short term) memories are both affected by scopolamine's interference with memory and cognitive function. In this study, mice were given 1 mg/kg of scopolamine to cause memory impairment.

Strong proof that oxidative stress plays a role in the aetiology of Alzheimer's disease has been revealed in numerous clinical trials. Elderly individuals may develop Alzheimer's disease as a result of oxygen-free radicals, which are linked to age-related declines in cognitive function. [20] According to El-Sherbiny et al. (2003), increased oxidative stress in the rat brain is a contributing factor to memory impairment in the scopolamine-induced animal model. In experimental models of Alzheimer's disease, there is evidence of increased oxidation of lipids, proteins, and deoxyribonucleic acid as well as changes in mitochondrial activity and a potential involvement for amyloid beta and its precursor protein in oxidative reactions. Furthermore, numerous clinical research have offered compelling evidence that oxidative damage plays a role in neurodegenerative illness. [26] The use of medications having antioxidant properties may help to maintain brain function. Superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase, glutathione reductase (GSH), and ascorbate are examples of antioxidant enzymes that are involved in reducing oxidative stress. In the part of the brain affected by Alzheimer's disease, antioxidant enzyme activity are diminished. Furthermore, the improvement of cognitive and/or psychomotor functions has been linked to the decrease in intracellular oxidised protein under these circumstances. [3] Because any such attribute of a therapeutic agent can be predicted to significantly boost the endogenous defence against oxidative stress, the augmentation of endogenous antioxidants by therapeutic compounds has recently piqued scientific attention. [9] In degenerative illnesses, these substances also lessen oxidative damage and aid in functional recovery. The EASF of P. alba shown a considerable memory enhancing activity in ORT and PAT during the search for natural compounds with memory enhancing activity utilising scopolamine-induced amnesic mice as an experimental model for Alzheimer's disease. Acute scopolamine injection causes memory impairment, which is linked to altered levels of SOD and GSH in the brain. [3] More specifically, it was discovered that Alzheimer's disease (AD) patients' complete brains were put through an oxidative test. Consumption of detoxifying endogenous antioxidants like SOD and GSH may result from such a peroxidation process and an excess of free radicals. The scopolamine (1 mg/kg)-treated mice with conditioned fear showed higher brain MDA levels, while SOD and GSH levels were decreased.

In the current investigation, the impact of EASF on mice's performance in an object identification test that has been thought of as a pure working memory task was investigated. Mice can distinguish between a novel thing and a familiar object 1 h or less after the familiar object is presented, but not 24 h later. [27] Investigations into the impact of EASF on information acquisition, memory consolidation that occurs shortly after acquisition, and information restitution were made. When pretreated with EASF (50 and 100 mg/kg), the results showed that mice in the elderly and scopolamine-treated group spent more time examining a novel object than a familiar object. The group that had been given scopolamine and was older saw a significant reduction in DI. When compared to the corresponding control, pretreatment with EASF (50 and 100 mg/kg) considerably raised the DI. When young mice were given EASF, there was no discernible impact on the DI.

In light of these findings, it may be concluded that EASF (50 and 100 mg/kg) improved retention in mice aged and treated with scopolamine who were given an object recognition challenge. EASF enhances the working memory consolidation phase and perhaps the acquisition phase, which are both affected by age and scopolamine in interoceptive memory deficiency models. The established nootropics utilised as a reference in this study, piracetam and donepezil, dramatically enhanced the DI as well.

In the passive avoidance test, the ameliorative effects of EASF on learning and memory were examined. The step-down latencies in mice receiving scopolamine were dramatically reduced. Treatment with EASF (25, 50, and 100 mg/kg) resulted in a notable rise in SDL in both young and old animals. The SDL in the group receiving scopolamine was dramatically reduced by pretreatment with EASF (25, 50, and 100 mg/kg). Thus, the deficit caused by

scopolamine was dramatically reversed by EASF. The SDL was also raised by the positive controls donepezil (1 mg/kg) and piracetam (200 mg/kg), which is in line with earlier results.

The EASF is nootropically active according to the requirements. The 8-day pretreatment with EASF shielded the rats against the memory impairment brought on by PAT's scopolamine. Scopolamine administration dramatically elevated MDA levels, a crucial LPO marker, and decreased GSH and SOD activity in mouse brain. In mice brains, 8 days of administration of EASF resulted in a notable decrease in MDA and a recovery of GSH and SOD activity. By reducing the decrease in the activities of GSH and SOD in mice brain, extract may provide a protective effect against oxidative damage brought on by scopolamine. The nootropic properties of Hibiscus sabdariffa Linn were documented by Joshi and Parle. The antioxidant, neuroprotective, pro-cholinergic, and anti-acetylcholine esterase properties may be responsible for the herb's memory-improving effects, indicating that certain species of Hibiscus may contain chemical components with nootropic properties. [28] The substantial anti-amnesic efficacy of neuroprotective substances in memory impairment brought on by scopolamine and ageing has long been known. The aforementioned behavioural and biochemical findings imply that EASF can enhance or mitigate spatial long-term and working memory through the control of the antioxidant system. The diverse chemical components of P. alba, including its glycosides, flavonoids, tannins, and saponins, may be responsible for the observed positive benefits.

## CONCLUSION

Our research suggests that a cognitive-enhancing impact may be caused by a combination of antioxidant and neuroprotective functions. Pisonia alba may therefore be helpful in the management or avoidance of a number of cognitive problems.

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