Original Article

Enhancing Cognitive Performance with Rejuvenation of Brain Antioxidant Markers and Acetylcholinesterase Activity by Ethanolic Extract of *Cucurbita pepo* L. Seeds in Scopolamine-Induced Model of Dementia in Rats

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Abstract

Background: *Cucurbita pepo* L. herb has been traditionally used for treating numerous disorders in Asian and African countries, including India. **Objective:** The aim of the present study was to evaluate the memory-enhancing activity of *C. pepo* L. seeds based on its antioxidant potential in cognitive impairment rat model induced by scopolamine. **Materials and Methods:** The experimental animals were treated with the ethanolic extract of *C. pepo* L. seeds (EECPS) (200, 400, and 800 mg/kg, p.o.). Piracetam (200 mg/kg, p.o.) was used as standard drug and given for 14 consecutive days. Cognitive impairment was evaluated by a passive avoidance apparatus and the Morris water maze test. The oxidative parameters in brain of rats were estimated to explore oxidative stress in experimental animals. Data were analyzed by using one-way analysis of variance followed by Student's *t*-test. **Results:** The phytochemical analysis revealed the presence of active ingredients such as terpenoids, phenols, alkaloids, and flavonoids in the EECPS. The total phenol content was estimated quantitatively in EECPS and found to be 8.37±0.2 mg gallic acid equivalent/g. The EECPS at a dose of 400 mg/kg has depicted a maximum increase in step-down latency and reduction in escape latency on behavioral tests, with decrease in oxidative stress by showing an increase in levels of superoxide dismutase, catalase, and glutathione and subsequently a decrease in the malondialdehyde level. Cholinesterase activity was also found to decrease with 400 and 800 mg/kg EECPS when compared with the scopolamine group. **Conclusion:** The results of the study clearly suggest that EECPS ameliorated spatial memory impairment induced by scopolamine, which could further attribute to their antioxidant properties.

Keywords: *Alzheimer's disease, ethanolic extract of Cucurbita pepo L. seeds (EECPS), scopolamine*

Introduction

A progressive disease of unknown etiology, i.e., Alzheimer, is one of the most devastating neurodegenerative disorders and common form of dementia. The prevalence of this deadly disease is widespread, especially in elderly population interfering with social functioning. It affects approximately 24.3 million people all over the world and 1.5 million in India.[1] According to global statistical data, it has become one of the fastest rising diseases. The number of people suffering from it is set to almost double in every 20 years, with an estimate 42.3 million living with dementia in 2020 and the number rising to 81.1 million by 2040.[2] The causes of this disease can vary resulting from neurodegeneration, i.e., brain cell death. It is generally characterized by the deposition of amyloid plaques, development of neurofibrillary tangles, and inflammation or neuronal loss in specific regions of the forebrain.[3] Progressive memory loss is the major symptom observed in people suffering from Alzheimer's disease (AD), followed by a cognitive decline.[4] The major risk factors include age, genetic disorders such as trisomy, positive family history, head injury, and numerous other miscellaneous reasons. However, the nature of the casual factors responsible for AD is poorly known, but free radicals and oxidative stress have been crucial factors in the pathophysiology of several neurodegenerative disorders, including AD as well.[5] The complex nature of AD should be understood by considering the essential pathways which are involved in the generation of reactive oxygen species (ROS) and consequently the oxidative stress that can also lead to nuclear degradation in neuronal population.[6] In its progression, lipid peroxidation, protein, and DNA oxidation also

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occur. In the reduction of oxidative stress, antioxidant enzymes such as superoxide dismutase (SOD), glutathione peroxidase, and catalase as well as low molecular weight reductants, alpha tocopherol, glutathione (GSH), and ascorbate are involved.[7] Apoptosis also appears to be a predominant factor in the loss of cells observed.[8] Thus all researchers working in this field strongly agree that oxidative stress is commonly associated with AD.

Loss of memory and cognitive impairments are strongly related to change in the activity of acetylcholine esterase (AChE).[9] AChE can increase the rate of fibrillation by binding amyloid-β-associated proteins as potent amyloid-promoting factors.[10] Inhibition of cholinesterase is the most employed treatment for treating the symptoms of AD and also acts as therapeutic targets for improving the cholinergic deficit.[11] Scopolamine-induced models, a muscarinic antagonist, for memory impairment have been widely implicated for the investigation of anti-dementia drugs.[12] It can block the cholinergic signaling through neuro-inflammation, apoptosis, and oxidative stress.[13] In addition, it can considerably enhance the activity of AChE and malondialdehyde (MDA) levels in the cortex and hippocampus, as well as oxidative stress in the brain.[6,14] Cure of most frequent cognitive disorders and neurodegenerative diseases such as amnesia, attention deficit, and AD are still a nightmare in the field of medicine. The use of herbal and natural extract in the treatment of psychiatric, neurological, and neurotoxicological disorders has increased tremendously due to their no or minimal side-effects.[12]

The pumpkin (*Cucurbita pepo* L.) is a very familiar fruit belonging to the family Cucurbitaceae popularly known as guard family.[15] *C. pepo* L. seeds are much important with Islamic and Quranic point of view and it is well known for traditional uses in Ayurveda and Chinese folk medicines.[16,17] Ethno-medicinal literature revealed that seeds of this important species have been used for treating numerous ailments such as stomach disorder, intestinal parasites, dizziness, kidney inflammation, and depression. It has been proved experimentally that pumpkin seeds oil possesses strong antioxidant properties.[18] Clinical studies employing pumpkin seeds showed that they are effective in the treatment and prevention of benign prostate hyperplasia, anthelminthic, heterophyiasis, prostate cancer, stomach cancer, and irritable bladder.[19,20] The seeds are used for bronchitis, to reduce thirst, as a diuretic and a stimulant, to cure painful chests, fever, and kidney problems.[21,22] Pharmacologically, it is used for different activities such as anti-hypercholesterolemia, anti-hypertensive, anti-inflammatory, anti-parasitic, anti-tumor, antioxidant, anti-diabetic, anti-carcinogenic, and anti-bacterial.[17] Different categories of phytoconstituents are found in abundance in *C. pepo* L. seeds such as flavonoids, alkaloids, polyphenols, triterpenoids, phytosterols, fatty acid components (such as palmitic, palmitoleic, stearic, oleic, linoleic, and gadoleic acid), and saponins, which may be responsible for its medicinal properties.[16,23] In addition, the seeds contain carotenoids as lutein and cucurbitosides which are acylated phenolic glycosides.[24] It is a good supplement of carbohydrate, minerals, proteins, and fat coupled with high mineral content. Also it is a good source of vitamin A, iron, phosphorus, and calcium.[25] Recently, it has also emerged as a very promising nutraceutical and multipurpose medicinal agent.

According to the WHO survey, more than 80% of the world's population trusts on complementary and alternative medicine for their medical care.[26] Thus, in the present study, our aim was to investigate and evaluate the neuroprotective effect of ethanolic extract of *C. pepo* L. seeds (EECPS) by its antioxidative potential in scopolamine-induced AD in rats.

Materials and Methods

Drugs and chemicals

Piracetam and scopolamine hydrobromide were obtained from Sigma-Aldrich, USA. 5,5'-dithiobis-(2-nitrobenzoic acid) (Ellman's reagent), trichloroacetic acid, and thiobarbituric acid (TBA) were purchased from Sigma-Aldrich (Bangalore, India) and other chemicals used in the present study were of analytical grade and purchased from Nice Pvt. Ltd, New Delhi, India.

Plant material and preparation of ethanolic extract

The seeds of *C. pepo* L. (pumpkin) were purchased from a local herbal store in October 2010. They were taxonomically identified and authenticated by the National Institute of Science Communication and Information Resources (NISCAIR), ref. no. NISCAIR/RHMD/consult/2010-11/1680/278, New Delhi, India.

The dried seeds of *C. pepo* L. were powdered coarsely in a mechanical grinder, passed through a sieve (40-mesh size), and preserved in an airtight container for further use. The dried samples of the seeds were weighed accurately (80 g) for Soxhlet assembly and were independently extracted with 95% ethanol (500 ml) at room temperature till exhaustion. The ethanol phase was collected after recovery, and the seeds' extracts were later evaporated to dryness under vacuum to give yellowish-brown-colored crude ethanol extract residue (yield 12.7% w/w). The dried ethanol extract was kept in a desiccator, for further use in experimental studies.

Phytochemical screening

Preliminary phytochemical screening of EECPS was performed for the determination of different classes of phytoconstituents such as alkaloids, phenol, triterpenoid, carbohydrates, flavonoids, steroids, and tannins using standard procedures.[27,28]

Estimation of total phenol content (TPC)

TPC in the EECPS was determined by the Folin–Ciocâlteau colorimetric method. First, the crude sample was prepared by liquefying 10 mg of EECPS (dry powder) in 10 mL of the solvent to yield a concentration of 1 mg/mL. Then 0.5 mL of this liquefied extract was added to 0.1 mL of Folin–Ciocâlteau reagent (0.5N) in a test tube and mixed thoroughly. After mixing, sodium carbonate (Na_2CO_3) 2.5 mL was added in the liquid mixture, shaken gently to mix them, and allowed to stand

for 30 min at room temperature. The absorbance of the mixture was measured at 760 nm in a UV–visible spectrophotometer. A calibration curve was plotted by using gallic acid as standard reference (concentration ranging from 0.01 to 0.05 mg/mL). TPC was expressed as gallic acid equivalents in milligrams per gram of the extract (GAE/g).[29]

Experimental animals

Wistar rats (150–180 g) of either sex were used for the experiments. All animals were kept for a period of 1 week to acclimatize with animal house conditions in a temperature- and light-controlled room (22°C, a 12-h cycle starting at 08:00 h). Three rats were housed per polycarbonate cage and allowed to feed and drink water *ad libitum*. Experiments were carried out between 08:00 am and 10:00 am. All the animals were kept at overnight (10 h) fasting with free access to water. Before administration of the dose, the body weight of each animal was determined and the dose was calculated according to the body weight. The extract was administered orally in a single dose (not more than 1 mL/100 g body weight body) using a gastric feeding tube. The protocol of the study was approved by the Institutional Animal Ethical Committee (Reg. no. 828/Ac/04/CPCSEA), and throughout the experiments, animals were processed in accordance with the suggested guidelines of Committee for the Purpose of Control and Supervision of Experiment on Animals (CPCSEA), Ministry of Forests and Environment, Government of India for Care of Laboratory Animals.

Acute toxicity study

Acute oral toxicity study of the EECPS was carried out using the "up-and-down" method in Wistar female rats (150–180 g). A total of five rats were randomly selected and fasted for overnight prior to the experiment with free access to water. The extract lethality was not available; so using the default progression dosing factor, doses were sequenced 1.75, 5, 17.5, 55, 175, 550, and 2000 mg/kg as recommended in OECD Guidelines 425. Rats were closely observed for 2 h post-treatment for any signs of toxicity as well as behavioral abnormalities. The rate of mortality shown within 24 h was recorded. The surviving rats were further observed for 7 days for any significant signs of delayed toxicity. The lethal dose 50 (LD_{50}) value was estimated by log-probit analysis.[30] One-tenth of the median lethal dose (LD_{50}) was taken as starting and effective dose for the elucidation of different pharmacological activities.

Grouping and treatment protocol

Cognitive impairment (learning and memory) was assessed with two behavioral models, i.e., passive avoidance apparatus and Morris water maze test. The Wistar rats were randomly allocated into six groups (six animals per group) for pharmacological screening model of learning and memory (passive avoidance and Morris water maze test) as follows:

Group 1: normal group, received normal diet and water;

Group 2: vehicle control (received CMC as vehicle);

Group 3: standard control (piracetam 200 mg/kg);

Group 4: low dose of EECPS (200 mg/kg);

Group 5: medium dose of EECPS (400 mg/kg);

Group 6: high dose of EECPS (800 mg/kg).

All the extract doses and standard drug were suspended in carboxymethyl cellulose sodium (as vehicle). The administration of test extract and standard drug were dosed (10 mL/kg of body weight) orally once daily for 14 successive days by using a 15-gage oral gavage needle. From 7th to 14th day, scopolamine hydrobromide (2 mg/kg, dissolved in an isotonic solution 0.9% NaCl) as a single dose (1 mL) was injected intraperitoneally (i.p.), half an hour before the behavioral tasks in groups 2–6 only.

Cognitive measurements (learning and memory)

Passive avoidance test

This behavior test based on negative reinforcement is used to evaluate long-term memory deficits. In this experiment, the box was illuminated with a 15 W bulb, and electric shock (20 V ac) was delivered to the stainless steel grid floor. Experimental training was given to animals in two similar sessions in which each rat was placed on the wooden platform (set in the center) of the grid floor. As soon as rats stepped down and placed all its paws on the grid floor, shock was delivered for a time period of 15 s and the step-down latency (SDL) was recorded. SDL can be defined as the time taken by rats to step down from wood platform to grid floor with all its paws placed on it. Animals showing SDL within the range of 2–15 s were used for the second session and the retention test. After completion of the first test, the second session was carried out after 90 s. During this period, the electric shock of 15 s was delivered if the rats stepped down before 60 s. However, if the rats did not step down for a period of 60 s, they were removed from the shock-free zone. After 24 h, retention test was carried out in a similar manner but electric shock was not passed to the grid floor. After placing each rat on the platform, the SDL was recorded, with an upper cut-off time of 300 s.[13]

Morris water maze test

The spatial memory of rat was assessed by using the method of Morris. A circular pool (with 130 cm diameter and height of 90 cm) was filled with mildly cold (20±1°C) water up to 60 cm (leaving 30 cm of the exposed wall inside the pool), in which 500 mL of milk had been mixed to make the surface opaque. The pool was divided into four equal quadrants, in which a white platform (6 cm in diameter and 56 cm in height) placed in the center of one quadrant was submerged 4 cm below the surface of water making it invisible. The location of each swimming rat was continuously monitored from the starting position to the centered platform. The rats were given two trial sessions every day for 14 consecutive days in the same manner. During these sessions of trials, the averaged time taken by the rats to swim to the platform [escape latency (EL)] was recorded. Once the

rat manages to locate the platform, it was permitted to remain on it for next 10 s. If rats were not able to locate the platform within 120 s, it was guided to the platform and remained on it for next 10 s and then removed from the water pool. After that, rats were given a second trial with an inter-trial interval of 20 m for 14 consecutive days. The entry point of rats into the pool and location of the platform were changed every day in a random order. The day prior to the experiment was dedicated to swim training for 60 s in the absence of the platform. Six rats were used per treatment group.[31]

Biochemical parameter assay

After completion of the behavioral tests, all the animals were sacrificed by cervical decapitation under the effect of light anesthesia (using sodium pentobarbital, 100 mg/kg b.w., i.p.,) on the last day of experiment. Immediately after decapitation, the whole brain was removed from the skull very carefully. The hippocampus was dissected out very carefully and homogenized in an ice cold sterile 0.1 M potassium phosphate buffer (pH 7.4). The homogenate was centrifuged at a speed of 3000 rpm for 10 min at 4°C, and the resultant cloudy supernatant liquid obtained was used for performing different biochemical estimations of AChE, SOD, catalase (CAT), GSH, and MDA levels.[13]

Biochemical estimations of oxidative stress in tissue

SOD assay

Tissue SOD was estimated by the standard method of Misra and Fridovich (1972). For this, the reaction mixture contained 1666 mL carbonate buffer (0.2 M, pH 10.2) with 0.134 mL of 10% homogenate. The enzyme reaction was started by addition of 0.2 mL of epinephrine (0.3 mM) in the reaction mixture. The change in absorbance of reaction mixture was recorded at 480 nm against the blank. SOD activity was expressed in units (µmol/mg protein).[32]

CAT assay

Catalase activity (CAT) was measured by the method described by Sinha (1972). In this procedure, the reaction mixture consisted of 0.75 mL phosphate buffer (0.1 M, pH 7.5), 0.2 mL H_2O_2 (50 mM), and 0.2 mL of 10% homogenate. Decrease in absorbance of reaction mixture was recorded at 570 nm. The catalase activity was expressed as µmol of H_2O_2/min/mg proteins.[32]

GSH assay

The GSH was assessed by the previously described method of Ellman (1961). For this test, 0.02 mL of 10% brain homogenate supernatant was mixed with 3 mL of Ellman reagent. The tubes were mixed properly and kept for 1 h at normal room temperature. The change in the absorbance of reaction mixture was determined at 412 nm on a spectrophotometer. The GSH activity was expressed as µmol/mg proteins.[32]

Lipid peroxidation assay

Lipid peroxidation is expressed indirectly in terms of MDA as described by El-Saadani *et al.* (1989). For this, the reaction mixture consisted of 1 mL trichloroacetic acid in 0.1 M HCl, 2 mL of 0.67% TBA, and 1 mL of 10% homogenate supernatant. After that, the reaction was carried out by heating at 95°C for 1 h and then cooled immediately at 25°C. The reaction mixture was centrifuged at 4000 rpm for 30 min. The resulting supernatant was used and absorbance was measured at 530 nm wavelength. Finally, MDA was quantified using an extinction coefficient of 1.56×105/M/cm and expressed as µmol of MDA/mg wet tissue.[33]

Measurement of AChE enzyme activity

The AChE activity was assessed as described by the method of Ellman *et al.* (1961) using acetylthiocholine (ATCL) as an artificial substrate. For this test, reaction mixture contained 25 µL of 15 mM ATC chloride (ATCh), 75 µL of 3 mM DTNB (5,5′-dithio-bis-2-nitrobenzoic acid), 75 µL of 50 mM Tris–HCl (pH 8.0), and 0.1% bovine serum albumin in the 96-well plate and incubated at 25°C for 5 min. Then absorbance was measured at 412 nm after the enzyme source (10 µL) was added in the reaction mixture. Then 25 µL of 10% brain homogenates was added and the value of absorbance was determined again after incubation at 25°C for 5 min. The enzyme activity is expressed in terms of mol/min/g of tissue protein. All determinations were carried out in triplicate.[13]

The enzyme activity is calculated using the following formula:
$R = 5.74 \times 10–4 \times A/CO$,

where R is the rate in moles of the substrate hydrolyzed/min/g tissue, A is the change in absorbance/min, and CO is the original concentration of the tissue (mg/mL).

Statistical analysis

The results were expressed as means ± SEM. Student's paired *t*-test was used for comparison between 1st and 14th day readings. One-way analysis of variance followed by Student's *t*-test and Tukey's *post hoc* test was done using Sigma Plot 12.5 software. If the probability of error is $P < 0.05$, the results were considered significant.

Results

Preliminary phytochemical screening

The EECPS was subjected to preliminary qualitative chemical analysis, and results indicated the presence of terpenoids, flavonoids, alkaloids, and phenolic compound in considerable quantity in the EECPS extract [Table 1].

Table 1: Phytochemicals analysis of EECPS

Sr. no.	Phytochemical test	Results
1	Flavonoids	Positive (+)
2	Alkaloids	Positive (+)
3	Terpenoids	Positive (+)
4	Phenols	Positive (+)
5	Saponins	Negative (−)
6	Steroids	Negative (−)

Total phenolic content

In our study, TPC of EECPS was represented in terms of GAE. The result of the present study showed that the phenol contents of the EECPS in terms of GAE were between 6.09±0.2 and 8.37±0.2 mg GAE/g.

Acute toxicity and selection of test dose

During the observation period, the EECPS did not produce any significant changes in both behavioral and motor-neuronal functions. The monitoring of skin and eyes of the rats also remained unchanged. Further, EECPS caused no significant change in exploratory behavior (rearing and grooming), and no mortality was noted throughout the observation period. The extract was found to be completely nontoxic, i.e., safe and the oral LD_{50} is considered to be greater than 2000 mg/kg in rats. Hence, on the basis of toxicity data, one-tenth of this dose is taken as the starting and lowest effective dose, i.e., 200 mg/kg body weight for the extracts.

Cognitive measurements (learning and memory)

Effect on SDL (using passive avoidance test)

SDL can be defined as the time taken by the rat in seconds to step down from the wooden platform to grid floor with all its paws on the grid floor. It reflected the long-term memory of animals. Significant increase in SDL value is an indication of improvement in memory. Scopolamine administration considerably decreases SDL (−26.34 s) from the normal value, which was an indication of dementia (neurodegeneration) [Figure 1]. EECPS (200, 400, and 800 mg/kg, p.o.) and piracetam (200 mg/kg, p.o.) administered orally in rats daily continuously for 14 days show significantly ($P < 0.05$) increased SDL when compared with the respective vehicle control groups after a single injection of scopolamine (2 mg/kg, i.p.) on 14th day before cognitive measurements tests [Table 2]. EECPS at doses of 400 and 800 mg/kg, p.o., shows significant ($P < 0.05$) improvement in memory and also reversed amnesia which was induced by scopolamine [Figure 2].

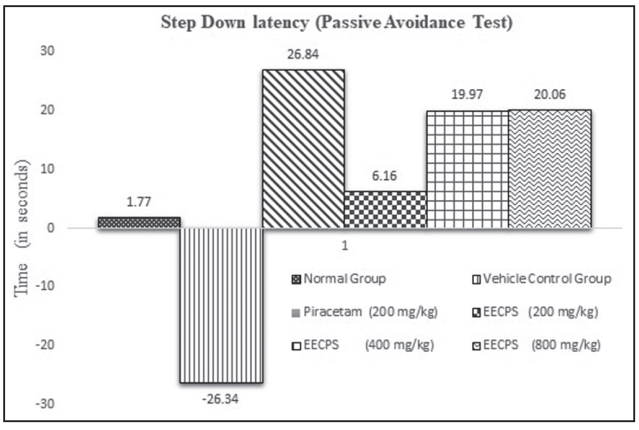

Figure 1: Difference of SDL time (using passive avoidance test) between 1st and 14th day

Effect on EL (using Morris water maze)

EL is defined as the time (in seconds) taken by rats to locate/reach the platform hidden below the water surface after it enters the water maze. Considerable reduction in EL value of retention indicated improvement in memory. Scopolamine administration considerably increases EL (+28.92 s) from normal value, which was an indication of memory loss (dementia) [Figure 3]. EECPS (200, 400, and 800 mg/kg, p.o.) and piracetam (200 mg/kg, p.o.) showed a significant ($P < 0.05$) reduction in EL on 14th day after the injection of scopolamine (2 mg/kg, i.p.) when compared with respective vehicle-treated control groups indicating significant improvement in memory. Moreover, EECPS extract at the dose of 400 and 800 mg/kg shows significant ($P < 0.05$) results of EL against scopolamine-induced oxidative stress and impairment in learning or memory. EECPS reversed memory deficits or amnesia induced by scopolamine successfully [Figure 4].

Biochemical estimation (SOD, CAT, GSH, and MPO) in rat brain

The results of biochemical estimation in the brain of rats indicate that EECPS (400 and 800 mg/kg, p.o.) exhibited increase in the activities of antioxidant enzymes, which was statistically significant ($P < 0.05$) when compared with the vehicle-treated control group [Table 2]. The extract at the doses of 400 and 800 mg/kg showed maximum significant ($P < 0.05$) increase in the enzyme activity when compared with the control group.

AChE enzyme activity

AChE activity measurement results in the hippocampus showed that the vehicle-treated group significantly ($^{\#}P < 0.05$) increased AChE activity after scopolamine administration when compared with the normal group. The rats treated with piracetam showed a significant decrease in AChE activity, whereas maximum inhibition of AChE was observed in the EECPS-treated group at 400 and 800 mg/kg when compared with the vehicle-treated group [Figure 5].

Discussion

AD is one of the most common neurodegenerative disorders characterized by early cognitive dysfunction and loss of learning ability along with aging.[9] The primary neuronal injury region involved in the pathophysiology of this disease is hippocampus.[34] One of the important animal models is scopolamine-induced dementia in rats which is quite similar to pathophysiology of AD in humans.[35] Scopolamine-induced dementia is mediated by cholinergic deficit through neuro-inflammation and oxidative stress associated with altered levels of antioxidant enzyme (SOD, CAT, GSH, and lipid peroxides) activities.[6] The cholinergic system plays an important role in learning and memory.[11] Loss of cholinergic neurons or reduced choline acetyltransferase activity in the cerebral cortex and hippocampus is consistent with findings in AD.[36] A new paradigm for treating this pathology was to target multiple underling molecular and cellular mechanisms,

Table 2: Biochemical estimations of antioxidant enzyme in rat brain

Parameters	Normal group	Vehicle control group	Standard group	EECPS (200 mg/kg)	EECPS (400 mg/kg)	EEPS (800 mg/kg)
Superoxide dismutase (SOD) (µmol/mg of protein)	32.45 ± 0.55	17.38 ± 0.21#	30.48 ± 0.46*	20.09 ± 0.62	28.98 ± 0.33*	28.87 ± 0.66*
Catalase (CAT), µmol of H_2O_2/min/mg proteins	56.83 ± 0.29	28.07 ± 0.29#	56.05 ± 0.31*	31.54 ± 0.88	52.22 ± 0.90*	46.52 ± 0.47*
Glutathione (GSH), µmol/mg of proteins	10.41 ± 0.44	05.61 ± 0.55#	09.76 ± 0.41*	06.31 ± 0.40	08.50 ± 0.26*	08.96 ± 0.39*
Malondialdehyde (MDA), µmol/mg of protein	08.78 ± 0.45	15.24 ± 0.31#	08.84 ± 0.22*	14.07 ± 0.21	09.93 ± 0.14*	09.19 ± 0.31*

#$P < 0.05$ shows the significant difference when compared with the normal group; *$P < 0.05$ shows the significant values when compared with the vehicle control group ($n = 6$)

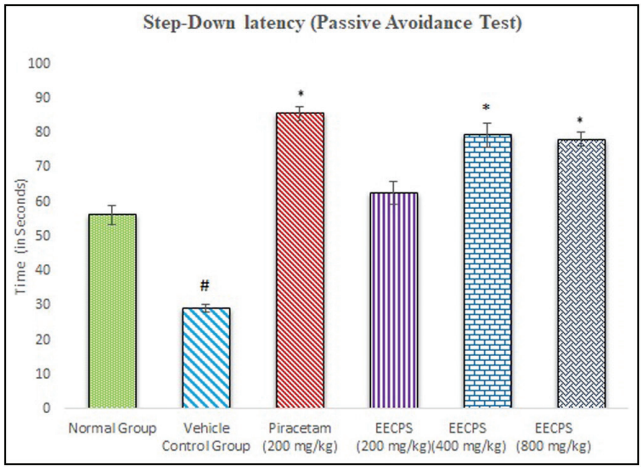

Figure 2: Effect of EECPS on SDL (using passive avoidance test) on 14th day. #*P* < 0.05 showed significant values when compared with the normal group. **P* < 0.05 shows the significant values when compared with the vehicle control group (*n* = 6)

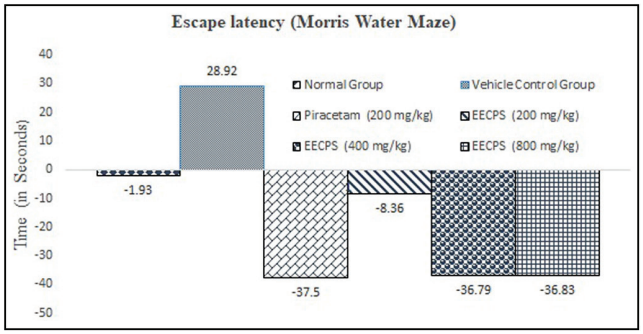

Figure 3: Difference of EL time (using Morris water maze test) between 1st and 14th day

instead of concentrating on single one. It has been proposed that exogenous antioxidants could effectively control the cumulative effects of oxidative damage in normal aging and neurodegenerative diseases.[33] After the extensive web research on medicinal plants for treating this disease, based on ethno-medicinal data *C. pepo* was selected because traditionally *C. pepo* could be very well associated with its ability to increase antioxidant capacity. Hence, the objective of the present study was to assess the neuroprotective activity of EECPS on learning and memory impairment observed in AD by using the Morris water maze and passive avoidance tests in rats. We also evaluated the neuroprotective mechanism of *C. pepo* L. by antioxidative parameters, for which estimating the SOD, CAT, GSH and lipid peroxides (by MPO) levels is also supported through AChE enzyme activity.

The passive avoidance test and Morris water maze test are the standard models for assessing spatial learning and memory. In both the mentioned models, oxidative stress was facilitated by a single dose of scopolamine to the particular level in rat brain, by virtue of which cell necrosis may take place by which SDL decreases and escape latency increases, respectively.[37] Treatment for 14 consecutive days of EECPS considerably increased the SDL and decreased the escape latency. EECPS at doses of 400 and 800 mg/kg showed the most significant alteration in SDL and escape latency, which may be attributed to free radical scavenging action of *C. pepo* L. seeds extract.

As reported in the literature, oxidative stress had been implicated in the neurodegenerative process in AD, which may be either due to free radicals production or loss of antioxidant defenses or even both.[38] The highly toxic ROS, such as superoxide anion, hydrogen peroxide, and hydroxyl radical, are found in specific areas of brain, which can lead to neurodegeneration, and is believed to be a major reason of AD.[39] In the antioxidant defense mechanisms, SOD, CAT, and GSH are the key enzymes that remove these ROS.[7] In the present experiment conditions, rats after scopolamine treatment showed a remarkable decrease in GSH, CAT, and SOD activities and increased the level of lipid peroxidation in the brain of rats.[12] The administration of EECPS significantly reversed the activity of GSH reductase, catalase, and SOD and inhibits the lipid peroxidation activity in the hippocampus region of rat brain. We suggests that the restoration of GSH, CAT, and SOD activities by EECPS might help in promoting scavenging of free radicals. Excitatory neurotransmitter, such as AChE, is more imperious for neuronal communication. It is responsible for causing degradation of AChE, and its level is higher in Alzheimer's patients.[26] The levels were measured in rat brain homogenates as a marker of central cholinergic status, which is compulsory in keeping typical cognitive functions.[40] Treatment with scopolamine increased the brain AChE level

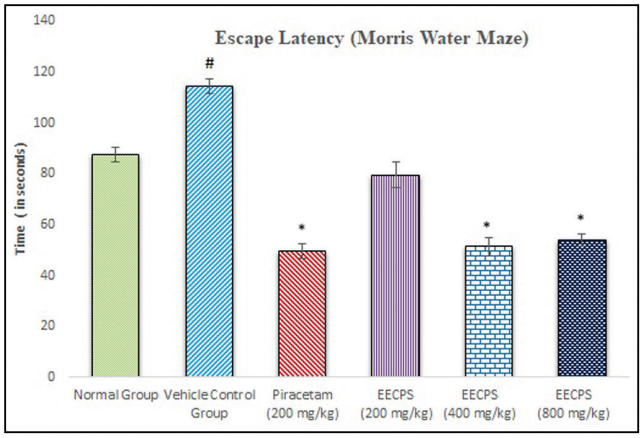

Figure 4: Effect of EECPS on EL (using Morris water maze test) on 14th day. #*P* < 0.05 shows the significant values when compared with the normal group; **P* < 0.05 shows the significant values when compared with the vehicle control group (*n* = 6)

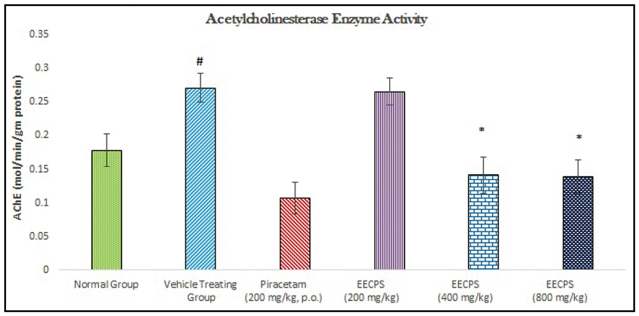

Figure 5: Effect of EECPS on hippocampus AChE enzyme activity in scopolamine-treated rats. All assays were performed in triplicate. **P*<0.05 represents significant difference with the vehicle-treated group. #*P*<0.05 represents significant difference with the normal group

when compared with the normal group, which confirmed this mechanism. The increased AChE level was also recovered appreciably by treatment with EECPS at doses of 400 and 800 mg/kg, which clearly showed that extract of seeds of this plant has potential nootropic activity.

The phenolic compounds are secondary metabolites found in plant kingdom and have received much attention as potential natural antioxidant in terms of their ability to act as radical scavengers against free radicals.[41] The antioxidant activity of phenolic contents is mainly due to their redox properties, hydrogen donors, and singlet oxygen quenchers.[42] Thus, antioxidant capacity of EECPS may be attributed mainly to its phenolic compounds as confirmed by phytochemical screening. The ability of antioxidant treatment to enhance cognition provides the direct linkage between free radicals/oxidative damage and decrease in cognitive function. The results clearly indicated that administration of the antioxidants through *C. pepo* seeds extract to rats can significantly improve cognitive performance in the Morris water maze and passive avoidance tests. It is well documented that the plant extracts reach the central nervous system by crossing the blood–brain barrier (BBB) through different pathways. In this way, as it can be seen, many active compounds have lipophilic or liposoluble property which possesses powerful anti-inflammatory or immune responses.[43] To exert memory-enhancing effect in the brain, the constituents of *C. pepo* L. should cross the BBB. The results of biochemical estimation also confirmed the enhanced antioxidant activity of *C. pepo* L. seeds extract; it is only possible when the active constituents of EECPS may have high penetration into the brain via crossing BBB. The major chemical constituents (i.e., polyphenols/phenols) of *C. pepo* L. seeds obtained by extraction with ethanol showed the cognitive-enhancing (antiamnesic) activity including neuroprotection by antioxidant mechanism. In our study, *C. pepo* showed free-radical scavenging and increased antioxidant enzymes activity, especially in the brain, which might be important in neuroprotective properties. These behavioral and biochemical results suggest that EECPS has the potential to alter the development and progression of AD.

Conclusion

The present study clearly reveals that EECPS appreciably attenuates scopolamine-induced dementia by producing antioxidant effect in the rat brain and mediating an interaction through AChE activity. This result suggests that *C. pepo* L. might offer a useful therapeutic choice for the treatment of AD. In this context, the present research work could serve as valuable resource for the development of ethno-medicine in the prevention as well as treatment of AD. Yet, advance studies are needed to characterize the active compound(s) responsible for the same. More comprehensive research which should focus on identifying active ingredients of plants along with their mechanism of action is further needed.

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Conflicts of interest

The authors have declared that there is no conflict of interest.

References

1. Raina SK, Raina S, Chander V, Grover A, Singh S, Bhardwaj A. Is dementia differentially distributed? A study on the prevalence of dementia in migrant, urban, rural, and tribal elderly population of Himalayan region in Northern India. N Am J Med Sci 2014;6:172-7.
2. Rizzi L, Rosset I, Roriz-Cruz M. Global epidemiology of dementia: Alzheimer's and vascular types. Biomed Res Int 2014;2014:908915.
3. Inestrosa NC, Alvarez A, Pérez CA, Moreno RD, Vicente M, Linker C, *et al*. Acetylcholinesterase accelerates assembly of amyloid-beta-peptides into Alzheimer's fibrils: Possible role of the peripheral site of the enzyme. Neuron 1996;16:881-91.
4. Jahn H. Memory loss in Alzheimer's disease. Dialog Clin Neurosci 2013;15:445-54.

5. Jomova K, Vondrakova D, Lawson M, Valko M. Metals, oxidative stress and neurodegenerative disorders. Mol Cell Biochem 2010;345:91-104.
6. Haider S, Tabassum S, Perveen T. Scopolamine-induced greater alterations in neurochemical profile and increased oxidative stress demonstrated a better model of dementia: A comparative study. Brain Res Bull 2016;127:234-47.
7. Das K, Choudhury RA. Reactive oxygen species (ROS) and response of antioxidants as ROS-scavengers during environmental stress in plants. Front Environ Sci 2014;2:1-13.
8. Elmore S. Apoptosis: A review of programmed cell death. Toxicol Pathol 2007;35:495-516.
9. Araujo JA, Studzinski CM, Milgram NW. Further evidence for the cholinergic hypothesis of aging and dementia from the canine model of aging. Prog Neuropsychopharmacol Biol Psychiatry 2005;29:411-22.
10. Ionita R, Postu PA, Beppe GJ, Mihasan M, Petre BA, Hancianu M, *et al*. Cognitive-enhancing and antioxidant activities of the aqueous extract from *Markhamia tomentosa* (Benth.) K. Schum. stem bark in a rat model of scopolamine. Behav Brain Funct 2017;13:5.
11. Talita H, Vieira F, Guimaraes IM, Silva FR, Ribeiro FM. Alzheimer's disease: Targeting the cholinergic system. Curr Neuropharmacol 2016;14:101-115.
12. Puri A, Srivastava P, Pandey P, Yadav RS, Bhatt PC. Scopolamine induced behavioral and biochemical modifications and protective effect of *Celastrus* paniculatous and Angelica glauca in rats. Int J Nutr Pharmacol Neurol Dis 2014;4:158-69.
13. Rahimzadegan M, Soodi M. Comparison of memory impairment and oxidative stress following single or repeated doses administration of scopolamine in rat hippocampus. Basic Clin Neurosci 2018;9:5-14.
14. Zhang Y, Wang Q, Chen H, Liu X, Lv K, Wang T, Wang Y, *et al*. Involvement of cholinergic dysfunction and oxidative damage in the effects of simulated weightlessness on learning and memory in rats. BioMed Res Int 2018;2547532:1-11.
15. Kirtikar KR, Basu BD. Indian Medicinal Plants. Published by Sudhindra Nath Basu, Allahabad, India; 1918.
16. Gutierrez RMP. Review of Cucurbita pepo (pumpkin) its phytochemistry and pharmacology. Med Chem 2016;6:012-021.
17. Adnan M, Gul S, Batool S, Fatima B, Rehman A, Yaqoob S, *et al*. A review on the ethnobotany, phytochemistry, pharmacology and nutritional composition of Cucurbita pepo L. J Phytopharmacol 2017;6:133-9.
18. Salehi B, Capanoglu E, Adrar N, Catalkaya G, Shaheen S, Jaffer M, *et al*. Cucurbits plants: A key emphasis to its pharmacological potential. Molecules 2019;24:1854.
19. Caili F, Huan S, Quanhong L. A review on pharmacological activities and utilization technologies of pumpkin. Plant Foods Hum Nutr 2006;61:73-80.
20. Yadav M, Jain S, Tomar R, Prasad GB, Yadav H. Medicinal and biological potential of pumpkin. Nut Res Rev 2010;23:184-90.
21. Ethiraj S, Balasundaram J. Phytochemical and biological activity of *Cucurbita* seed extract. J Adv Biotechnol 2016;6:813-21.
22. Kaur N, Kishore L, Singh R. Attenuation of STZ-induced diabetic nephropathy by Cucurbita pepo L. seed extract characterized by GCMS. J. Food Biochem 2017;41:1-13.
23. Rajasree RS, Sibi PI, Francis F, William H. Phytochemicals of Cucurbitaceae family—A review. Int J Pharmacogn Phytochem Res 2016;8:113-23.
24. Ratnam N, Vandana, Md Najibullah, Md Ibrahim. A review on Cucurbita pepo. Int J Pharmacogn Phytochem Res 2017;9:1190-4.
25. Adebayo OR, Farombi AG, Oyekanmi AM. Proximate, mineral and anti-nutrient evaluation of pumpkin pulp (*Cucurbita pepo*). J Appl Chem IOSR-JAC 2013;4:25-8.
26. Uddin SM, Mamun AA, Hossain MS, Ashaduzzaman M, Noor MAA, Hossain MS. Neuroprotective effect of Phyllanthus acidus L. on learning and memory impairment in scopolamine-induced animal model of dementia and oxidative stress: Natural wonder for regulating the development and progression of Alzheimer's disease. Adv Alzheimer's Dis 2016;5:53-72.
27. Trease GE, Evans WC. Textbook of Pharmacognosy. 15th ed. London: Balliere Tindall; 2002.
28. Edeoga HO, Okwu DE, Mbaebie BO. Phytochemical constituents of some Nigerian medicinal plants. Afr J Biotechnol 2005;4:685-88.
29. Johari MA, Khong HY. Total phenolic content and antioxidant and antibacterial activities of *Pereskia bleo*. Adv Pharmacol Sci 2019;2019:7428593.
30. Akindele AJ, Adeyemi OO. Evaluation of the antidiarrhoeal activity of *Byrsocarpus coccineus*. J Ethnopharmacol 2006;108:20-5.
31. Wang J, Wang X, Lv B, Yuan W, Feng Z, Mi W, *et al*. Effects of *Fructus akebiae* on learning and memory impairment in a scopolamine-induced animal model of dementia. Exp Ther Med 2014;8:671-5.
32. Katerji M, Filippova M, Duerksen-Hughes P. Approaches and methods to measure oxidative stress in clinical samples: Research applications in the cancer field. Oxid Med Cell Longev 2019;1279250:1-29.
33. Oni MO, Ogungbite OC, Oguntuase SO, Bamidele OS, Ofuya TI. Inhibitory effects of oil extract of green *Acalypha* (Acalypha wilkesiana) on antioxidant and neurotransmitter enzymes in Callosobruchus maculatus. J Basic Appl Zool 2019;80:1-13.
34. Sanabria-Castro A, Alvarado-Echeverría I, Monge-Bonilla C. Molecular pathogenesis of Alzheimer's disease: An update. Ann Neurosci 2017;24:46-54.
35. Navarro NM, Krawczyk MC, Boccia MM, Blake MG. Extinction and recovery of an avoidance memory impaired by scopolamine. Physiol Behav 2017;171:192-8.
36. Nigam A, Kulshreshtha M, Panjwani D. Pharmacological evaluation of *Hibiscus* abelmoschus against scopolamine-induced amnesia and cognitive impairment in mice. Adv Hum Biol 2019;9:116-23.
37. Budzynska B, Boguszewska-Czubara A, Kruk-Slomka M, Skalicka-Wozniak K, Michalak A, Musik I, *et al*. Effects of imperatorin on scopolamine-induced cognitive impairment and oxidative stress in mice. Psychopharmacology (Berl) 2015;232:931-42.
38. Perry G, Cash AD, Smith MA. Alzheimer disease and oxidative stress. J Biomed Biotechnol 2002;2:120-3.
39. El-Sherbiny DA, Khalifa AE, Attia AS, Eldenshary Eel-D. *Hypericum perforatum* extract demonstrates antioxidant properties against elevated rat brain oxidative status induced by amnestic dose of scopolamine. Pharmacol Biochem Behav 2003;76:525-33.
40. Craig LA, Hong NS, McDonald RJ. Revisiting the cholinergic hypothesis in the development of Alzheimer's disease. Neurosci Biobehav Rev 2011;35:1397-409.
41. Kasote DM, Katyare SS, Hegde MV, Bae H. Significance of antioxidant potential of plants and its relevance to therapeutic applications. Int J Biol Sci 2015;11:982-91.
42. Dai J, Mumper RJ. Plant phenolics: Extraction, analysis and their antioxidant and anticancer properties. Molecules 2010;15:7313-52.
43. Balmus IM, Ciobica A. Main plant extracts' active properties effective on scopolamine-induced memory loss. Am J Alzheimers Dis Other Demen 2017;32:418-28.