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Investigation of nootropic potential of ethanol extract of *Elettaria cardamomum* Maton fruit (Cardamom): a spice of India

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Abstract

Background: Cognitive impairment mainly affects learning and problem solving abilities of the person. Traditional medicines, due to fewer side effects, more efficacy and lesser cost are still the choice of treatment in India. The main objective of the present study is to explore the nootropic potential of ethanol extract of *Elettaria cardamomum* Maton fruits. The present study was conducted by using exteroceptive behavioral models like elevated plus maze, passive avoidance apparatus and object recognition task at doses 100, 300 and 500 mg/kg.

Results: Ethanol extract of *E. cardamomum* fruits significantly decreased transfer latency and increased step down latency and discrimination index respectively when compared to normal control groups. Piracetam and diazepam exhibited respective rise and fall in memory of the animals. The effect of plant extract on total serum cholesterol, acetylcholinesterase and malondialdehyde were more effective at two higher doses. However, it decreased serum glucose levels insignificantly whereas a significant rise in brain GSH levels was observed with increasing dose of the extract.

Conclusions: The study concludes that the nootropic effect of ethanol extract may be attributable to its anti-oxidant, anti-cholinesterase and cholesterol as well as glucose lowering potential.

Keywords: *Elettaria cardamomum*, Memory, Cognitive impairment, Amnesia: Nootropics

Background

Alzheimer's disease (AD), one of the most common cognitive disorders, characterized by progressive neurodegeneration, prevails in around 35 million people worldwide, of which 4.5 million Americans are affected annually [1]. However, the estimated figure for AD patients in India is less than 3.5 million, which is relatively smaller as compared to United States of America [2]. Oxidative stress is a major implication to these disorders as it plays a vital role in various pathological events such as mutagenesis, aging and neurodegenerative disorders [3]. Moreover, neuromodulators, diverse neurotransmitters and receptor systems are included in the cognitive viz., acetylcholine, nor-epinephrine, dopamine, serotonin, GABA and histamine [4, 5].

Piracetam is the chiefly used nootropic agent [6], along with aniracetam, pramiracetam [7] and choline esterase inhibitors like donepezil which are primarily being used for enhancing mood, gastro protective memory and behavior. However, due to side effects their use has been limited [8, 9]. According to World Health Organization (WHO), more than 3.3 billion people are resident of developing countries and rely on traditional herbal medicines for their primary healthcare needs [10]. Ayurveda, the oldest Indian medical system in the world, reports a group of plants called '*medhyas*' which possess neuromodulatory activity, of which, *Shankhapushpi* (leaf), *Jataamansi* and *Ashwagandha*, are the most extensively used plants [11].

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Elettaria cardamomum Maton (Zingiberaceae), commonly referred to as Cardamom in English and Choti ilaichi in Hindi is a widely accepted condiment which is commonly used for culinary purposes throughout India [12, 13]. It contains cineole (present in volatile oil) and borneol compounds [14] but apart from its aromatic character it also possesses anti-inflammatory, anticancer [15], anti-oxidant, analgesic, antispasmodic [16], antibacterial [17], gastro protective and anti-ulcerogenic activities [18, 19].

The present work was designed to justify the traditional use of *E. cardamomum* as neuropharmacological agent since no such reports had been reported in the literature. The study was attempted to corroborate the effectiveness of Indian traditional herbal plant *E. cardamomum* for the treatment of memory enhancement. Additionally, the study may be extended to delineate the possible mechanistic pathway responsible for memory enhancement so that an effective drug molecule can be evaluated.

Materials and methods

Plant material

Selection and identification of plant material

The plant *Elettaria cardamomum*, used in the present study has been selected by meticulous literature analysis of classical text books, peer reviewed papers and scientific databases. The fresh fruits of cardamom were procured from Kurukshetra, Haryana, India. The authentication of collected sample was done by Dr. H.B. Singh, Chief Scientist & Head, Raw Materials Herbarium and Museum (RHMD), National Institute of Science Communication and Information Resources (NISCAIR), New Delhi (Ref. NISCAIR/RHMD/Consult/2011-12/1939/239). Collected fruits were cleaned thoroughly in order to prevent any contamination.

Preparation of the extract

Seeds of cardamom were separated from undesirable materials using oscillating shakers. The dried plant material was ground to powder of 60 mesh size by using hand mill. The coarse powder (1200 gm) thus obtained was placed in soxhlet's extractor and exhaustedly extracted using ethanol (60–80˚C). The crude ethanol extract of *Elettaria cardamomum* (EEC) was concentrated at 40˚C using a Rota evaporator. The crude extract (135 gm) thus obtained was labeled and preserved in a freezer at -20˚C until further use. The extractive value (11.25 % w/w) was found by using the formula= (Crude extract obtained /Total powder taken)x100.

Animals

Swiss albino mice of either sex weighing 18–22 gm (aged 10–12 weeks) were used, which were kept in Animal House, Institute of Pharmaceutical sciences, Kurukshetra University, Kurukshetra. They had free access to water and food and were subjected to the natural dark-light cycle at the interval of 12 h. They were acquainted to the laboratory environment for a minimum period of one week before carrying out the cognitive studies. All experiments were performed from 09 a.m. to 04 p.m. during the day. Institutional Animal Ethics Committee (IAEC) (Reg. No 562/02/a/CPCSEA) approved the experimental protocol (No. 340 A/18) of the present study and animals were treated according to the guidelines of CPSCEA, Ministry of Forest and Environment, Government of India.

Drugs, chemicals and instruments

Piracetam (UCB India Ltd., India), diazepam (Calmpose, Ranbaxy, India), ethanol (SD Fine Chemicals Pvt. Ltd.), acetylthiocholine iodide, 5,5'-dithiobis-2-nitrobenzoic acid (DTNB), thiobarbituric acid (TBA) (Himedia Co. Ltd., India), cholesterol diagnostics kit (Erba diagnostics Mannheim GmbH Mallaustr, Germany), glucose diagnostic kit (Bayer diagnostic, Gujarat, India), U.V. Spectrophotometer (Systronics UV-Spectrophotometer 2202, Systronic India Ltd, India), Rota evaporator (Heidolph Labrota 4011 digital, Germany), Centrifuge machine (R-8 C, Mumbai) and auto analyzer (ERBA Chem-7, Raj Biosis Private Limited, India), Soxhlet's extractor (Laborate Technocracy, Ambala, Haryana, India). All the other chemicals and solvents used in the present study were of analytical grade.

Initial phytochemical tests

Chemical tests were carried out on ethanol extract for the qualitative determination of phytochemical constituents as described by Khandelwal [20].

Acute toxicity studies

Guidelines (No. 423) of Organization for Economic Co-operation and Development (OECD) were followed to detect the possibility of acute toxicity in laboratory animals. The animals were treated with EEC at the following four doses (5, 50, 300 and 2000 mg/kg). The treated animals were kept under continuous supervision for behavioral and autonomic profiles or any signs of toxicity during this period.

Vehicle

Plant extract (EEC) was suspended in 2 % (w/v) gum acacia at the time of administration and was administered per oral to animals. Diazepam and piracetam were dissolved in normal saline and injected by intraperitoneal route.

Drug protocol

100, 300 and 500 mg/kg of EEC was given via oral gavage to different groups of mice for twelve days to perform various memory models and for biochemical estimations. Piracetam (400 mg/kg, i.p.), used as reference standard was given as positive control and was injected for 12 successive days. All control groups received normal saline for 12 days. After 1.5 h of the last dose of EEC, diazepam (1 mg/kg, i.p.) was administered to experimental animals to induce amnesia. 45 min after the diazepam was injected into the animals, they were subjected to exposure of training session. The retention of memory was measured after 24 h (13th day) of extract administration.

Exteroceptive behavioral studies

Elevated plus maze activity [21]

An elevated plus maze stood 25 cm above the ground had two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm × 15 cm). These arms were projected from a platform located in the center (5 cm × 5 cm). The test consisted of placing the mice on the extreme end of the open arm and measuring the time taken by it to move from there into any of the arm which was covered, by making use of its four paws termed as, Transfer Latency (TL). If the mice could reach to one of the covered arm of the platform before 90 s, the time it took to do so was recorded, else it was placed towards the covered arm and time taken to do so was assigned as 90 s. 10 s after the TL was recorded on the first day, each animal was transferred to its case and was again placed on the same platform after 24 h to record the retention of this learned task (memory). TL of the first and second day could become the measurement of parameters for acquisition and retrieval respectively, with a condition that each mouse has to be used once.

Passive avoidance paradigm activity [21]

A passive avoidance apparatus based on the principle of negative reinforcement is designed to measure the long term memory of mice. The apparatus is designed in the form of a rectangular box with its three sides made up of wood and fourth one of Plexiglass. The bottom consisted of a wooden platform in the center (10 cm×7 cm×1.7 cm) surrounded by a steel grid in all the four sides to deliver a current (20 V, AC). The mice were placed in the center of the bottom of the box and box was illuminated with a 15 W electric bulb and the Step Down Latency (SDL) was recorded. SDL is the time that a mouse takes to change its position from the central wooden platform to the grid with all its paws on it. Mice with the SDL of 2–15 s in the first test were chosen for the second trial which was performed 90 min after the first one. Mice with SDL less than 60 s were placed on the second wooden platform for another 15 s. Those mice which did not change their position from the platform to the grid within 60 s were removed from the central wooden platform and were placed into the same box on the central platform after 24 h of the first trial maintaining same environment except for the flow of (20 V, AC) current into the grid in the bottom.

Object recognition task [22]

This test is carried out to determine the object recognition ability of mice after a given period of time. An open circular field of height 400 mm and diameter 480 m was used to determine the ability of mice to recognize the object. Three non-identical objects (sphere, cylinder and cone) made up of aluminum and which were neither could be moved by mice nor could be used by them to hide behind, were implied. The objects did not have any natural significance and were novel for the mice. The mice were put face to face with two objects in the first trial (T1) and the third object in the trial meant for recognition trial (T2). To vanish the familiar odor, the objects were washed with tap water and detergent after each trial was performed. In the first two days the mice were put in the apparatus twice a day, 3 min in the morning and 3 min in the afternoon preceding it so that they become familiar with the environment within. Two objects were placed equidistant from the mice within the apparatus, the mice having its face towards the wall in front of the person performing the trial. Maximum time taken for the experiment was 3 min. Pairs of two trials T1 and T2 were done to train the animals, the second trial being done after the first one after one hour. The first experimental trial was designed in two ways that the two identical objects A1 and A2 were placed at the symmetrical position 120 mm away from the center of the apparatus towards the wall. The second trial T2 consisted of placing two different objects, one novel one and the other identical tool that in T1, at the same position where the identical objects were placed in T1 trial. The time taken by mice to identify the object identical to that placed in trial T1 was noted. If the animals take more time to differentiate between the two objects or to identify the familiar objects, it indicates reduced recognition memory. The mouse is said to explore the objects if it directs its nose to the object at a distance of not more than 2 cm and/or touching the object with muzzle. However, if the mouse sits on the object it is not said to explore it.

Biochemical estimations

Collection of blood and brain samples [21]

On the last day of the study, the animals were anesthetized, following which they were sacrificed, 1.5 h after the last dose of control/standard drugs or extract was

given to them. The animals were sacrificed using cervical dislocation under light chloroform anesthesia, their whole brain was removed consciously from skull and the blood of their trunk was collected. Total cholesterol and blood glucose level of mice were determined after centrifugation of blood at 3000 rpm for 15 min, to obtain its plasma followed by its chemical analysis.

Brain homogenate was prepared by homogenizing the weighed fresh whole brain in a glass homogenizer at -20˚C in an ice bath, in 10 times (w/v) of sodium phosphate buffer (0.1 M, pH 7) as a medium. Brain malondialdehyde (MDA) level, acetyl-cholinesterase (AChE) activity and glutathione (GSH) levels were estimated by centrifugation of the homogenate at 3000 rpm at 40˚C for 10 min and analyzing the resultant cloudy supernatant.

Estimation of brain AChE activity

The method demonstrated by Ellman et al., 1961 with slight modification was used for brain AChE activity. DTNB solution was prepared fresh (10 mg DTNB in 100 ml of Sorenson phosphate buffer, pH 8.0) and was added to 0.5 ml of the cloudy supernatant up to 25 ml in a volumetric flask. After pipetting 4 μl twice in two test tubes and adding 1 ml of substrate solution (75 mg of acetylcholine iodide per 50 ml of distilled water) in both of them and two drops of eserine solution into one of them and incubating for 10 min at 30˚C, they were subjected to colorimetric analysis. The DTNB was reduced to thionitobenzoic acid by some chemicals in the brain homogenate and non-enzymatic hydrolysis of substrate due to which yellow color was obtained. The instrument was calibrated and the alteration in absorbance per min was measured at 420 nm [23, 24].

Estimation of total cholesterol levels [25]

Serum total cholesterol was estimated by CHOD-PAP method (Allain et al., 1974). To prepare the standard and test solutions 20 μl of standard cholesterol and serum were mixed with 1000 μl of the working reagent. Blank was prepared by adding 20 μl of distilled water to 1000 μl of working reagent. After incubating both standard and test solutions at 37˚C for 10 min, the auto-analyzer was used to measure the absorbance at 510 nm and 630 nm (Filter 1 and Filter 2) against the blank.

Quantitative estimation of glucose in the blood [26]

Blood glucose level was estimated by GOD-POD method using semi auto-analyzer. The standard and test sample were prepared by respectively mixing 10 μl of standard glucose and serum with 1000 μl of the working reagent. The blank was prepared by mixing together 10 μl of distilled water and 1000 μl of working reagent. After incubating these solutions for 1/4 h at 98.7˚F they were analyzed through the UV-visible Spectrophotometer, the absorbance being measured at 510 nm and 630 nm (Filter 1 and Filter 2).

Estimation of brain MDA level [27]

Lipid peroxidation is measured with the help of MDA, a thiobarbituric acid reactive substance (TBARS) using UV-visible Spectrophotometric method. 500 μl of thiobarbituric acid (TBA) reagent, 1.5 ml of 15 % trichloroacetic acid (TCA) and 500 μl supernatant of tissue homogenate were mixed using 10 ml screw-cap pyrex centrifuge tube and the mixture was heated on water bath for 3/4 h and cooled in an ice bath subsequently and the extraction of chromogen was carried out by adding 3 ml of n-butanol and centrifuging the mixture. The pink colored organic phase thus obtained was analyzed spectrophotometrically taking 512 nm as $_{max}$. $\varepsilon = 1.56 \times 10^5\ M^{-1}\ cm^{-1}$ formula was used to calculate the quantity of lipid peroxidation which was expressed as nmol of MDA per g of wet tissue.

Estimation of brain reduced GSH level [28]

20 % TCA was added to the supernatant homogenate and proteins were separated from it by centrifugation. 3 ml of phosphate buffer (pH 8.4), and 2 ml of DTNB was added to 0.25 μl of this supernatant. The yellow colored solution thus obtained was analyzed spectrophotometrically using 412 nm as $_{max}$ against a blank, within 15 min. $\varepsilon = 13.6 \times 103\ M^{-1}\ cm^{-1}$ formula expressed as nmol/g of wet tissue was used to calculate GSH content.

Statistical analysis

The data was analyzed by the One way ANOVA followed by Dennett multiple comparison tests. Mean ± SEM were used to express all the outcomes. The critical range for significant difference between two groups of observations was taken as $p < 0.05$. All the treated groups were compared with the respective control groups.

Results

Preliminary phytochemical screening

The preliminary phytochemical screening of EEC showed the presence of various phytoconstituents as summarized in the Table 1.

Acute toxicity studies

EEC administered at the dose levels (5, 50, 300 and 2000 mg/kg) produced no signs of toxicity when administered orally. Even the highest dose of extract did not show any toxic symptoms. Also, no mortality was seen in any animal at all these doses of EEC.

Table 1 Preliminary phytochemical investigation of EEC

Sr. no.	Phytoconstituents	Results
1.	Carbohydrates	-
2.	Glycosides	+
3.	Alkaloids	+
4.	Terpenoids	+
5.	Saponins	-
6.	Gums	-
7.	Steroids	+
8.	Proteins	+
9.	Flavonoids	++

+: present; -: not detected

Elevated plus maze model

On the first day (12th day of treatment) TL reflected learning behavior, whereas on the next day (13th day of the treatment) TL reflected retention of information (Fig. 1). Effective memory retrieval was indicated by a decrease in TL values of retrieval test as compared to the acquisition session and vice-versa. EEC (at all doses) and piracetam treatment exhibited a significant ($p < 0.05$) decrease in TL indicating improvement in learning and memory as compared to the control animals. The effect was highest with piracetam treatment. EEC showed fall in TL in a dose dependent manner, however the effect was more profound at 500 mg/kg dose. Diazepam treatment showed increase in TL in both the sessions i.e., learning and memory, in comparison to vehicle control.

Passive avoidance paradigm activity

Increase in SDL value indicated improvement of memory whereas low SDL value represented memory impairment. EEC increased SDL in animals as compared to vehicle control groups dose dependently however, the effect was more at doses 300 and 500 mg/kg (Fig. 2). Diazepam treated mice showed a decrease in SDL indicating amnesic activity whereas piracetam revealed increase in SDL and exhibited maximum improvement when compared to all other groups.

Object recognition task

Increase in DI indicated enhanced recognition memory. EEC administered orally for 12 days increased the DI indicating improvement in memory of mice at higher doses i.e., 300 and 500 mg/kg (Fig. 3). Diazepam produced impairment in memory as indicated by reduced DI whereas piracetam revealed increase in the DI when compared to the normal control.

Biochemical parameters

Estimation of AChE activity

Acetylcholine pathway is considered as the most important conduit in regulation of memory functions. EEC produced significant reduction in brain AChE activity in dose dependent manner as compared to normal control group, when measured using Ellman's kinetic colorimetric method. Interestingly, EEC at 100 and 300 mg/kg showed insignificant results whereas at 500 mg/kg, it produced a significant ($p < 0.01$) fall in brain AChE activity (Table 2). Diazepam treated group increased AChE activity suggesting an impaired cholinergic system.

Effect on total cholesterol level

Piracetam and EEC (at all doses) showed a significant reduction in serum total cholesterol levels as compared to normal control treated mice when treated for 12 successive days (Table 2). The reduction was more in

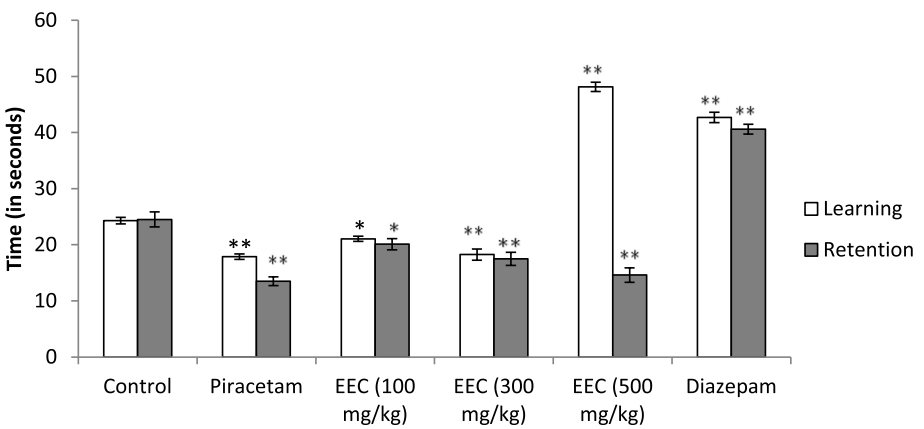

Fig. 1 Effect of EEC on transfer latency (TL) using elevated plus maze model. All values are mean ± SEM (n = number of animals in each group). Data was analyzed using one-way ANOVA followed by Dunnett test. *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ vs. normal control group

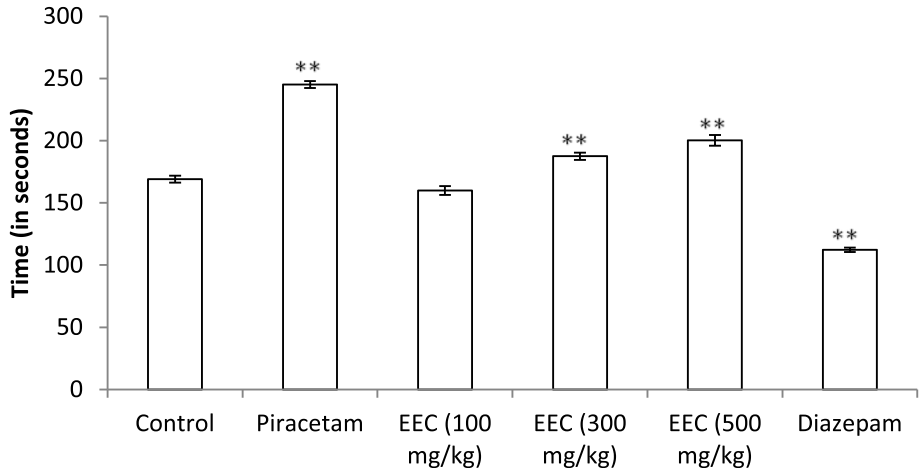

Fig. 2 Effect of EEC on step down latency (SDL) using passive avoidance paradigm.All values are mean ± SEM (n = number of animals in each group). Data was analyzed using one-way ANOVA followed by Dunnett test. *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ vs. normal control group

piracetam treated animals. The effect of EEC at dose 100 mg/kg was found to be less than the higher doses (300 and 500 mg/kg). But, diazepam treated animals exhibited an increase in cholesterol levels.

Effect on blood glucose level

The effects of EEC on serum glucose levels were found to increase but as evident from the Table 2, the effects were insignificant whereas the standard drug treated animals revealed a significant decrease in glucose levels. And similar to the effect on serum cholesterol level, diazepam control groups presented a marked increase in glucose levels.

Effect on brain MDA level

A reduction in brain MDA levels is a marker of enhanced memory protective activity. As evident from Table 2, EEC at highest dose 500 mg/kg showed significant though slight decrease in the MDA levels when compared to normal control group. However, the results were insignificant for EEC at doses 100 and 300 mg/kg. The negative control group (diazepam) showed increase in levels of brain MDA. Piracetam on the other hand, unexpectedly increased brain MDA levels.

Effect on brain reduced GSH level

When the animals were treated with EEC (at all doses), a rise in GSH was observed in a dose dependent manner which infers its ameliorative action on memory and learning. However, in diazepam treated animals GSH level was found to be reduced (Table 2).

Discussions

In India, despite its high population, AD is found to be less prevalent, may be due to regular intake of some

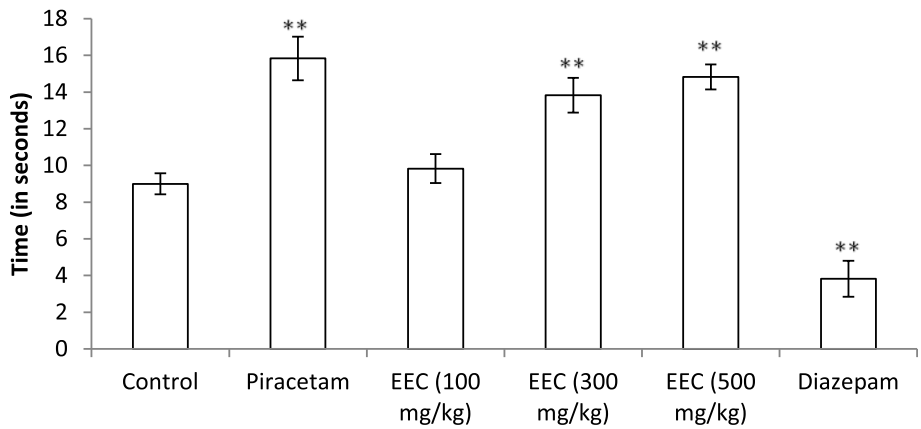

Fig. 3 Effect of EEC on discrimination index (DI) using object recognition taskAll values are mean ± SEM (n = number of animals in each group). Data was analyzed using one-way ANOVA followed by Dunnett test. *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ vs. normal control group

Table 2 Effect of EEC on biochemical parameters administered orally for 12 consecutive days

Groups	n	Biochemical parameters (on 13th day)				
		AChE activity (U/g)	Cholesterol (mg/dl)	Glucose (U/g)	MDA (μmol/g of tissue)	GSH (μmol/g of tissue)
Control (Normal saline)	6	16.9 ± 1.09	114 ± 2.17	132.06 ± 4.98	0.240 ± 0.016	1.47 ± 0.10
Piracetam (400 mg/kg, i.p.)	6	10.41 ± 0.61**	81.33 ± 2.90**	106.89 ± 4.03**	0.99 ± 0.01**	2.09 ± 0.079**
EEC (100 mg/kg, p.o.)	6	14.90 ± 0.69	102.42 ± 1.54*	124.46 ± 4.89	0.201 ± 0.009	1.96 ± 0.09**
EEC (300 mg/kg, p.o.)	6	13.58 ± 0.58	96.5 ± 1.48**	119.89 ± 4.03	0.192 ± 0.01	1.98 ± 0.03**
EEC (500 mg/kg, p.o.)	6	11.90 ± 0.73**	89.0 ± 4.12**	117.26 ± 4.25	0.172 ± 0.02*	2.12 ± 0.01**
Diazepam (1 mg/kg, i.p.)	6	29.72 ± 0.88**	118.3 ± 3.79	142.05 ± 5.08	0.244 ± 0.025	1.17 ± 0.02*

All values are mean ± SEM (n = number of animals in each group). Data was analyzed using one-way ANOVA followed by Dunnett test. *** $p < 0.001$, ** $p < 0.01$ and * $p < 0.05$ vs. normal control group
EEC: ethanolic extract of *Elettaria cardamomum*; AChE: acetylcholinesterase; MDA: malondialdehyde; GSH: glutathione

nutrients [4, 22]. Epidemiological studies suggested that high peripheral cholesterol pools increases vulnerability to AD [29, 30] and the countries with high calorie diet shows higher propensity to AD [31]. Histologically, extracellular protein deposits (β- amyloid plaques) in blood vessels and intraneuronal neurofibrillary tangles are manifested in AD. Increased Aβ in cellular and animal models of AD was found to be correlated with accumulation of abnormally high cholesterol level [32, 33]. Similarly, some research investigations revealed that hyperglycemia and hypoinsulinaemia could increase the neuronal damage produced by hypoxia, hypoglycemia or β-amyloid depositions [34]. Interestingly, EEC treated animals exhibited significant reduction in both serum cholesterol and glucose levels as compared to control groups. So, *E. cardamomum* may be regarded as a potential anti-Alzheimer candidate.

Memory impairments are allied to selective and irreversible deficiency in cholinergic transmission in brain. Also, senile dementia of AD is characterized by enhanced cholinergic loss and decreased choline acetyltransferase activity [22, 35]. Therefore, cognitive impairment is associated with disturbed cholinergic function. In our research, EEC decreased the brain AChE level dose dependently which may be the reason for improvement in memory and learning of experimental animals.

Activation of GABA-A and GABA-B receptors are documented to be involved in the processes of learning and memory [36]. BZD receptor agonists like alprazolam and diazepam have been shown to induce anterograde amnesia in rodents [37, 38] and humans [39] which may be responsible for the accessory symptoms of AD whereas GABA-A antagonist, bicuculline when injected prior to training enhanced memory in chicks [40] and rats [41]. Diazepam produced significant and comparable amnesia in mice in the present study.

Reactive oxygen species (ROS) and other oxidative metabolic byproducts have been shown to be neurotoxic [42, 43] and aged rats showed improvement in cerebellar physiology and motor learning when administered antioxidant rich diets [44]. MDA is a reactive electrophilic aldehyde which is produced as a result of lipid peroxidation of polyunsaturated lipids by ROS in an organism. It serves as a biological marker of oxidative stress as high levels of MDA are an indication of increased oxidative stress [45, 46]. Similarly, reduced GSH in brain is a marker of augmented oxidation reactions. GSH is a cellular thiol present in mammalian cells in concentrations upto 12 mM. GSH system is an antioxidant cellular defense system against ROS and its hindrance can lead to various deleterious effects including cessation of cell proliferation [47]. Since EEC considerably decreased the MDA and increased GSH levels, it can be inferred that EEC exhibits potent anti-oxidant activity. Also the surplus presence of flavonoids in EEC (as concluded from the preliminary phytochemical studies) further confirms this theory as flavonoids are known to possess potent anti-oxidant activity [48].

Inflammation (a consequence to oxidative stress [49]) may predispose to AD and its progression over age. Indomethacin, a potent non-steroidal anti-inflammatory agent augmented memory protection against electroconvulsive shock induced retrograde amnesia and amyloid deposits in the brain [50, 51]. Therefore, anti-inflammatory action of *E. cardamomum*, as documented by Majdalawieh and Carr, 2010, may also be attributable to the observed memory enhancing activity [15]. Thus, a combination of anti-oxidant, anti-inflammatory, anti-stress and neuroprotective task of *E. cardamomum* could all be leading to the memory enhancing effect.

EEC contains various chemical constituents like flavonoids, glycosides, alkaloids, triterpenoids and steroids. Memory enhancing effect of extract may be due to combined effect of various constituents present in the plant. Flavonoids have been reported to give protection against the memory impairment related to aging as well as associated with AD induced dementia [52]. Alkaloids act as nootropics either through inhibition of AChE [53] or by potentiating the GABA level [54]. The memory

improving effect of *E. cardamomum* may be due to presence of alkaloids, flavonoids and other anti-oxidants.

Conclusions

In conclusion, the present work gives preliminary data that the EEC possess potent nootropic principles, which enhances memory that may be attributable to its anti-inflammatory, anti-oxidant and neuroprotective activities. Also, its peripheral cholesterol and glucose lowering tendency suggests its potential to decrease progression of cognitive impairment. The findings of this study suggests that *E. cardamomum* may be used for the development of a new herbal derived medicament in the treatment of diverse cognitive disorders. However, further insight into the molecular details could explain the possible mechanism of action and the active principle responsible for the exhibited activity.

Abbreviations

EEC: Ethanol extract *Elettaria cardamomum*; AD: Alzheimer's disease ; AChE: Brain Acetylcholinesterase ; MDA: Malondialdehyde; GSH: Glutathione; TL: Transfer Latency ; SDL: Step Down Latency ; DI: Discrimination Index ; IAEC: Institutional Animal Ethics Committee ; CPCSEA: Committee for the Purpose of Control and Supervision on Experiments on Animals; SEM: Standard error of Mean ; ANOVA: Analysis of Variance

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Authors' contributions

Sapna Saini conducted all the experiments. Manjusha Choudhary supervised the experimental-work including selection of study, finalizing plan of work, *in-vivo* studies and interpretation of data. Ankur Garg did the writing and editing work. All the authors read and approved the final manuscript.

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Availability of data and materials

Data and materials will be provided on request.

Declarations

Ethics approval and consent to participate

The experiments conducted on animals complied with ARRIVE guidelines and carried out in accordance with the committee formulated for the Breeding of the Experiments on Animals (Control and Supervision) Rules, 1998, which was amended in 2001 and then in 2006 and associated guidelines. Laboratory animals were duly approved from IAEC (Institutional Animal Ethical Committee) and obtained from Institutional central animal facility (Approval Letter no. 340 A/18) as per the guidelines of CPCSEA (Committee for the Purpose of Control and Supervision on Experiments on Animals) Govt. of India. The authors have obtained written informed consent to use the animals in the study from the owner of the animals. An ARRIVE checklist has been attached as a separate file regarding the ethics approval and consent to participate.

Consent for publication

The authors have taken consent for publication from all those are involved in the study directly or indirectly.

Competing interests

There is no conflict of interest among the authors.

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