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Original Article

Effect of *Elettaria cardamomum* hydroethanolic extract on learning and memory in Scopolamine induced amnesia

Teena Kunwar*, Neeraj Kumar, Preeti Kothiyal

Shri Guru Ram Rai Institute of Technology and Science, Uttarakhand, India

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ABSTRACT

Elettaria cardamomum, commonly known as green cardamom in traditional system of medicine has antibacterial, antioxidant, antiasthmatics and digestive properties. The present study was designed to assess the effect of hydroethanolic extract of *Elettaria cardamomum* fruit on learning and memory, brain cholinesterase levels and associated altered brain oxidative stress markers in scopolamine treated mice. The extract was administered orally in three doses (250, 500 and 1000 mg/kg p.o) for a period of 15 days. Piracetam, 500mg/kg p.o, was used as standard treatment. Scopolamine was administered in the dose of 1.0 mg/kg intraperitoneally. The Morris water maze and elevated plus maze were used to assess cognitive functions. At the end of the study, effect of extract was assessed on brain cholinesterase levels and oxidative stress markers like lipid peroxidation, reduced glutathione, catalase and superoxide dismutase in the brain tissue of mice. The Scopolamine-treated group (negative control) showed significantly impaired acquisition and retention of memory as compared to the saline treated group (vehicle control). Pre-treatment with *Elettaria cardamomum* extract (500 and 1000 mg/kg) for 15 days significantly reversed Scopolamine induced amnesia as evidenced by increased time spent in target quadrant in Morris water maze test and decreased transfer latency in elevated plus maze test compared to the negative control. Scopolamine administration caused significant increase in brain cholinesterase levels which were attenuated by *Elettaria cardamomum* treatment. Pre-treatment with *Elettaria cardamomum* extract (500 and 1000 mg/kg p.o.) resulted in a significant decrease in lipid peroxidation and increase in reduced glutathione, catalase and superoxide dismutase levels as compared to the negative control. These results suggest that *Elettaria cardamomum* may improve learning and memory of amnesic mice and this effect can be attributed to decreased oxidative stress and reduction in brain cholinesterase levels.

Keywords: Alzheimer's disease, Amnesia, Elevated plus maze, Morris water maze, Lipid peroxidation, Catalase, Glutathione, Superoxide dismutase

INTRODUCTION

Memory is the ability of an individual to record sensory stimuli, events, information, etc., retain them over short or long periods of time and recall the same at a later date when needed. Poor memory, lower retention and slow recall are common problems in today's stressful and competitive world.[1] Age, stress and emotions are conditions that may lead to memory loss, amnesia, anxiety, high blood pressure, dementia, to more ominous threat like schizophrenia and Alzheimer's disease (AD) wherein the person is not able to make full use of his or her potentials.[2] As per the estimation of World Health Organization, globally, 5% of men and 6% of women above 60 years of age are suffering from Alzheimer's type of dementia. According to Alzheimer's disease international, in 2010, 35.6 million people were living with dementia worldwide. This figure would increase to 65.7 million by 2030 and 115.4 million by 2050. Therefore it is imperative to curb the progress of cognitive decline before it crosses the threshold to dementia.[3]

Ayurveda is full of evidence regarding use of medicinal plants in cognitive decline. The drugs are either used alone or in poly herbal formulations. *Elettaria cardamomum* (EC) seed extract is one of the ingredients of the polyherbal formulation, Abana (Himalaya Drugs), which is used as a memory enhancing remedy in dementia.[4] Moreover, EC has been proven to have anticholinesterase activity in vitro.[5] It is also a

***Corresponding Author Address: Teena Kunwar,** Shri Guru Ram Rai Institute of Technology and Science, Uttarakhand, India; E-mail: teenakunwar@gmail.com*

rich source of flavonoids[6], which are known for their antioxidant potential and beneficial effect on memory and learning[7]. The present study was designed to investigate the effect of EC hydroethanolic extract on learning and memory in Scopolamine (SCOP) induced amnesia and to evaluate its effect on brain acetylcholinesterase (AChE) activity and antioxidant defence by assessment of lipid peroxidation (LPO), catalase (CAT), superoxide dismutase (SOD) and reduced glutathione (GSH) levels.

MATERIALS AND METHODS

Plant collection & extraction: The fruits of EC were collected from the local market of Dehradun, Uttarakhand. The authenticity of EC was confirmed at Botanical survey of India, Dehradun. Fresh samples of the plant fruits were air dried at ambient room temperature and powdered in a grinder. One gram of sample was weighed and extracted with 90% ethanol. The sample was boiled for 15-20 minutes and cooled. The samples were then filtered using muslin cloth and the filtrate was collected and stored in refrigerator. The percentage yield from the 90% hydroalcoholic solvent was obtained.[5] The phytochemical screening of the extract was performed as per the standard methods.[8] Acute oral toxicity of the extract was determined in accordance with OECD 423 guidelines.[9]

Animals: Swiss Albino Mice (20–30g) of either sex, three to four months of age were procured from the Departmental Animal House of SGRRITS Dehradun, (Uttarakhand) India. Animals were housed in an air-conditioned animal room at 23 ± 2°C with 12/12 h light/ dark photo period, free access to water and standard laboratory chow. The animals were acclimatized to the laboratory conditions for at least seven days prior to the behavioural experiments. The experiments were carried out between 0900 h and 1800 h.

The care of laboratory animals and all the procedures involving animals were performed in strict accordance with the guidelines of Committee for the Purpose of Control & Supervision of Experiments on Animals (CPCSEA), Ministry of Forests and Environment, Government of India. The Institutional Animal Ethics Committee (IAEC) approved the experimental protocol prior to the commencement of experiments.

Experimental Induction of Amnesia: Each animal was dosed for 15 days as per the stated treatment. On the day of the retrieval trial, all animals were subjected to SCOP (1 mg/kg i.p.) 60 min after the drug administration, except the vehicle control. The transfer latency (TL) in elevated-plus maze (EPM) and time spent in target quadrant (TSTQ) in Morris water maze (MWM) was evaluated 45 min after the SCOP administration.

Experimental protocol: The animals were randomly divided into 12 groups with each group comprising 6 animals. The treatment profile of the groups was as follows:

Groups for Elevated-Plus Maze

- Group 1: Vehicle (Normal Saline 10ml/kg p.o) treated
- Group 2: Vehicle (Normal Saline 10ml/kg p.o) + Scopolamine (1mg/kg i.p.) treated
- Group 3: Piracetam (500mg/kg p.o) + Scopolamine (1mg/kg i.p.) treated
- Group 4: EC Extract (250mg/kg p.o) + Scopolamine (1mg/kg i.p.) treated
- Group 5: EC Extract (500mg/kg p.o) + Scopolamine (1mg/kg i.p.) treated
- Group 6: EC Extract (1000mg/kg p.o) + Scopolamine (1mg/kg i.p.) treated

Groups for Morris Water Maze

- Group 7: Vehicle (Normal Saline 10ml/kg p.o) treated
- Group 8: Vehicle (Normal Saline 10ml/kg p.o) + Scopolamine (1mg/kg i.p.) treated
- Group 9: Piracetam (500mg/kg p.o) + Scopolamine (1mg/kg i.p.) treated
- Group 10: EC Extract (250mg/kg p.o) + Scopolamine (1mg/kg i.p.) treated
- Group 11: EC Extract (500mg/kg p.o) + Scopolamine (1mg/kg i.p.) treated
- Group 12: EC Extract (1000mg/kg p.o) + Scopolamine (1mg/kg i.p.) treated

Behavioural models for evaluation of learning & memory:

Elevated Plus Maze: The elevated plus maze served as the exteroceptive behavioural model to evaluate the learning and memory in mice. TL i.e. the time taken by the animal to move into any one of the covered arms with all its four legs was recorded on the first day. If the mouse did not enter into one of the covered arms within 90s, it was gently pushed into one of the two covered arms and the TL was assigned as 90s. The mouse was allowed to explore the maze for 10s and then was returned to its home cage. Memory retention was examined 24 h after the first day trial on the second day. TL measured on plus maze on first day served as an index of learning and acquisition, whereas TL on 2nd day served as an index of retrieval and memory.[10]

Morris Water Maze: Morris water maze was employed to assess learning and memory of the animal. It is a swimming based model where the animal learns to escape on to a hidden platform. Acquisition trials comprised of four consecutive trials on each day with an interval of five minutes,

during which each animal was allowed to escape on to the hidden platform and was allowed to remain there for 20 seconds. In case the animal was unable to locate the hidden platform within 90 seconds, it was gently guided by hand to the platform and allowed to remain there for 20 seconds. Escape latency time (ELT) i.e. the time taken to locate the hidden platform in water maze, was noted as an index of acquisition and learning. In preliminary study, trial was conducted to familiarize the mouse with the task and was not counted. Mouse was subjected to acquisition trials for four consecutive days. Retrieval trial was conducted on the 5^{th} day. During retrieval trial platform was removed and each mouse was allowed to explore the pool for 90 seconds. Mean time spent by the mouse in each of four quadrants was noted. The mean time spent by the mouse in target quadrant (Q4) for searching the hidden platform was noted as an index of retrieval. The experimenter always stood at the same position. Care was taken that relative location of water maze with respect to other objects in the laboratory, was not disturbed during the total duration of study.[11]

Biochemical parameters for evaluation of learning and memory: Animals were sacrificed by cervical dislocation; brains were removed and homogenized in the suitable buffer. The homogenates were then centrifuged at 3000rpm for 15 min. The supernatant was collected and used for the following biochemical measures:

- *Estimation of brain cholinesterase activity:* Brain cholinesterase activity was estimated by the method of Ellman GF *et al*. In this method, esterase activity is measured by providing an artificial substrate, acetylthiocholine (ATC). Thiocholine released due to the cleavage of ATC by AChE is allowed to react with the –SH reagent, 5,5'-dithiobis-(2-nitrobenzoic acid) (DTNB), which is reduced to thionitrobenzoic acid, a yellow coloured anion with an absorption maxima at 412nm. The concentration of thionitrobenzoic acid, detected using a UV spectrophotometer, is taken as a direct estimate of the AChE activity. [12]
- *Estimation of lipid peroxidation:* LPO was estimated by the method of Ohkawa *et al.* The method estimates Malondialdehyde (MDA), a product of LPO. One molecule of MDA reacts with two molecules of Thiobarbituric acid (TBA) under mildly acidic conditions to form a pink color chromogen, whose intensity is measured in spectrophotometer at 535nm.[13]
- *Estimation of catalase:* CAT activity was measured by the method of Aebi. CAT exerts a dual function, because it catalyses the following reaction:

Catalase

$2H_2O_2 \xrightarrow{\text{----------}} 2H_2O + O_2$

$ROOH+AH_2 \xrightarrow{\text{----------}} H_2O + ROH + A$

In the UV range, hydrogen peroxide shows a continued increase in absorption with decreasing wavelength. The decomposition of hydrogen peroxide can be followed directly by the decrease in absorbance at 240nm. The difference in the absorbance per unit time serves as a measure of the CAT activity.[14]

- *Estimation of superoxide dismutase:* The SOD activity in supernatant was measured by the method of Misra and Fridovich. SOD is a metalloprotein and is the first enzyme involved in the antioxidant defence against reactive oxygen species. SOD scavenges the superoxide ions produced as cellular byproducts. It has the ability to inhibit autooxidation of epinephrine to adenochrome at pH 10.2. This inhibition can be measured with spectrophotometer at 480nm. [15]
- *Estimation of reduced glutathione:* GSH was estimated by the method of Ellman GL. GSH is a major non-protein thiol and an endogenous antioxidant that counter balances free radical mediated damage. It protects the normal cell structure and function by maintaining redox homeostasis, quenching free radicals and by participating in detoxification reaction. Glutathione consists of sulhydryl groups. DTNB, a disulphide compound gets easily attacked by tissue sulfydryl groups and forms a yellow coloured anion which is measured with spectrophotometer at 412nm.[16]

Statistical Analysis: The statistical analysis was carried out using Prism Graph Pad 6.0 Software. All values were presented as Mean $\pm$ SEM. The statistical significance of difference between means was calculated by one way Analysis of Variance (ANOVA), followed by Tukey`s multiple comparison test in all behavioural and biochemical evaluations except for escape latency in Morris water maze where 2 way ANOVA was used followed by Bonferroni's posttests. Difference level at $P<0.05$ was considered as statistically significant.

RESULTS

Physical Characteristics & Percentage Yield of EC Extract: The yield and physical characteristics of hydro-ethanolic extract of EC fruits are described in Table 1.

Preliminary phytochemical screening of EC Extract: The phytochemical analysis of the EC extract was performed as per standard tests.[8] The results of the tests are given in Table 2.

Acute Oral Toxicity Study: Hydroethanolic extract of EC fruits was evaluated for acute oral toxicity as per OECD 423 guidelines. The observations of various evaluation parameters are enumerated in Table 3.

On the basis of acute oral toxicity study, hydroethanolic extract of EC was deemed to be extremely safe even at high dose of 5000mg/kg. The EC extract doses selected for the present study were 250mg/kg (EC250), 500mg/kg (EC500) and 1000mg/kg (EC1000).

Behavioural Evaluation

- *Effect of EC extract on Transfer Latency of scopolamine treated mice in elevated plus maze:* Administration of EC extract (250mg, 500mg, and 1000mg p.o.) significantly ($p<0.001$) attenuated scopolamine induced rise in TL (Figure 1) as compared to negative control group (NS+SCOP) indicating protection from scopolamine induced learning and memory impairment. The effects of EC500 and EC1000 were observed to be comparable to standard treatment of PIR while effects of EC250 were significantly ($p<0.05$) lesser than that of all the other treated groups. There was no significant difference between EC500 and EC1000.
- *Effect of EC extract on Escape Latency and Time spent in target quadrant of scopolamine treated mice in Morris water maze:* Administration of EC extract (250mg, 500mg, and 1000mg p.o.) significantly enhanced learning during acquisition trials and resulted in decrease in ELT by day 4 (Figure 2). Further, it significantly ($p<0.001$) increased TSTQ (Figure 3) as compared to NS+SCOP indicating protection from scopolamine induced learning and memory impairment. The effects of EC500 and EC1000 were observed to be on par with the standard treatment of PIR while effects of EC250 were significantly ($p<0.01$) lesser than that of all the other treated groups. There was no significant difference between EC500 and EC1000.

Biochemical Estimation

- *Effect of EC extract on brain AchE activity of scopolamine treated mice:* Scopolamine produced a significant ($p<0.001$) increase in brain AChE activity in comparison with vehicle control (Figure 4). However, treatment with EC extract (250mg, 500mg and 1000mg p.o.) significantly ($p<0.001$) inhibited the scopolamine induced rise in brain AChE activity as compared to NS+SCOP. All doses of EC extract produced significantly ($p<0.01$) better results than the standard treatment of PIR which did not have any beneficial effect on AChE levels.
- *Effect of EC extract on LPO activity of scopolamine treated mice:* Scopolamine produced a significant ($p<0.001$) increase in brain oxidative stress as determined by LPO levels in comparison with vehicle control (Figure 5). However, pre-treatment with EC extract (250mg, 500mg and 1000mg p.o.) significantly ($p<0.001$) inhibited the scopolamine induced rise in brain oxidative stress. The effects of EC500 and EC1000 were observed to be on par with the standard treatment of PIR.
- *Effect of EC extract on CAT activity of scopolamine treated mice:* Scopolamine produced a significant ($p<0.001$) decrease in CAT levels in comparison with vehicle control (Figure 6). However, pre-treatment with EC extract (250mg, 500mg and 1000mg p.o.) significantly ($p<0.001$) protected the brain from scopolamine induced decrease in brain CAT levels. The effects of EC250 were observed to be on par with the standard treatment of PIR while that of EC500 and EC1000 were significantly better than both EC250 ($p<0.01$) and PIR ($p<0.001$) treated group.
- *Effect of EC extract on SOD levels of scopolamine treated mice:* Scopolamine produced a significant ($p<0.001$) increase in brain oxidative stress as determined by LPO levels in comparison with vehicle control (Figure 7). However, pre-treatment with EC extract (250mg, 500mg and 1000mg p.o.) significantly ($p<0.001$) inhibited the scopolamine induced rise in brain oxidative stress. The effects of EC500 and EC1000 were observed to be on par with the standard treatment of PIR.
- *Effect of EC extract on GSH levels of scopolamine treated mice:* Scopolamine produced a significant ($p<0.001$) decrease in GSH levels in comparison with vehicle control (Figure 8). However, pre-treatment with EC extract (250mg, 500mg and 1000mg p.o.) significantly ($p<0.01$) protected the brain from scopolamine induced decrease in brain GSH levels. Although the standard treatment of PIR also significantly ($p<0.001$) attenuated the effects of scopolamine on GSH, the effect of EC500 and EC1000 was significantly better than PIR treated group ($p<0.01$) and EC250 ($p<0.001$).

DISCUSSION

Two widely accepted behavioural models for assessment of learning and memory i.e. Morris water maze and elevated plus maze were used in

the present study. Scopolamine interferes with memory and cognitive function in both humans and rodents by blocking muscarinic receptor in the brain. [17] In the present study scopolamine administration resulted in decrease in TSTQ in Morris water maze and increase in transfer latency in Elevated plus maze thereby demonstrating failure to recall the skills acquired during acquisition trials. Meanwhile, PIR and EC extract treated animals exhibited well-formed memory which, though not equivalent to normal saline (NS) treated group, was still better than NS+SCOP treated group. Medium and high dose of EC extract (500mg, 1000mg p.o.) produced significant improvement in learning and memory which was equivalent to PIR treated group. No significant difference was found between medium and high dose of EC extract. Thus, the effects of EC extract increased in a dose dependent manner which reached their peak at medium dose after which no significant benefit was observed by giving a higher dose of EC extract making EC500 the most beneficial dose of EC extract in improving learning and memory.

To assess the mechanism behind the effectiveness of EC extract on learning and memory, its effect on brain AchE as well as its antioxidant potential were evaluated. It is a well-known fact that cholinergic neuronal system plays an important role in the cognitive deficits associated with AD.[18] Dysfunction of the cholinergic system is implicated in major neurological disorders. EC extract significantly decreased AchE activity in amnesic mice, indicating its potential in attenuating the severity of diseases associated with impairment in learning and memory.

Several epidemiological studies suggest that inclusion of antioxidant rich foods in diet is helpful in improving cognitive performance in humans. [19] Furthermore, dietary intake of flavonoids has been inversely related to the risk of dementia. [20] LPO plays a major role in oxidative stress. [21] It has been reported that MDA levels are generally higher in AD. In present study, EC extract produced significant decrease in levels of brain LPO as compared to NS+SCOP indicating reduction in oxidative stress which may be associated with improvement in memory and learning observed in behavioural experiments. Further, levels of three endogenous antioxidants CAT, SOD and GSH were assessed in brain. All three were found to increase in EC extract treated animals as compared to NS+SCOP, thus alluding to the role of EC extract's antioxidant potential in the improvement of learning and memory.

CONCLUSION

The purpose of present study was to evaluate the effect of *Elettaria cardamomum* hydroethanolic extract on learning and memory in Scopolamine induced amnesia and to find the possible mechanism of action involved in its effect on learning and memory.

From the above results and discussion it can be concluded that:

- *Elettaria cardamomum* shows promise as a natural memory booster in scopolamine induced amnesia.
- The effect of EC extract increases in a dose dependent manner with medium dose of EC extract (500mg/kg) producing most beneficial effects on memory and learning.
- The beneficial effect of EC extract maybe attributed to its anti-cholinesterase and anti-oxidant activity with significant rise in the levels of endogenous antioxidants like Catalase, Glutathione and Superoxide dismutase.
- Thus, the present study supports the concept that onset of neurodegenerative disease may be delayed or mitigated with the use of dietary polyphenols that protects against oxidative stress and neurodegeneration.

In the light of the above, it may be worthwhile to explore the potential of this plant in the management of cognitive dysfunction.

Competing interests: The author(s) declare that they have no competing interests.

Tables 1: Physical Characteristics & Percentage Yield of EC Extract

Extract	Colour	Odour	% Extractive value
90% hydroethanolic	Dark Green	Characteristic	5.7%

Table 2: Results of Preliminary phytochemical screening of EC Extract

Phyto-constituents	Test	Presence (+)/ Absence (-) in EC extract
Alkaloids	Wagner's Test	+
Carbohydrates	Benedict's test	+
Glycosides	Borntrager's Test	+
Saponins	Froth Test	-
Phytosterols	Salkowski's Test	+
Fixed oils & fats	Stain Test	+
Resins	Acetone-water Test	+
Phenols	Ferric Chloride Test	+
Tannins	Gelatin Test	-
Flavonoids	Alkaline Reagent	+
Proteins	Xanthoproteic Test	+
Amino acids	Ninhydrin test	+

Table 3: Observations in Acute Toxicity Study

Evaluation Parameters	Monitoring Time Period	EC 300mg/kg	EC 2000mg/kg	EC 5000mg/kg
Grooming	First 30 min	+	+	+
	Periodically for 4 hrs	+	+	+
	After 24 hrs	+	+	+
	For 14 days	+	+	+
Hyperactivity	First 30 min	-	-	-
	Periodically for 4 hrs	-	-	-
	After 24 hrs	-	-	-
	For 14 days	-	-	-
Sedation	First 30 min	-	-	-
	Periodically for 4 hrs	-	-	-
	After 24 hrs	-	-	-
	For 14 days	-	-	-
Respiratory Arrest	First 30 min	-	-	-
	Periodically for 4 hrs	-	-	-
	After 24 hrs	-	-	-
	For 14 days	-	-	-
Convulsions	First 30 min	-	-	-
	Periodically for 4 hrs	-	-	-
	After 24 hrs	-	-	-
	For 14 days	-	-	-
Increased Motor Activity	First 30 min	-	-	-
	Periodically for 4 hrs	-	-	-
	After 24 hrs	-	-	-
	For 14 days	-	-	-
Decreased Motor Activity	First 30 min	+	+	+
	Periodically for 4 hrs	-	-	-
	After 24 hrs	-	-	-
	For 14 days	-	-	-
Death	First 30 min	-	-	-
	Periodically for 4 hrs	-	-	-
	After 24 hrs	-	-	-
	For 14 days	-	-	-
Body Wt. Changes	During 14 days	-	-	-
(+) Presence of Activity, (-) Absence of Activity				

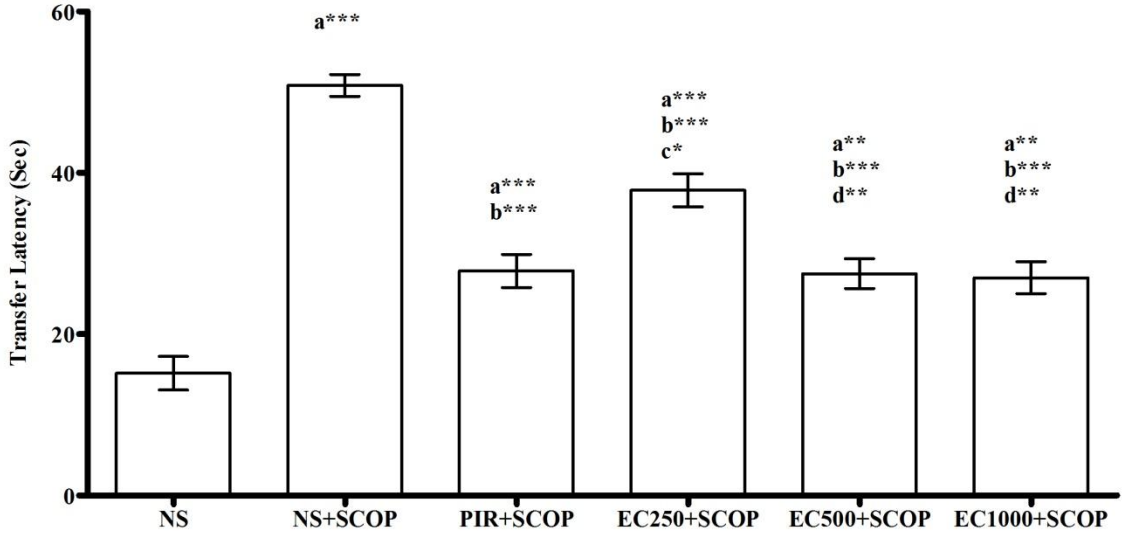

Figure 1 Effect of EC extract on Transfer Latency of scopolamine treated mice in Elevated Plus Maze. 'a' indicates significance versus vehicle control(NS), 'b' indicates significance versus negative control, 'c' indicates significance versus PIR treated group, 'd' indicates significance versus EC250 treated group. *represents $p<0.05$, ** represents $p<0.01$, *** represents $p<0.001$.Values are expressed as Mean±SEM at n=6.

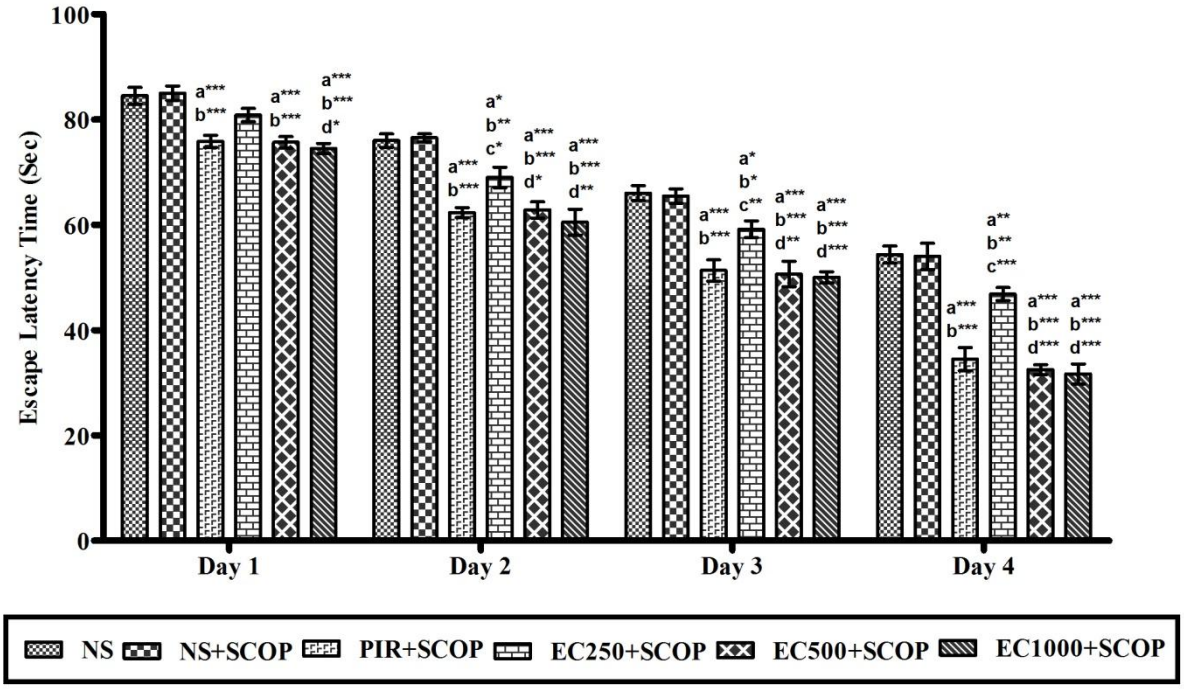

Figure 2 Effect of EC extract on Escape Latency Time in Morris water maze.'a' indicates significance versus vehicle control(NS), 'b' indicates significance versus negative control, 'c' indicates significance versus PIR treated group, 'd' indicates significance versus EC250 treated group. *represents $p<0.05$, ** represents $p<0.01$, *** represents $p<0.001$.Values are expressed as Mean±SEM at n=6.

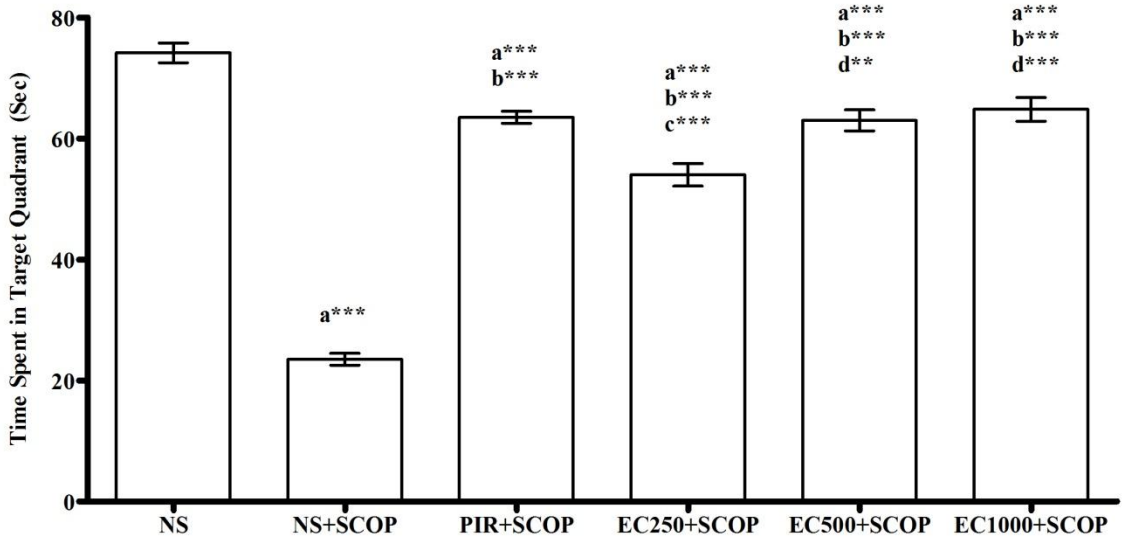

Figure 3 Effect of EC extract on Time spent in target quadrant in scopolamine treated mice in Morris water maze. 'a' indicates significance versus vehicle control(NS), 'b' indicates significance versus negative control, 'c' indicates significance versus PIR treated group, 'd' indicates significance versus EC250 treated group. ** represents $p<0.01$, *** represents $p<0.001$.Values are expressed as Mean±SEM at n=6.

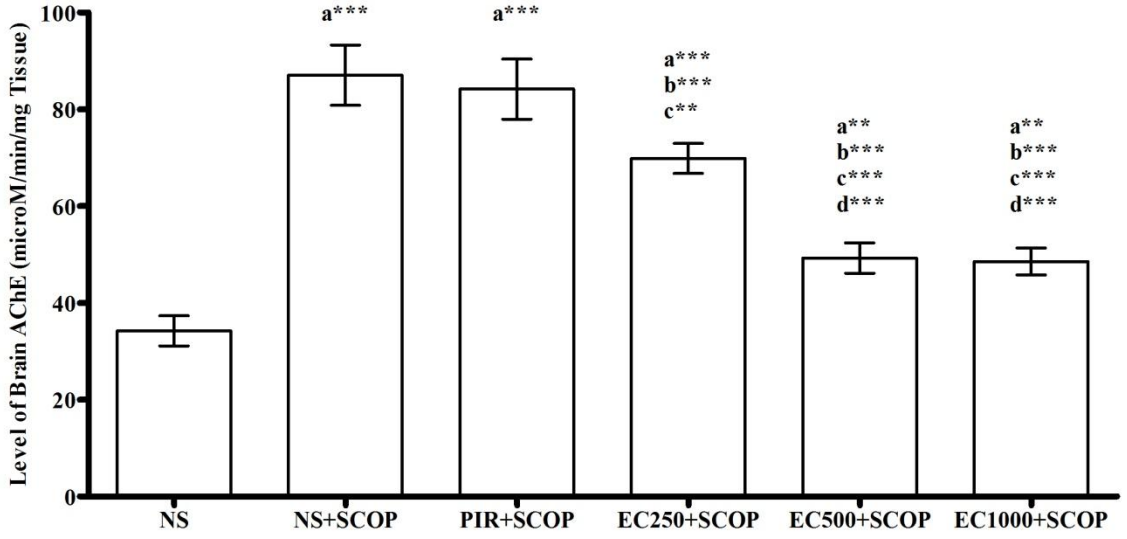

Figure 4 Effect of EC extract on levels of brain cholinesterase levels in scopolamine treated mice. 'a' indicates significance versus vehicle control(NS), 'b' indicates significance versus negative control, 'c' indicates significance versus PIR treated group, 'd' indicates significance versus EC250 treated group. ** represents $p<0.01$, *** represents $p<0.001$.Values are expressed as Mean±SEM at n=6.

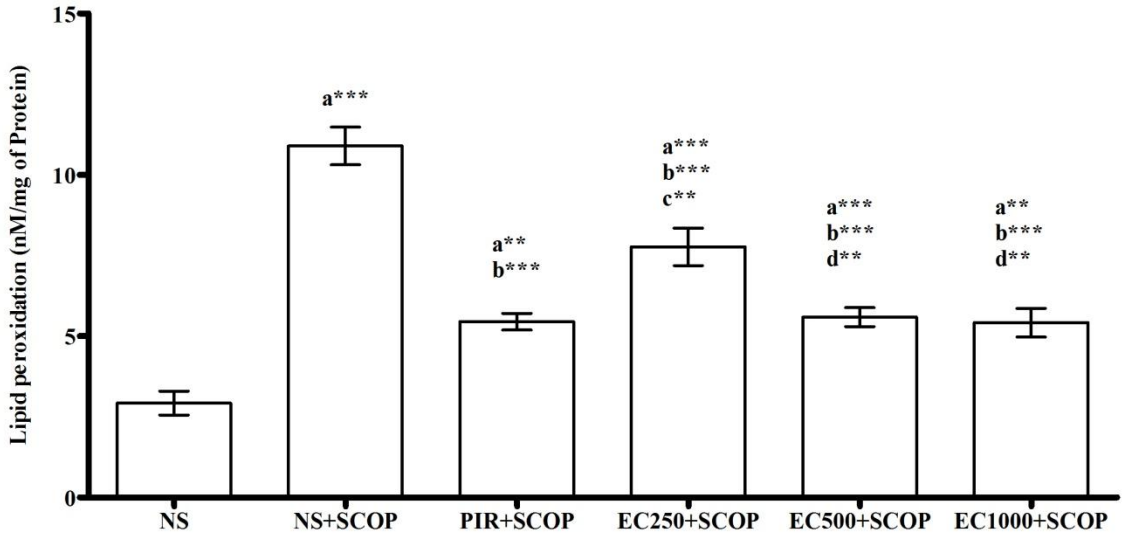

Figure 5 Effect of EC extract on levels of LPO levels in scopolamine treated mice. 'a' indicates significance versus vehicle control(NS), 'b' indicates significance versus negative control, 'c' indicates significance versus PIR treated group, 'd' indicates significance versus EC250 treated group. ** represents p<0.01, *** represents p<0.001.Values are expressed as Mean±SEM at n=6.

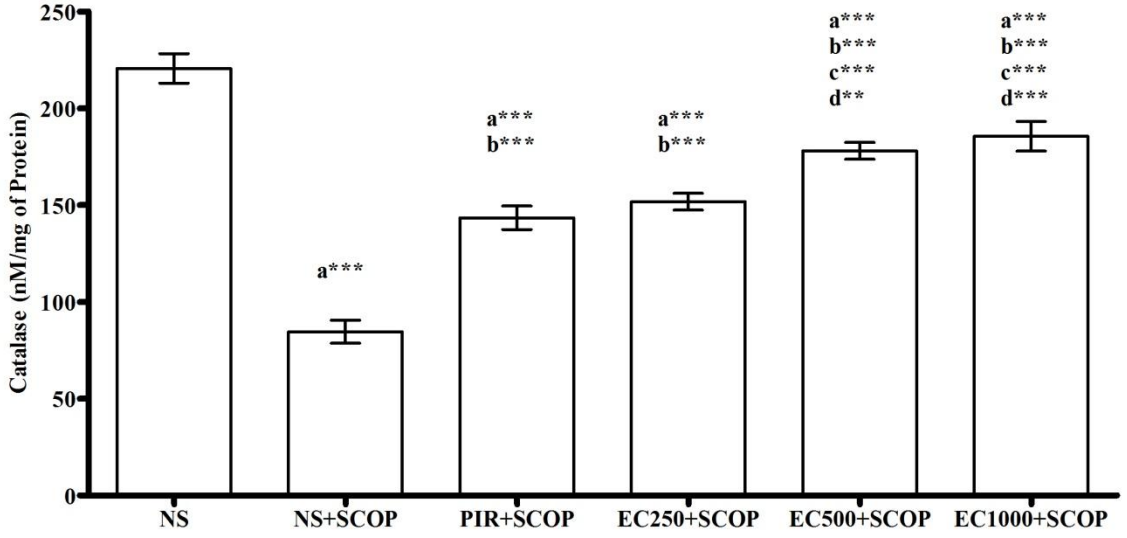

Figure 6 Effect of EC extract on levels of CAT levels in scopolamine treated mice. 'a' indicates significance versus vehicle control(NS), 'b' indicates significance versus negative control, 'c' indicates significance versus PIR treated group, 'd' indicates significance versus EC250 treated group. ** represents p<0.01, *** represents p<0.001.Values are expressed as Mean±SEM at n=6.

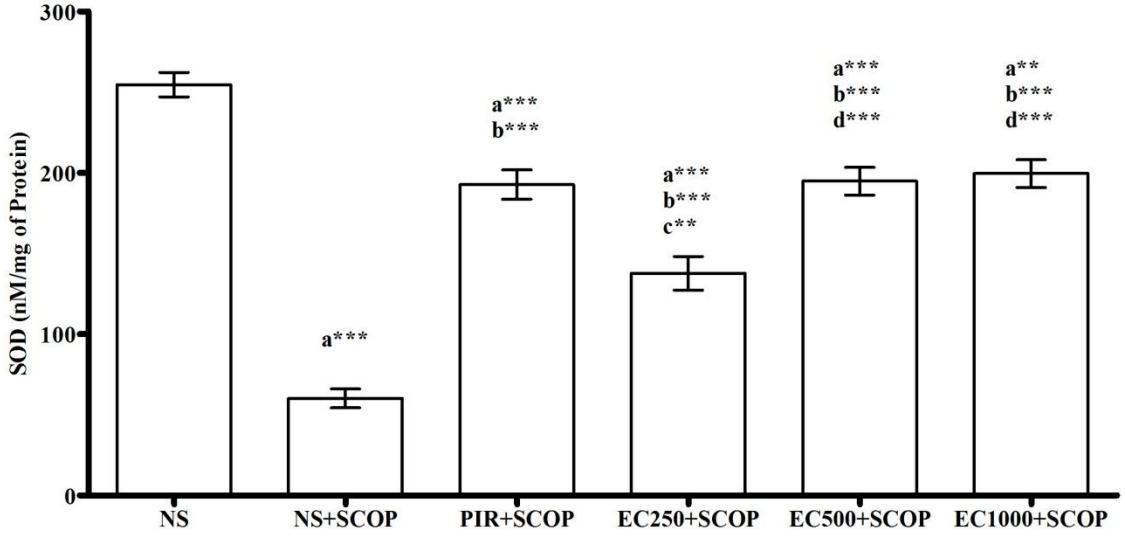

Figure 7 Effect of EC extract on levels of SOD levels in scopolamine treated mice. 'a' indicates significance versus vehicle control(NS), 'b' indicates significance versus negative control, 'c' indicates significance versus PIR treated group, 'd' indicates significance versus EC250 treated group. ** represents p<0.01, *** represents p<0.001.Values are expressed as Mean±SEM at n=6.

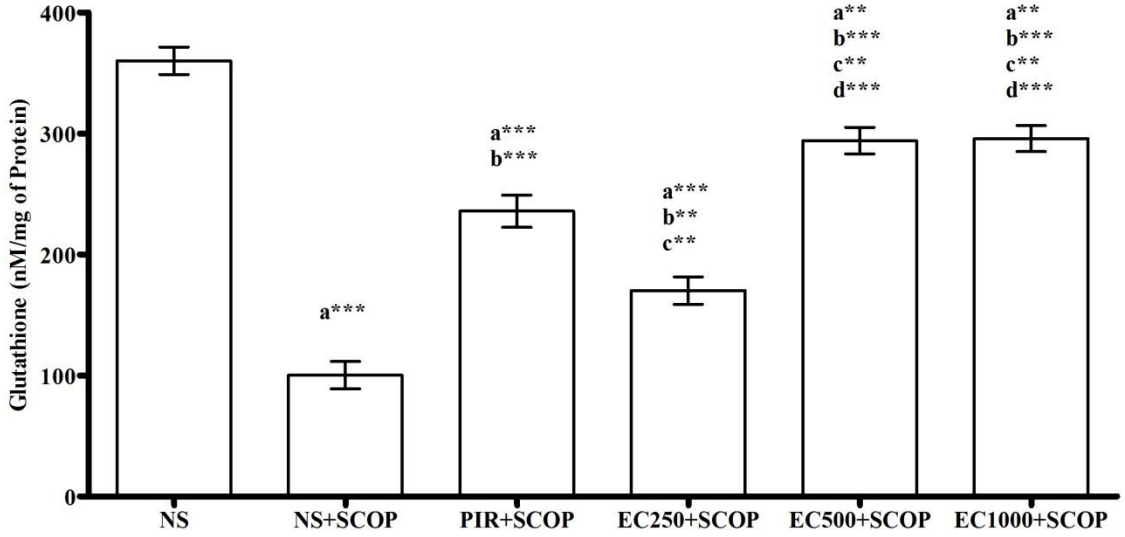

Figure 8 Effect of EC extract on levels of GSH levels in scopolamine treated mice. 'a' indicates significance versus vehicle control(NS), 'b' indicates significance versus negative control, 'c' indicates significance versus PIR treated group, 'd' indicates significance versus EC250 treated group. ** represents p<0.01, *** represents p<0.001.Values are expressed as Mean±SEM at n=6.

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