Vol 3|Issue 1| 2013 | 1-10.

e-ISSN 2248 - 9169
Print ISSN 2248 - 9150

International Journal of
Experimental Pharmacology

www.ijepjournal.com

INVESTIGATION OF NEUROPROTECTIVE EFFECTS OF *CONYZA CANADENSIS* ETHANOLIC EXTRACT ON SCOPOLAMINE-INDUCED COGNITIVE IMPAIRMENT AND OXIDATIVE STRESS IN SWISS MICE

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ABSTRACT

Alzheimer's disease (AD) is a progressive neurodegenerative disorder characterized by cognitive and memory deterioration associated with shrinkage of brain tissue, with localized loss of neurons mainly in the hippocampus and basal forebrain, with central cholinergic loss neurotransmitter-acetylcholine and also reported to be associated with accumulation of β amyloid plaques leading to oxidative stress and inflammation**.** The current study aims at the measurement of the certain biochemical parameters such as concentrations of red blood cell choline, glycerophosphocholine (GroPCho), phosphocholine (PCho) and lipid-bound choline in blood and AchE estimation in the brain homogenate .The Behavioural tests such as Rectangular maze test, Morris Water maze test and Locomotor activity were also conducted to evaluate the learning and memory parameters. Red blood cell choline in the *Conyza canadensis* Extract (CCE) treated group(18.7±2.3 nmol/ml) was significantly lower than in the Scopolamine treated group(41.4± 9.2 nmol/ml) ($p<0.023$). The Red blood cell lipid-bound choline (1387 ±58 nmol/ml) in the Disease control(Scopolamine) group was significantly lower ($p<0.0022$) than in the CCE treated group (1667±65). It was noticed that there were no significant differences in PCho, GroPCho, Plasma choline and lipid-bound choline between the groups. CCE treatment significantly inhibited the brain AchE level (26.91±8.10) compared to the scopolamine treated group (9.5±1.87). The CCE treated group has shown significant ($P < 0.05$) decrease in transfer latency when compared to disease control group in Rectangular maze test and Morris water maze test. On detection of locomotor activity using actophotometer, the CCE treated mice showed significant transfer latency on 7th day (168.29±14.6) when compared to the Scopolamine treatment group (135.78± 14.9). The above results indicate memory enhancing capacity of the *Conyza canadensis* ethanolic herbal extract.

Keywords: Phosphatidylcholine, Choline(Cho), Red blood cell, *Conyza canadensis*, Alzheimer's disease (AD), Scopolamine, Morris water maze.

INTRODUCTION

Alzheimer's disease (AD) is a progressive neurodegenerative brain disorder that is characterized by the presence of excessive amounts of neuritic plaques containing amyloid β protein and abnormal tau protein filaments in the form of neurofibrillary tangles. Loss of cholinergic cells, particularly in the basal forebrain, is accompanied by loss of the neurotransmitter acetylcholine [1]. A decrease in acetyl choline in the brain of patients

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with AD appears to be a critical element in producing dementia [2]. Scopolamine, a muscarinic cholinergic receptor antagonist, has been widely adopted to study cognitive deficits in experimental animals. After intraperitoneal (i.p.) injection of scopolamine, the cholinergic neurotransmission was blocked, leading to cholinergic dysfunction and impaired cognition in rodents [3]. Recently, it has been reported that memory impairment induced by scopolamine in rats is associated with altered brain oxidative stress status [4]. Therefore, rats with scopolamine-induced memory deficits were used as an animal model for screening antidementia drugs [5]. Choline (Cho), a major dietary nutrient is utilized for the synthesis of acetylcholine (ACh) in cholinergic cells and is an important component of the cell membrane. The levels of venous Cho leaving the brain is greater than arterial Cho suggesting that there is an overall loss of free Cho from the central nervous system (CNS) [6] and that Cho is actively transported out of the brain [7]. Cellular uptake of Cho is the rate-limiting step for ACh synthesis and a deficiency of Cho could lead to compensatory breakdown of PtdCho, a necessary component of the cell membrane.Aging is associated with extensive loss of the long cholinergic projections from the septum and the basal forebrain [8]. In AD this loss is drastically increased and there is a severe depletion of cholinergic cells in AD [9]. The net transport of Cho out of the brain, and the dual role of Cho for ACh and membrane synthesis have led Blusztajn and Wurtman and Jenden to postulate that functional deficiency of Cho might be due to abnormality in the absorption or transport of Cho in the cholinergic cells.The catabolism of lipid-bound Cho in membrane results in a net loss of Cho from the cell, possibly leading to premature death. In earlier clinical studies ,a small sample of patients with AD compared to neurologic controls (primarily patients with multi- infarct dementia),it was found that Cho was elevated in the red blood (RBC) of over 50% of patients with AD [10]. High RBC Cho with AD had been previously reported by others while some found no difference between AD and control populations [11]. Ethanolic extract of *Conyza canadensis* herb (commonly known as Canadian fleabane, Coltstail, Horseweed) is choosen to detect its potential as an Antiamnesic with Antiacetylcholinesterase activity. The ethanolic extract of whole herb *Conyza canadensis* exhibit a significant anti-inflammatory effects on rats[12].The reported activities of this herb which made to carry out this study are Antistress,Anxiolytic, CNS stimulant, Hypocholesterolemic, Antioxidant, COX-1 Inhibitor and Immunomodulator activity[13] .In our study, Group of mice under Scopolamine treatment had elevated RBC Cho because of decreased transport of Cho with approximately a ten-fold difference in comparision with *Conyza canadensis* Extract(CCE) treated group .The assumed mechanism for the elevation of RBC Cho was degradation of membrane phospholipids,defective outward transport leading to accumulation of Cho within the cell [14]. In group of mice receiving CCE treatment it was found that lower Cho concentrations was because of renormalization of Cho transport. We now report the results of a study comparing RBC and plasma Cho in between the two groups.. In addition, In this study a comparision between RBC lipid-bound Cho (primarily PtdCho), the Cho-bound monoester phosphocholine (PCho) and diester glycerophosphocholine (GroPCho) in these groups.The other biochemical parameter assessed is AchE level determination. By employing Ellman method, acetylcholinesterase, a cholinergic marker was estimated in the whole brain homogenate. Behavioural tests such as Rectangular maze test, Morris water maze test and Locomotor activity were done on groups of mice to analyse the role of CCE in improving spatial memory.

MATERIALS AND METHODS

Animals

Swiss mice of male sex weighing 20–25 g were used in the this study. They had free access to food and *water ad libitum* and were maintained under standard laboratory conditions with alternating light and dark cycles of 12 h each. They were acclimatized to laboratory conditions before behavioral studies. All the readings were taken during the same time of the day i.e., between 10 am and 2 pm. The Institution Animals Ethics Committee (IAEC) has approved the experimental protocol, and care of animals is done as per guidelines of CPCSEA, Department of Animal Welfare, and Government of India [15].

Drugs

Scopolamine (Cadila Healthcare pvt. Ltd) and Donepezil (Alkem laboratories Ltd.) were purchased. The whole herb of *Conyza canadensis* is collected from the Thirupathi area located in Andhra Pradesh and duly certified by a famous botanist of Post doctorate fellow degree. Scopolamine and Donepezil were diluted with vehicle (0.1% CMC).

Experimental Design

The animals (n = 24) were divided into four different groups of 6 animals per each group. Scopolamine (1.4 mg/kg) as a disease inducer was administered to all groups through intraperitoneal (i.p) route after drugs administration to all the groups except control group. The same procedure was carried out for 9 days (as seen in Table 1).

Drug administration to various groups

Group-I	Control	Vehicle (0.1% CMC).
Group-II	Disease control	Scopolamine (1.4 mg/kg) i.p.
Group-III	Standard	Donepezil (5mg/kg) oral + Scopolamine (1.4 mg/kg) i.p.

Group-IV	Test	*Conyza canadensis* extract (500mg/kg) oral + Scopolamine (1.4 mg/kg) i.p.

Microwave assisted extraction of *Conyza canadensis* herb

Microwave assisted extraction is chosen for the higher yield of polyphenolic compounds,terpenoidal compounds,alkaloids from the herb in shorter duration. This Extraction procedure has high efficiency,less solvent volume and requires Shorter duration in comparision to Heatflux Extraction and Soxhlet extraction. In this study this system is used to extract phenolic compounds and sphingolipids using 37.5ml 0f 50%methanol in water as solvent under MAE conditions like 80^oC temperature,50%microwave power,30s irradiation time and 1:10w/v of solid/liquid ratio. The supernatant is collected, placed in 15ml glass centrifeuge tubes. CCE has to be stored at -70^0c prior to analysis.By Chromatographic techniques, the chemical compounds such as Harmine, D-limonene, Gallic acid,Apigenin,β sitosterol were found to be present in the extract.

Acute Toxicity Studies

Swiss albino mice are randomly distributed into 1control group and 4 treated groups, containing 6 animals per group. Test Group1, 2, 3, 4 were orally administered 100,500,1000,3000mg/kg Plant extract (CCE). The control group received vehicle alone, the animals observed for first 72hrs and 7days for any signs of behavioural changes, toxicity, mortality, changes in body weight. There were no signs of toxicity after 72hrs of observation and variation in organs weight is not prominent.The LD50 value in this study was found to be above 3000mg/kg.

Behavioural Tests

1. Rectangular Maze Test

Assessment of learning and memory can be effectively done using this method. The maze consists of completely closed rectangular box with an entry and reward chamber partitioned with wooden slats into blind passages leaving just twisting corridor leading from the entry to the reward chamber present in opposite ends.On the 1^{st}day as a training session all the mice were familiarized with rectangular maze for a period of 10 min for 2 h. Well-trained animals were choosen for the experiment.On the 3^{rd} day the mouse was placed in the entry chamber and the timer was activated as soon as the mouse leaves the entry chamber. The time taken for the mouse to reach the reward chamber was taken as the latency time. 4 readings are taken and average of reading gives learning score. Lower scores indicate efficient learning and higher scores indicates poor learning in animals.The time taken by the animals to move from the entry chamber to the reward chamber was noted on day 1, 3, 5, 7, and 9 [16].

2. Morris Water Maze Test

Morris water maze was used to assess learning and memory with several advantages over other models such as absence of motivational stimuli such as food and water deprivation, electrical stimulations, and buzzer sounds [17,18]. The Morris water maze is a circular tank (90 cm in diameter and 50 cm in height) filled with water at 22 ±1°C. All animals were habituated to the water maze by lowering each mice into the apparatus for 90 s without escape. First, animals were trained to locate the platform. Commencing on the2^{nd} day, mice were tested in the hidden-platform version of the task on seven consecutive days (three trials per day). The submerged platform (transparent perspex: 16 cm ×16 cm) was placed in the centre of one quadrant of the maze at 1.5 cm below water level. Mice were placed gently in the water maze at a start point in the mid of the rim of a quadrant without the platform. The starting points for the 3 trials of a mice is changed randomly so that the same start point was not used twice in a mice during a session. After reaching the platform, the mice has to stay on it for 30 s; the next trial is started 1 min following this 30s period. If an animal failed to find the platform in 90 s, it was placed on the platform again for 30 s. Parameters such as time needed to reach the platform, swim speed and swim distance were measured. The above 8days of trials are the training session. On the ninth day, the platform was removed and the animals were tested as described for the habituation session. The parameter assessed during this 90-s spatial probe was the time spent in each quadrant. On day 10, the animals were observed for three additional trials in the visible-platform version of the task. Mice were tested as described for the hidden-platform task, with the exception that the platform was made clearly visible and positioned 1.5 cm above water level in the quadrant opposite to its original location [19]. During these acquisition and retrieval sessions of the maze, the animals were tracked using a computer aided videotracking system (VideoMot, TSE, Germany) and their behaviour was analysed.

3. Locomotor activity

Most of the CNS drugs influence the locomotor activities in man & animals.The locomotor activity of herbal extract and drugs can be studied using actophotometer which operates on photoelectric cells connected in circuit with a counter, when the beam of light falling on photocell is cut off by the animal, then a count is recorded. Animals are placed individually in the activity cage and the activity was monitored for 10min. The test is done before 30 min and after the drug and in some groups *Conyza canadensis* extract administration. The photo cell count is noted and The decrease or increase in locomotor activity is calculated using the photo cell count .

Histopathological Studies

After 8-day treatment, the brains of different groups were perfusion-fixed with 4% paraformaldehyde in

0.1M phosphate buffer. The brains were removed and postfixed in the same fixative overnight at 48^{o}C. The brains were then routinely embedded in paraffin and stained with Hematoxylin-Eosin. The hippocampal lesions were assessed microscopically at 40 magnification [20].

Dissection and Homogenization

On day 9, after behavioral assessments, animals were scarified by cervical dislocation. The brains were removed. Each brain was separately put on ice and rinsed with ice-cold isotonic saline. A (10% w/v) homogenate was prepared in 0.1M phosphate buffer (pH 7.4). The homogenate was centrifuged at 3000 rpm for 15 minutes and aliquots of supernatant were separated and used for biochemical estimation .

Biochemical Tests

1. AchE Estimation

The cholinergic marker, acetylcholinesterase levels in the brain homogenate was estimated using Ellman method. Ellman's reagent is DTNB-5, 5_-dithiobis(2-nitrobenzoate). The homogenate was incubated for about 5 min with 2.7ml of $PO4^{2-}$ buffer and 0.1ml of DTNB. To this 0.1ml of freshly prepared acetylthiocholine iodide (pH 8) was added and the absorbance was read at 412nm [21, 22].

2. Determination Of Choline Levels

In this study a comparision was made between RBC Cho, GroPCho, PCho and PtdCho and plasma PtdCho and Cho levels in 33 mice receiving Scopolamine treatment compared to 26 age-matched mice receiving *Conyza canadensis* Extract(CCE) treatment. All these forms of choline levels were measured using a sensitive gas-chromatography-mass-spectrometry (GCMS) method [23].

a) Measurement of Red Blood Cell Choline

Packed RBC can be obtained when heparinized eppendroff tube containing blood is centrifuged at 3000rpm at 2°C for 10 min. Thus obtained RBC were washed with 4 volumes of saline and again recentrifuged. The packed RBC (0.1 ml) were treated with 1ml of 0.4 M HClO4 and 2 nmol Cho ,an internal standard (I.S.). The sample was mixed thoroughly and allowed to stand for 10 min.The suspensions were centrifuged at 12,000rpm for 10 minutes. Cho levels in the supernatant was measured by GCMS analysis . Because heat or freezing increases RBC Cho levels, all samples were immediately placed in ice and were measured afresh with in 10min. This short time interval will not significantly change RBC Cho .

b) Measurement of Phospholipid-Bound Choline

RBC (100 µl) were mixed with 4ml of 2:1 CHCL3/ CH3OH mixture and 0.4ml distilled water thoroughly and centrifuged at 480 rpm. The organic phase containing lipid-bound Cho (primarily sphingomyelin & PtdCho) and the aqueous phase containing free Cho, GroPCho and PCho were separated. To measure lipid-bound Cho, organic phase of 1 ml is taken and evaporated to dryness. To this 0.5ml of 6M HC1 was added and heated for 1 hour at 100°C to hydrolyze the lipid-bound Cho. To the above solution 0.285ml of 10 M NaOH , 40nmol of Cho as Internal standard and 1ml of 0.4 M HC1O4 (1 ml) were added and the supernatant obtained is subjected to GCMS analysis.

To measure GroPCho, 0.1 ml of aqueous phase was added to 0.1ml of 20 µM Cho and 0.1ml of 3 M HCI , heated at 100°C for 30 minutes and cooled to room temperature. To the above cooled mixture0.1ml of 4 M NaOH, 1 ml of 0.4 M HClO4 was added and allowed to stand for 5 min. After thorough mixing, the suspension was centrifuged at 12,000 rpm for 10 minutes. The supematant is subjected to GCMS analysis as previously described. The Cho measured earlier was subtracted from the Cho measured by cleavage from GroPCho. To measure PCho, 0.2ml of Aqueous phase was added to 0.7ml of 1M (TAPS Buffer-N-tris[hydroxymethyl]methyl-3-aminopropane sulfonic acid) at pH 8.5 along with 0.1ml of 20µM Cho and 0.1 ml of 20 mg/ml alkaline phosphatase (Sigma). This mixture was mixed and incubated at 38°C for 2 h and later cooled to room temperature. This was later cooled to room temperature and 1 ml of 0.4 M HC104 was added, mixed and allowed to stand for 5 min. After thorough mixing, the suspension was centrifuged at 12,000rpm for 10 min. Choline concentration was measured in the supernatant by GCMS Analytical method. The Cho measured firstly was subtracted from the Cho measured by cleavage from PCho.

The Scopolamine treated group and age-matched *Conyza canadensis* treated group were compared with Student's t-test and analysis of variance(ANOVA).Because data appeared to follow a bimodal distribution, Fisher's exact test was used to compare Cho levels among two groups. Data was expressed as mean ±standard Deviation (sample size).

RESULTS

Behavioural Tests

1. Rectangular Maze Test

The activity of *Conyza canadensis* Extract(CCE) 500mg/kg was evaluated using rectangular maze.The mice in all treatment groups except scopolamine-treated group showed lower transfer latency on 7th day and 9th day compared to 5th day of the same group as well as with the scopolamine group (as shown in Fig 1). This indicates memory enhancing capacity of the *Conyza Canadensis* whole herb extract. Donepezil (5 mg/kg) treated for successive 8 days acts as positive control, possessed significant ($P < 0.05$) decrease in transfer latency when compared to normal control and disease control (scopolamine) using Dunnet's test. These results are demonstrated in the Table 1.

Table 1. Results of Rectangular Maze Test

Days	Control(vehicle)	Scopolamine	Donepezil	*Conyza canadensis* extract(CCE) 500mg/kg
1	102.48 ± 8.65	100.56± 8.45	101.49±8.23	99.85±5.67
3	99.57± 7.45	104.39± 7.22	100.45±8.11	99.58±7.13
5	97.65± 6.78	111.62± 6.33	96.42±6.45	96.53±0.45
7	95.62± 5.98	118.46± 7.56	92.56±6.98	92.85±6.58
9	93.56± 5.23	124.78± 7.22	90.48±5.34	89.41±6.52

Figure 1. Depicting the significance between CCE treatment and Scopolamine treatment using Rectangular Maze test

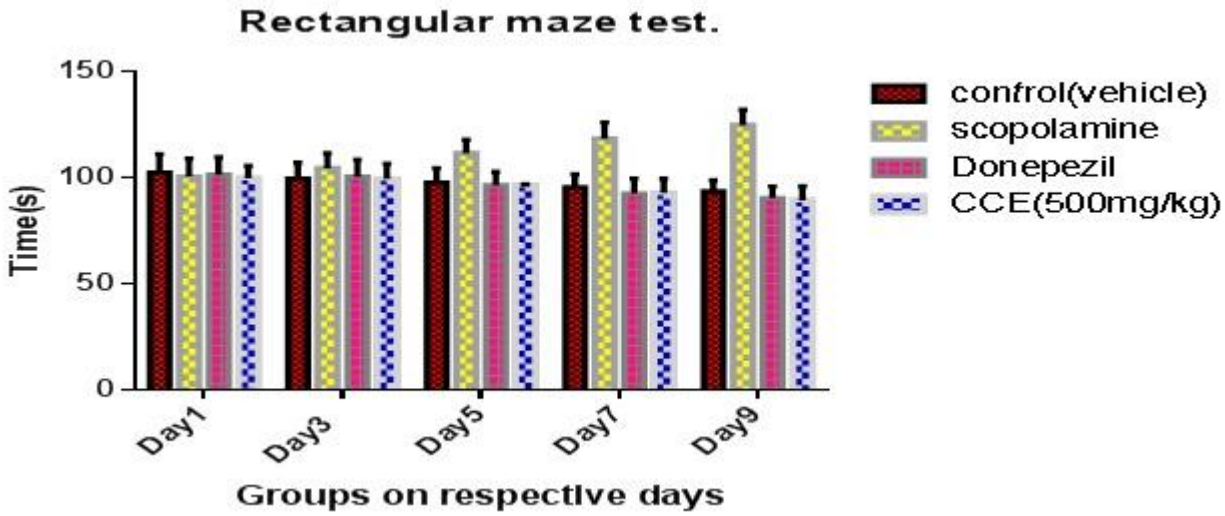


Table 2. Results of Morris Water Maze Test

Days	Control(vehicle)	Scopolamine	Donepezil	*Conyza canadensis* extract (CCE) 500mg/kg
1	103.48 ± 6.48	100.56± 7.22	103.38±5.87	101.45±6.39
3	98.54± 5.78	105.37± 6.33	100.45±6.33	96.51±7.49
5	93.34± 5.45	107.35± 7.56	94.39±6.98	90.48±6.48
7	90.26± 5.67	110.56± 7.22	89.45±5.34	85.64±6.52
9	88.76± 5.38	114.51± 6.54	74.43±6.42	76.49±6.35

Figure 2. Depicting the significant latency time difference among various groups

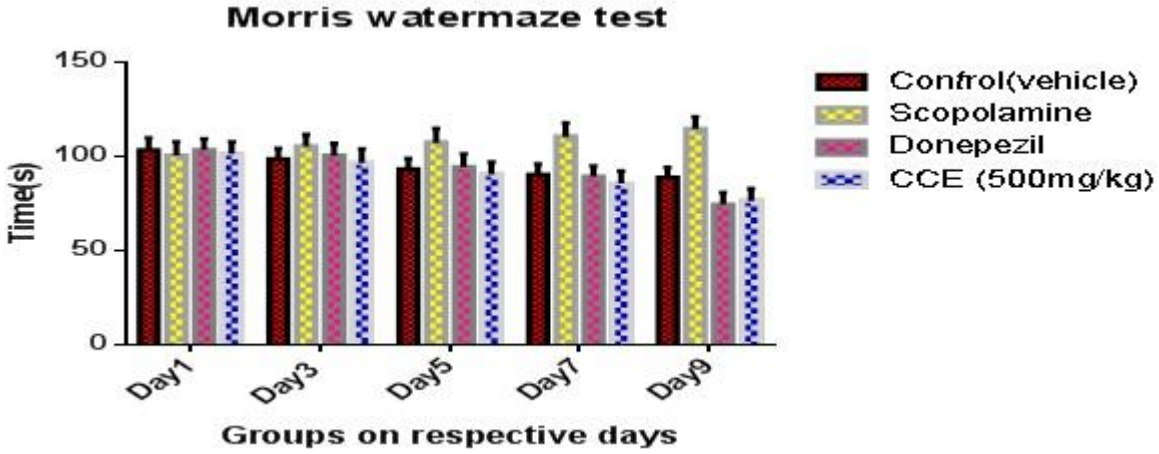


Table 3. Results of Locomotor activity

Days	Control(vehicle)	Scopolamine	Donepezil	*Conyza canadensis* extract (CCE) 500mg/kg
1	198.54 ± 16.20	193.36± 13.5	196.54±15.9	192.47±15.2
3	194.34± 15.20	178.34± 16.4	188.45±14.6	184.34±13.6
5	193.67± 18.1	148.28± 17.3	174.39±13.5	175.27±15.3
7	192.28± 13.5	135.78± 14.9	168.34±17.4	168.29±14.6
9	195.39± 16.5	122.56± 15.4	145.68±13.7	160.35±13.7

Figure 3. Depicting the decrease in number of crossings(with in 10min) among various groups

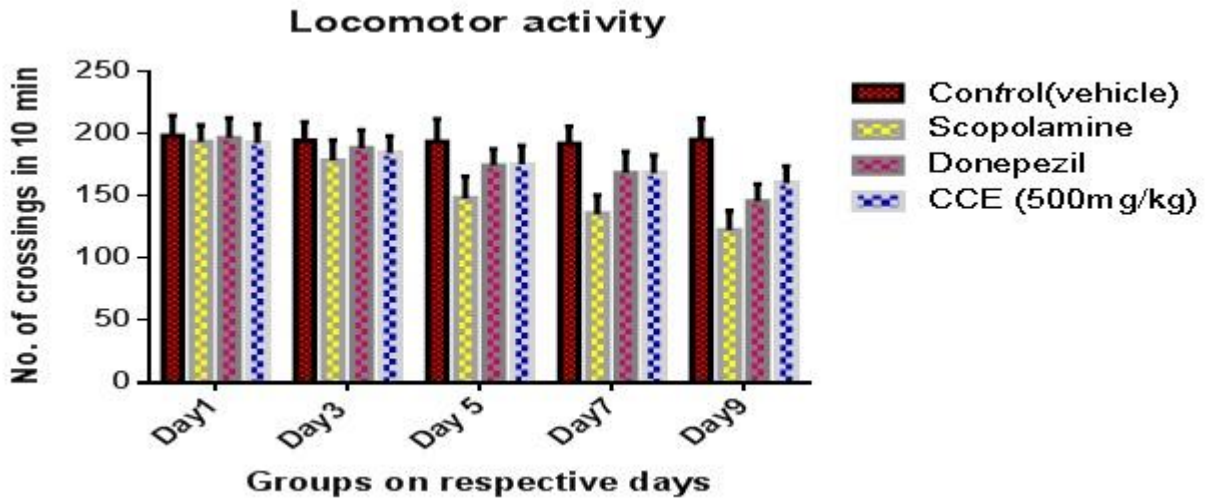


Table 4. Results of AchE Estimation

S.NO	Groups	%Inhibition Of AchE
1	Control	15.64±6.2
2	Scopolamine	9.5±1.87
3	Donepezil	30.16±2.78
4	CCE(500mg/kg)	26.91±8.10

Figure 4. **Depicting the comparison of brain AchE level among the groups**

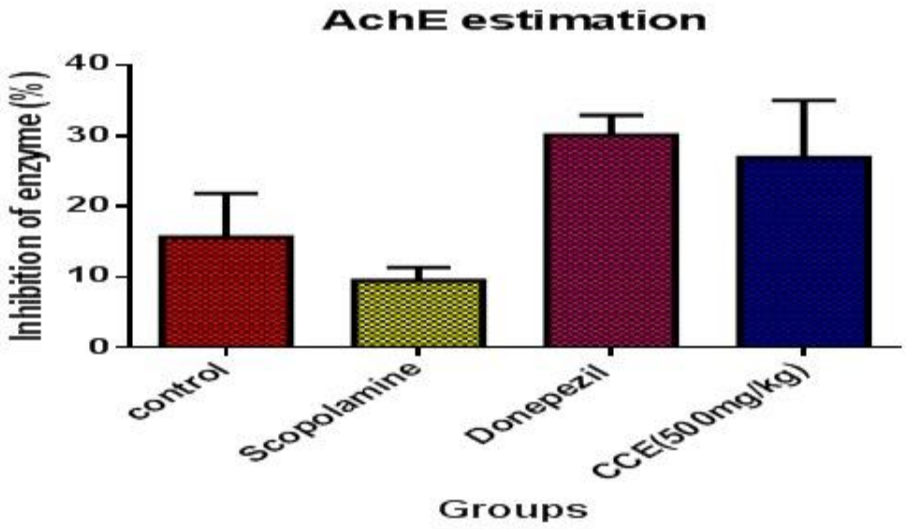


Table 5. Comparison of RBC Choline compounds between two groups

Variable	Disease Control(Scopolamine) Group(n = 33)	*Conyza canadensis* Extract(CCE) treated Group (n = 26)	p Value Student's t-Test
RBC Cho	41.4 ±9.2	18.7 ± 2.3	p<0.023
Plasma Cho	11.5 ± 0.77	11.7 + 0.76	p<0.86
RBC GroPCho	18.2±3.0	15.9 ± 2.5	p<0.55
RBC PCho	34.0 ± 4.3	26.2 ±3.0	p<0.14
RBC PtdCho	1387±58	1667±65	p<0.0022
Plasma PtdCho	2119 ±142	2364 ± 188	p<0.47

Figure 5. Depicting the alteration in Choline levels among CCE treated and Scopolamine treated groups

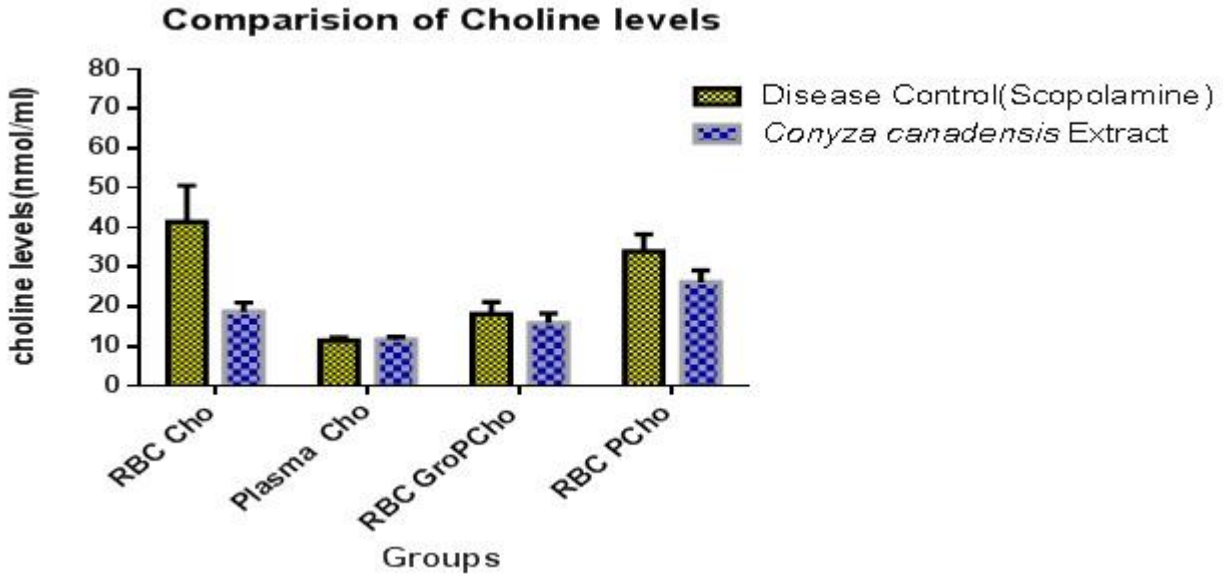


Figure 6. Depicting the levels of RBC and Plasma Phosphotidyl Choline levels among the two groups

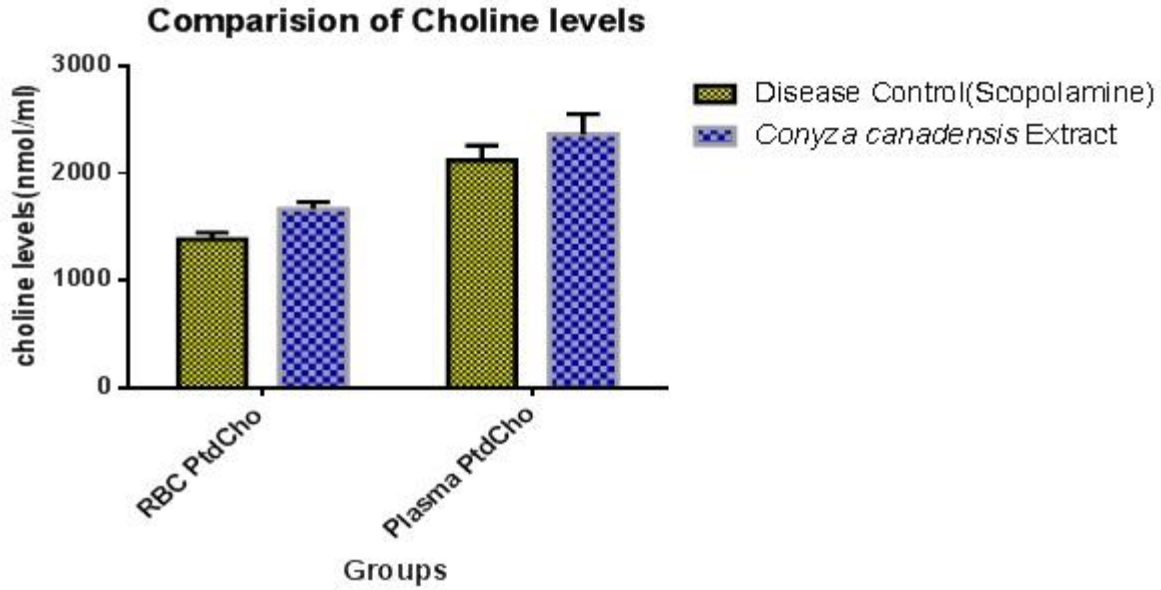


Figure 7. Histological sections of brain tissue showing neurological lesions of Normal control

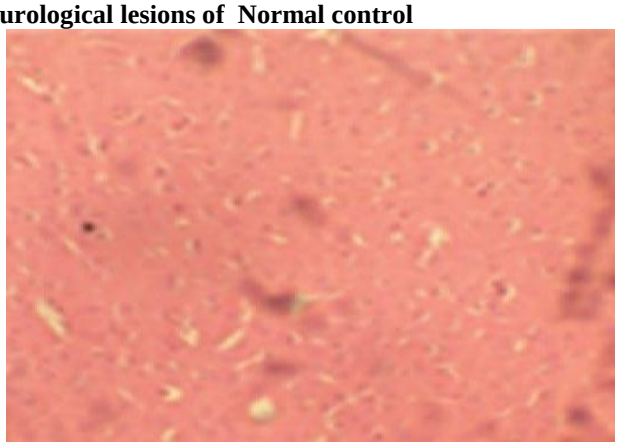


Figure 8. Histological sections of brain tissue showing neurological lesions of Scopolamine (Disease control)

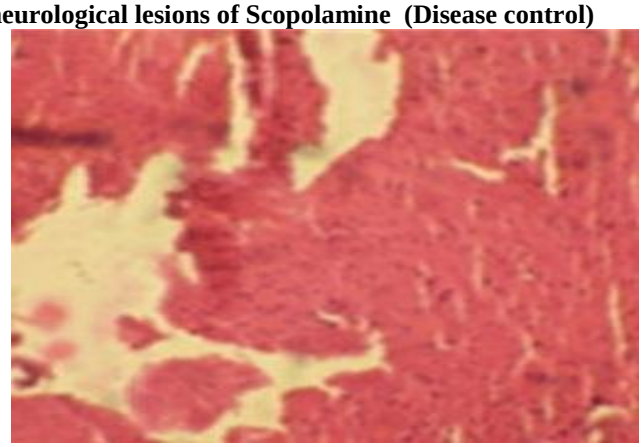


Figure 9. Histological sections of brain tissue showing neurological lesions of Donepezil (Standard)

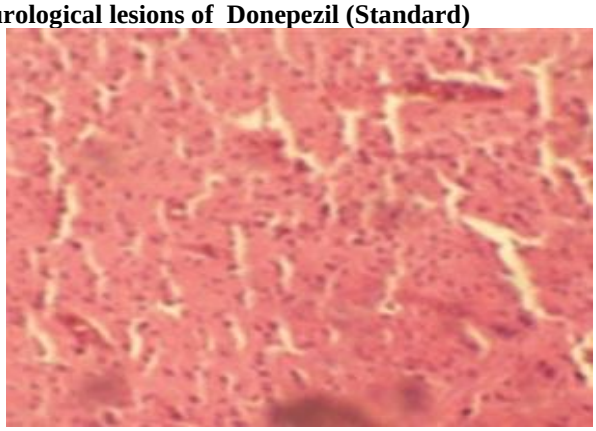
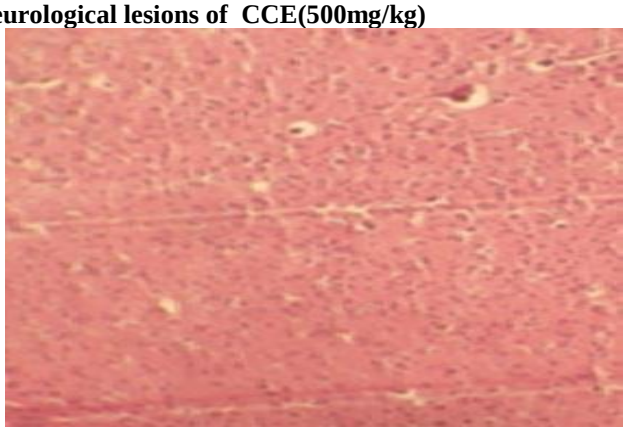


Figure 10. Histological sections of brain tissue showing neurological lesions of CCE(500mg/kg)



2. Morris Water Maze Test

The activity of *Conyza canadensis* Extract (CCE) 500mg/kg was evaluated using Morris water maze. The mice treatment groups except scopolamine-treated group showed significant reduction in transfer latency on 4th day with platform and on 5th day without platform (as shown in Fig 2). This indicates memory enhancing capacity of the *Conyza Canadensis* whole herbal extract. Donepezil (5 mg/kg) treated for successive 8 days acts as positive control, possessed significant ($P < 0.05$) decrease in transfer latency when compared to disease control (scopolamine) using dunnet's test. These results are demonstrated in the Table 2.

3. Locomotor activity

The activity of *Conyza canadensis* Extract (CCE) 500mg/kg was evaluated using photoactometer. The mice showed significant transfer latency on 7th day compared to the 9th day in all treatment groups except scopolamine treated group (as shown in Fig 3). This Donepezil (5mg/kg) treated successive 8 days acts as positive control, possessed significant ($P < 0.05$) decrease in number of crossings which is comparable to the other treatment groups. These results are demonstrated in the Table 3.

Biochemical Tests

1) AchE Estimation

Scopolamine treatment significantly increased the brain AchE level compared to control group (as seen in Fig 4). Standard drug (donepezil) and test drug (*Conyza canadensis* Extract) treatment significantly inhibited the AchE level in brain homogenate compared to their corresponding scopolamine treated groups. These results are demonstrated in the Table 4.

2) Choline levels in the Scopolamine treated group Vs CCE treated group

The mean RBC Cho for the Scopolamine treated group was 41.4±9.2 nmol/ml ,compared to 18.7±2.3 nmol/ml for the CCE treated group and this was statistically significant using Student's t-test, $t(57)= 2.38$, $p = 0.023$. However, the plasma Cho was almost identical in the two groups i.e., 11.5 ± 0.77 nmol/ml in Scopolamine treated group and 11.7 + 0.76 nmol/ml in CCE treated group. GroPCho, PCho and PtdCho were compared in 33 Scopolamine treated group versus 26 CCE treated group.These results are demonstrated in the table 5. The GroPCho was not statistically different between the two groups (as seen in Fig 5). The PCho was higher in the group receiving Scopolamine treatment and this reading

approached but did not reach statistical significance. The CCE treated ones had significantly higher RBC PtdCho compared to the *Conyza canadensis* treated ones. The plasma PtdCho did not significantly differ between the groups (as shown in Fig 6). Also, no significant correlations were found between RBC Cho and RBC parameters including hematocrit or mean cell volume (MCV). The overall correlation coefficient was calculated for Cho, GroPCho, PCho and PtdCho. The GroPCho and the PCho had a Pearson correlation coefficient of 0.2852 which was not significant ($p = 0.26$). The correlation for Cho and GroPCho was 0.5424 with significance $p = 0.024$, whereas correlation coefficient for the RBC Cho and PCho did not reach significance 0.2729 ($p = 0.29$). The PtdCho varied inversely with the Cho but this correlation was not significant.

3) Histopathological studies

The hippocampal lesions assessed microscopically at 40 magnification revealed significant decrease in the lesion size with CCE treated group when compared with Scopolamine treated group , Histopathological studies. Figures 7-10 were normal control, scopolamine (disease control), donepezil (standard), CCE (500mg/kg), respectively, representing the histological sections of the brain tissue showing neurological lesions (Fig 7-10).

DISCUSSION AND CONCLUSION

The results in this study demonstrate a significant elevation of RBC Cho in Scopolamine treated group compared to age-matched CCE treated group. It was found that *Conyza canadensis* treatment played a significant role in the elevation of RBC Cho whereas neither plasma Cho nor plasma lipid-bound Cho showed a significant difference among the groups. In contrast, RBC lipid-bound Cho was significantly lower in Scopolamine treated group compared to CCE treated group. The inverse relationship between RBC PtdCho and RBC Cho suggests that the elevated intracellular Cho might be due to the release of Cho from membrane bound PtdCho. The elevated RBC Cho is thought to be associated with abnormal transport of Cho. These metabolic changes in RBC may reflect similar changes within neurons which could have pertinence to the loss of cholinergic cells in AD. Recently there has been evidence for changes in phospholipid concentration in AD. Using both in vitro [24] and in vivo [25] methods, Pettegrew *et al*. [26] and Brown et al. [27] showed an increase in the phosphomonoester NMR peak (which includes PCho) in the brains of patients with AD. Still others have shown in autopsy studies increases in phosphodiesters but no alteration in phosphomonoesters [28]. In Pettegrew's studies, the changes in phosphomonoesters occurred in areas of the brain where plaques and tangles were not present, suggesting that a generalized abnormality in phospholipids may have preceded the development of Alzheimer changes. In our study, we found an elevation in the RBC phosphomonoester PCho although this elevation did not quite reach statistical significance, Studies of phospholipid metabolism in AD have not yet delineated an etiology for these changes. The mechanisms for the changes in phospholipids in AD need further investigation. The elevated RBC Cho or diminished lipid-bound Cho may reflect changes in phospholipid metabolism that predispose to AD. In this study, it was found that mice under scopolamine treatment were more than 4 times as likely as CCE treated mice to have elevated RBC Cho and 2 times as likely to have diminished PtdCho. The memory loss effect of scopolamine treated group is more prominent compared to the control group. From the behavioral test, that is, rectangular maze test and Morris water maze test, it observed that there was a general decrease in the transfer latency in all CCE treated groups compared to the scopolamine-treated group. The above evidences highlights the role of *Conyza canadensis* whole herbal extract to prevent predisposition to AD.

In conclusion, the present study demonstrates that *Conyza canadensis* whole herbal extract had potential therapeutic effects on improving the antiamnesic activity in mice as it was proved in behavioural tests and biochemical tests and probably through inhibiting lipid peroxidation, augmenting endogenous antioxidant enzymes, and decreasing acetylcholinesterase (AChE) activity in brain.

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