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Research Article

MEMORY ENHANCING ACTIVITY OF *CISSAMPELOS PARIERA* IN MICE

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ABSTRACT

The present study was undertaken to investigate the effects of *Cissampelos pariera* on learning and memory in mice. Elevated plus maze and passive avoidance paradigm were employed to test learning and memory. Three doses (100, 200 and 400 mg/kg, p.o.) of hydroalcoholic extract were administered for 7 successive days in separate group of animals. The dose of 400-mg/kg p.o. of CPE significantly improved learning and memory of mice. Furthermore, this dose significantly reversed the amnesia induced by scopolamine(0.4 mg/kg, p.o.) and ageing induced amnesia. To delineate the mechanism by which CPE exerts nootropic activity, the effect of CPE on whole brain AChE activity was also assessed. CPE also decreased whole brain acetyl cholinesterase activity. Anti –inflammatory and antioxidant properties of *C.pariera* may be contributing favorably to memory enhancement effect. Here, Piracetam (200 mg/kg, i.p) was used as a standard nootropic agent. Hence *C.pariera* appears to be a promising candidate for improving memory and it would be worthwhile to explore the potential of this plant in the management of dementia and Alzheimer's disease. However, further studies are necessitated to identify the exact mechanism of action.

Keywords: *Cissampelos pariera,* Alzheimer's disease

INTRODUCTION

Alzheimer's disease [AD] is a progressive neurodegenerative brain disorder that is slow in onset but leads to dementia, unusual behavior, personality changes and ultimately death[1]. AD is characterized by the presence of excessive amounts of neurotic plaques containing amyloid β protein loss of cholinergic markers in brain. Loss of cholinergic cells particularly in the basal forebrain, is accompanied by loss of the neurotransmitter acetylcholine[2]. A decrease in acetyl choline in the brain of patients with AD appears to be a critical element in producing dementia [3]. The cause of AD is not known clearly. Recently, the mainstay treatments for the AD are acetylcholinesterase inhibitor which increase the availability of acetylcholine at cholinergic synapses. AChE inhibitors from general chemical classes such as physostigmine, tacrine, galantamine and heptylphysostigmine have been tested for the symptomatic treatment of AD[4]. However, non selectivity of these drugs, their limited efficacy, and poor bioavailability, adverse cholinergic side effects in the periphery, narrow therapeutic ranges and hepatotoxicity are among the sever limitations to their therapeutic success[5].Therefore it is worthwhile to explore the utility of traditional medicines for the treatment of various cognitive disorders[6].

In our screening program to search for AChE inhibitors from plants a crude 50% hydroalcoholic extract of roots of *C. pariera* (Family:Menispermiaceae) exhibited significant AchE inhibitory activity (at 400mg/kg p.o.) The root contains several pharmacologically important alkaloids such as pelosine, bebeerine, hyatine, hyatinine cycleanine, hyatidin bulbocapnine, cissamine, cissampareine ,curine and tetrandrine. In some species of Papaveraceae family, AchE inhibitory activity has been detected and has been traced to benzylisoquinoline alkaloids [7]. In the traditional system of medicine, the roots of *C. pariera* (Family: Menispermiaceae) have been in clinical use for centuries. Traditionally, the roots have anti- inflammatory, expectorant, diuretic, anti-asthmatic activities. Immunohistochemical studies revealed the existence of chronic inflammation in certain regions of the brain in AD patients. Since inflammation can be damaging to the host tissue, anti-inflammatory drugs might be beneficial in controlling the progression of AD[8]. In the light of above the present study was undertaken to investigate the effects of *C.pariera* roots on cognitive functions and cholinesterase activity in mice.

MATERIALS AND METHODS

Plant material

The roots of *C. pariera* were collected from Agriculture College, Pune, in December 2008.The plant material was identified and authenticated taxonomically at Regional Research Institute (AY), Kothrud, Pune. A voucher specimen (214) of collected sample was deposited in the institutional herbarium for future reference.

Preparation of extracts

Roots of *C. pariera* were washed with distilled water to remove the dirt and soil and shade dried. The dried material were powdered and passed through a 10-mesh sieve. The coarsely powdered material was extracted with ethanol (50%v/v) by using Soxhlet's Extraction method. The extracts were filtered and concentrated at high vacuum (yield 3.4%w/w)

Drug treatment

For the pharmacological tests, the obtained extract was suspended in double distilled water containing carboxy methyl cellulose (1%w/v CMC) in doses of 100,200,400-mg/kg p.o. The doses were fixed based on earlier studies on the 50% aqueous ethanolic extract of *C. pariera* roots extract (CPE) were administered at up to 2 g/kg to individual mice in-group[9,10,11,12]. There was no mortality due to treatment up to end of the observation period. The *C.pariera* drug extract caused no abnormality or death during the course of treatment.

Animals

Swiss mice of either sex weighing18-20g (younger ones, aged 8 weeks) or 22-25 g (older ones, aged 28 weeks) were used in present study. They had free access to food and water and were maintained under standard laboratory conditions with alternating light and dark cycles of 12 h each. They were acclimatized to laboratory conditions for 5 days before behavioral studies. All the readings were taken during the same time of the day i.e. between 8 a.m. and 11 a.m. The Institution Animals Ethics Committee (IAEC) had approved the experimental protocol, and care of animals was taken as per guidelines of CPCSEA, Department of Animal Welfare, and Government of India.

Drugs and chemicals

The drug used in this study was obtained from following drug houses. Scopolamine hydrobromide (Sigma-Aldrich, USA), 5,5-dithiobis-2-nitrobenzoic acid (DTNB), acetylcholine iodide, eserine (Hi Media, India), Piracetam (UCB India Ltd.)

Vehicle

The plant extract (CPE) was suspended in 1%w/v CMC and administered orally in mice. Scopolamine hydrobromide and

Piracetam were dissolved separately in normal saline and injected i.p. Volume of oral administration and i.p. injection was 1ml/100 g of mouse.

Exteroceptive behavioral models

Elevated plus maze

The elevated plus maze served as the exteroceptive behavioral model (wherein the stimulus existed outside the body) to evaluate learning and memory in mice. The apparatus consisted of two open arms (16 cm × 5 cm) and two covered arms (16 cm × 5 cm ×12cm). The arms extended from a central platform (5cm×5cm) and the maze was elevated to a height of 25 cm from the floor. On the first the day, each mouse was placed at the end of open arm, facing away from central platform. Transfer latency (TL) was taken as the time taken by the mouse to move into any one of the covered arms with all its four legs. TL was recorded on the first day for the each animal. The mouse was allowed to explore the maze for another 2 min. and returned to its home cage. Retention of this learned task was examined 24 h after the first day trial.

Mice were divided into 15 groups and each group consisted of a minimum of 6 animals separate animals were used for each experiment.

Group I: It represented the control group for young mice. Vehicle was administered orally for seven successive days and transfer latency was measured after 90 min of administration on seven day and again after 24 hr i.e. on eighth day.

Group II: It represented the positive control group for young mice. Piracetam (200 mg/kg i.p.) was injected to young mice for seven successive days and transfer latency was measured after 60 min of administration on seven day and again after 24 hr i.e. on eighth day.

Group III, IV and V: CPE (100, 200, 400 mg/kg, p.o.) were administered orally to the young mice for seven successive days to young mice. TL was noted after 90 min of administration on seven day and again after 24 hr i.e. on eighth day.

Group VI: Piracetam (200 mg/kg, i.p.) was injected for seven successive days to young mice. At 60 min after the injection of piracetam on the seventh day, scopolamine 0.4 mg/kg, i.p. was administered. TL was noted after 45 min of administration of scopolamine and again after 24 hr i.e. on eighth day.

Group VII: Scopolamine (0.4 mg/kg) was injected i.p. to young mice and transfer latency was measured 45 min after injection and again after 24 hr (i.e. on eighth day)

Group VIII, IX and X: CPE (100, 200, 400 mg/kg, p.o.) were administered orally to the young mice for seven successive days and Scopalamine (0.4 mg/kg) was injected i.p. to young mice at 90 min. after administration of extract on day seven. TL was noted 45 min. after injection and after 24h (i.e. on eighth day)

Group XI: It represented the control group for older mice. Vehicle was administered orally for seven successive days and transfer latency was measured after 90 min of vehicle administration on seven day and again after 24 hr (i.e. on eighth day.)

Group XII: It represented the positive control for older mice. Piracetam (200 mg/kg i.p.) was injected to older mice for seven successive days and transfer latency was measured after 60min of i.p. Injection on seventh day and after 24 hr (i.e. on eighth day.)

Group XIII, XIV and XV: CPE (100, 200 and 400 mg/kg, p.o.) were administered orally to the aged mice for seven successive days. TL was noted after 90 min. of the extract administration on the day seven and after 24 hr (i.e. on eighth day)

Passive avoidance paradigm

Passive avoidance behavior based on negative reinforcement was used to examine the long-term memory. The apparatus consisted of a box (27 cm × 27 cm×27cm) having three walls of wood and one wall of Plexiglas, featuring a grid floor (made up of 3 mm stainless steel rods set 8 mm apart), with the wooden platform (10 cm × 7 cm ×1.7cm) in the center of the grid floor. The box was illuminated with a 15W bulb during experimental period. Electric shock (20 V, AC) was delivered to the grid floor. Training was carried out in two similar sessions. Each mouse was gently placed on the wooden platform set in the center of grid floor. When the mouse stepped down placing all its paws on the grid floor, shocks were delivered for 15 sec and step-down latency (SDL) was recorded. SDL was defined as the time (in seconds) taken by the mouse to step down from the wooden platform to grid floor with all its paws on the grid floor. Animals showing SDL in the range of 2-15 s during the first test were used for the second session and the retention test .The second session was carried out 90 min. after the first test. When the animals stepped down before 60 s, electrical shocks were delivered for 15 sec. During the second test, animals were removed from shock free zone, if they did not step down for a period of 60 s. Retention was tested after 24 h in a similar manner, expect that the electric shocks were not applied to the grid floor observing an upper cut-off time of 300s.

Mice were divided into 15 groups and each group consisted of a minimum of 6 animals. Separate animals were used for each experiment

Group I: It represented the control group for young mice. Vehicle was administered orally for seven successive days. Shock was delivered for 15 secs after 90 mins of vehicle administration on the day seven and SDL was noted after 24 h (i.e. on eighth day).

Group II: It represented the positive control group for young mice. Piracetam (200 mg/kg i.p.) Was injected to young mice for seven successive days. Shock was delivered for 15 secs after 60 mins of i.p. injection on the day seven and SDL was noted after 24 h (i.e. on eighth day).

Group III, IV, and V: CPE (100, 200, 400 mg/kg, p.o.) were administered orally to the young mice for seven successive days to young mice. Shock was delivered for 15 secs after 90 mins of extract administration on the day seven and SDL was noted after 24 h (i.e. on eighth day).

Group VI: Piracetam (200 mg/kg, i.p.) was injected for seven successive days to young mice. At 60 min after the injection of piracetam on the seventh day, scopolamine 0.4 mg/kg, i.p. was administered. Shock was delivered for 15 secs after 90 mins of extract administration on the day seven and SDL was noted after 24 h (i.e. on eighth day).

Group VII: Scopolamine (0.4 mg/kg) was injected i.p. to young mice and transfer latency was measured 45 min after injection and again after 24 hr (i.e. on eighth day.)

Group VIII, IX and X: CPE (100, 200, 400 mg/kg, p.o.) were administered orally to the young mice for seven successive days and Scopalamine (0.4 mg/kg) was injected i.p.to young mice at 90 min. after administration of extract on day seven. TL was noted 45 min. after injection and after 24hr (i.e. on eighth day)

Group XI: It represented the control group for older mice. Vehicle was administered orally for seven successive days and transfer latency was measured after 90 min of vehicle administration on seven day and again after 24 hr (i.e. on eighth day.)

Group XII: It represented the positive control for older mice. Piracetam (200 mg/kg i.p.) was injected to older mice for seven successive days and transfer latency was measured after 60 min of i.p. Injection on seventh day and again after 24 hr (i.e. on eighth day)

Group XIII, XIV and XV: CPE (100, 200 and 400 mg/kg, p.o.) were administered orally to the aged mice for seven successive days. TL was noted after 90 min. of the extract administration on the seven-day and SDL was recorded after 24 hr (i.e. on eighth day)

Estimation of brain AChE activity

The time frame of cholinesterase activity estimation was similar to the behavioral tests i.e.8am -11 am on each day. On the ninth day animals were euthanized by cervical dislocation carefully to avoid any injury to tissues. The whole brain AChE activity was measured

using the Ellman method[13]. The end point was the formation of the yellow color because of the reaction of thiocholine with dithiobisnitrobenzoate ions. The rate of formation of thiocholine from acetylcholine iodide in the presence of tissue cholinesterase was measured using spectrophotometer. The resulting yellow color is due to reduction of DTNB by certain substances in the brain homogenate and due to non-enzymatic hydrolysis of substrate. After having calibrated the instrument, change in absorbance per min of sample was read at 420 nm [14].

$$\text{Rate} = \frac{\text{Change in the absorbance / min}}{\text{Co}} \times (5.74 \times 10^{-4})$$

Where,

Rate = Moles substrate hydrolyzed per min per gram of tissue

Co = Original concentration of brain tissue (mg/ml)

Group I: Served as control and treated with vehicle

Group II: It was treated with Piracetam (200 mg/kg, p.o.)

Group III, IV and V: They were treated with CPE (100, 200 and 400mg/kg, p.o.) resp. for seven days and Acetyl cholinesterase levels were determined.

Statistical analysis

All the results were expressed as mean ± standard error (SEM). Data were analyzed using one-way ANOVA followed by Dunnett's test and Student's unpaired t-test. $p<0.001$ and $p<0.05$ were considered as statistically significant.

RESULTS

Effect on transfer latency (By elevated plus maze)

Transfer Latency (TL) of first day (on seventh day of drug treatment) reflected acquisition of learning behavior of animals. Where as TL of next day reflected retention of information or memory. CPE (100 mg/kg) administered for 7 days orally did not have any significant effect on TL of seventh day and eighth day in elevated plus maze test. The young and older animals treated orally with 200 mg/kg and 400 mg/kg showed remarkable reduction ($p<0.05$, $p<0.001$) in TL of seventh day as well as eighth day, indicating significant improvement in learning and memory (Fig 1 and 2). Scopolamine hydrobromide (0.4 mg/kg, i.p.) injected before training significantly increased ($p<0.001$ TL on days seven and eight indicating impairment in learning and memory (Fig 3). The CPE at higher dose levels (200 and 400 mg/kg, p.o. for 7 successive days) successfully reversed memory deficits induced by scopolamine ($p<0.001$). Piracetam (used as the positive control) at a dose of 200 mg/kg, i.p. Also improved learning and memory in both young and older mice and reversed the amnesia induced by scopolamine as expected.

Effect on step-down latency (Using passive avoidance paradigm)

Step-down Latency (SDL) of second day/eighth day of drug treatment reflected the long-term memory of animals. CPE (100 mg/kg, p.o.) did not exert any significant effect on SDL of young mice as compared to control group (fig 4). On the other hand, the higher doses of 200 and 400 mg/kg of the extract administered orally in young mice for 7 days markedly ($p<0.05$, $p<0.001$) increased SDL as compared to the control group of young mice, Scopolamine (0.4 mg/kg, i.p.) significantly decreased SDL as compared to control group of young mice, indicating impairment of memory (amnesia). CPE (200 and 400 mg/kg, p.o.) administered for 7 days significantly reversed amnesia induced by both scopolamine (Fig.5). The groups of mice, which were treated with piracetam (200 mg/kg, i.p.) for seven successive days showed improvement ($p<0.001$). In memory of young as well as older mice and reversed amnesia induced by scopolamine. Older mice showed significantly ($p<0.001$). Low SDL there by indicating that ageing had produced amnesia in these animals. CPE (200 and 400mg/kg, p.o.) successfully reversed ($p<0.001$) aging induced amnesia (Fig.6).

Effect on whole brain acetylcholinesterase levels

The lowest dose of CPE (100 and 200 mg/kg, p.o.) did not produce any effect on cholinesterase activity in young and aged mice. However, in higher doses (400 mg/kg, p.o.) CPE showed remarkable reduction in brain cholinesterase activity in young and aged mice as compared to resp. control groups by using Ellman's kinetic colorimetric method.

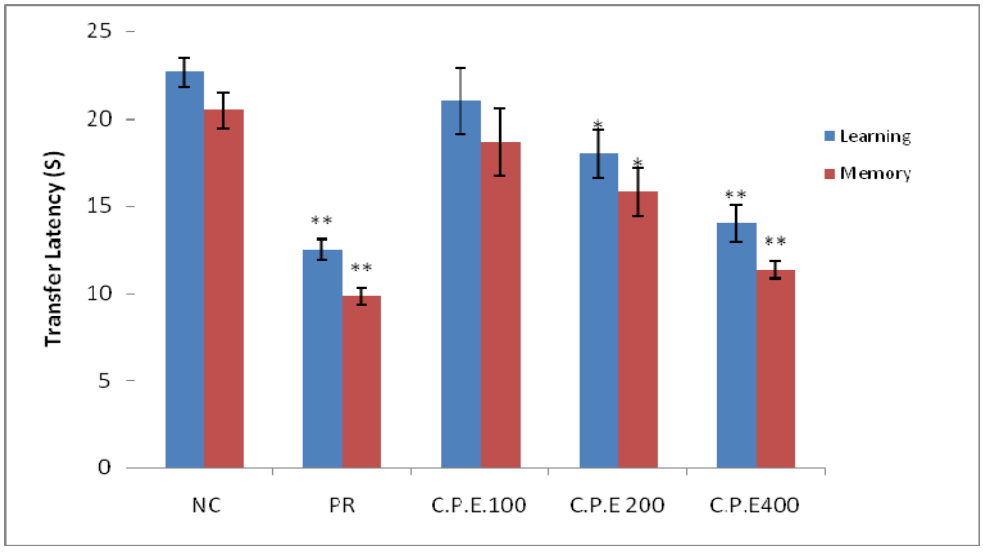

Fig.1: Effect of hydroalcoholic extract of *C. pariera* on transfer latencies of young mice in elevated plus maze

NC – normal control, C.P.E - Hydroalcoholic Extract of *C. pariera,* PR- Piracetam

Percentage expressed in MEAN±SEM (n=6), ANOVA followed by Dunnett's Test

*** p< 0.05, ** p< 0.001 when compared with normal control.**

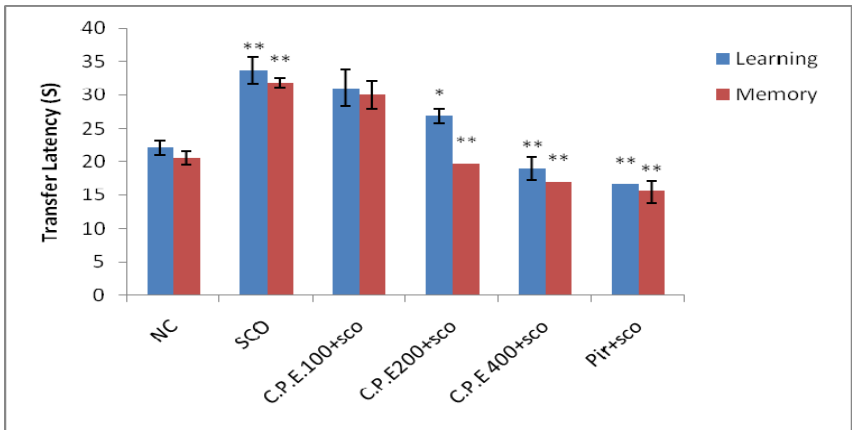

Fig. 2: Effect of hydroalcoholic extract of *C. pariera* on transfer latencies of scopolamine induced amnesic mice

NC – normal control, SCO – Scopolamine, C.P.E - Hydroalcoholic Extract of *C. pariera*, PR- Piracetam. Percentage expressed in MEAN±SEM (n=6), ANOVA followed by Dunnett's Test

*** p < 0.05, ** p < 0.001 when compared with scopolamine**

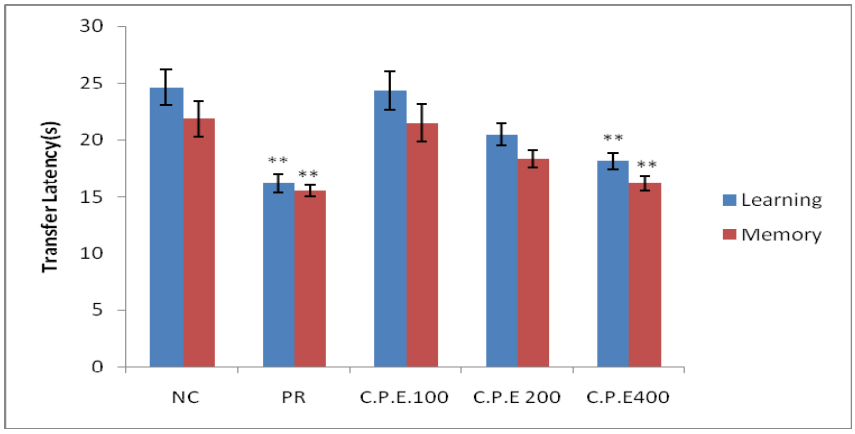

Fig. 3: Effect of hydroalcoholic extract of *C. pariera* on transfer latencies of aged mice

NC – normal control, C.P.E- 4.

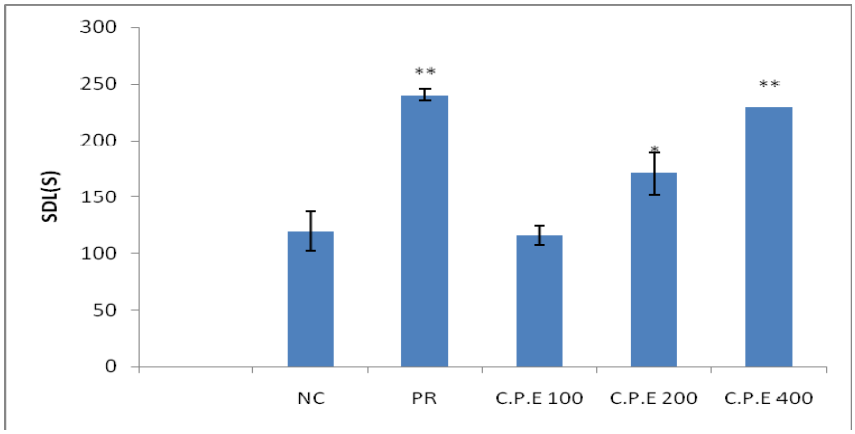

Fig. 4: Effect of hydroalcoholic extract of *C. pariera* on passive avoidance behavior in young mice

NC – normal control, C.P.E - Hydroalcoholic Extract of *C. pariera*, PR- Piracetam.

Percentage expressed in MEAN±SEM (n=6) ANOVA followed by Dunnett's Test

*** p < 0.05, ** p < 0.001 when compared with normal Control**

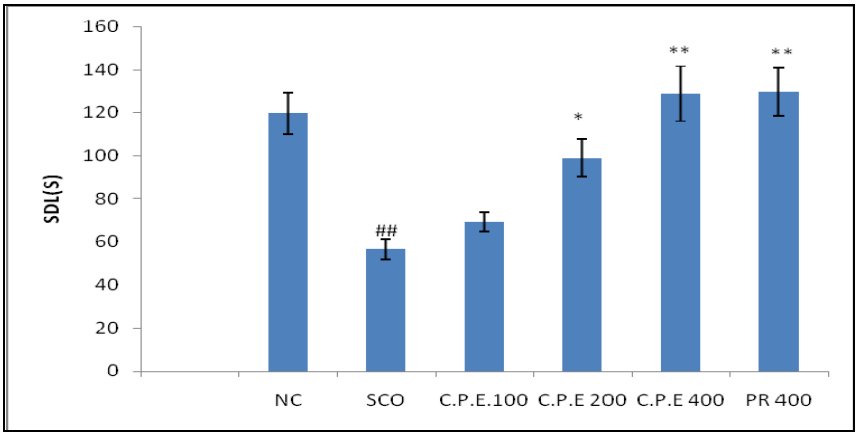

Fig. 5: Effect of hydroalcoholic extract of *C. pariera* on passive avoidance behavior in scopolamine induced amnesia in young mice

NC – Normal control, SCO – Scopolamine, C.P.E - Hydroalcoholic Extract of *C. pariera*, PR- Piracetam. Percentage expressed in MEAN±SEM (n=6), ANOVA followed by Dunnett Test

p<0.001, when compared with normal control. * p < 0.05, ** p < 0.001 when compared with scopolamine

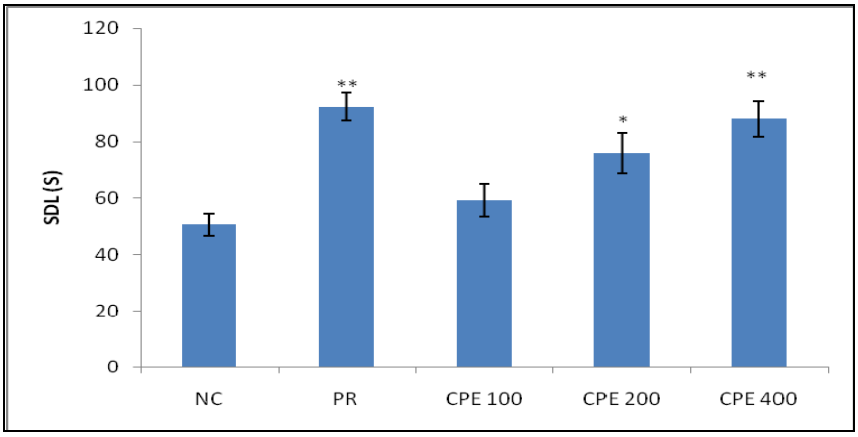

Fig. 6: Effect of hydroalcoholic extract of *C. pariera* on passive avoidance behaviour in aged mice

NC – normal control, C.P.E- Hydroalcoholic Extract of *C. pariera* PR- Piracetam Percentage expressed in MEAN±SEM (n=6), ANOVA followed by Dunnett'sTest * p < 0.05, ** p < 0.01 when compared with normal control.

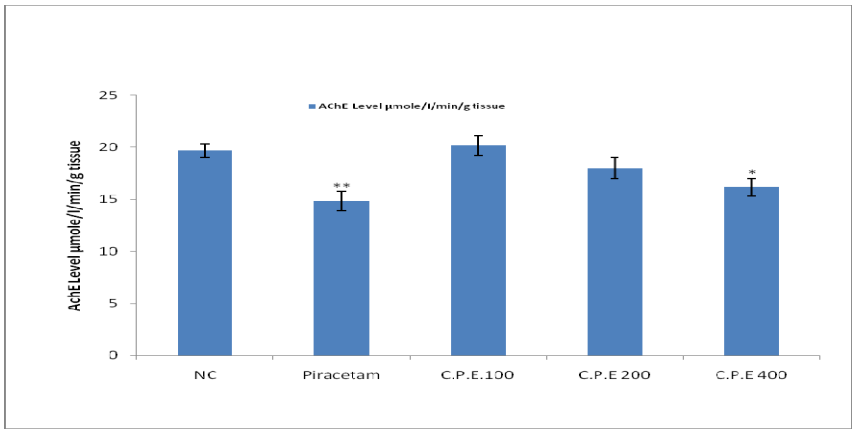

Fig.7: Effect of hydroalcoholic extract of *C. pariera* on braincholinesterase activities of aged mice

NC – normal control, C.P.E - Hydroalcoholic Extract of *C. pariera* PR- Piracetam

Percentage expressed in MEAN±SEM (n=6) ANOVA followed by Dunnett Test.

*** p < 0.05 when compared with control, ** p < 0.01 when compared with control**

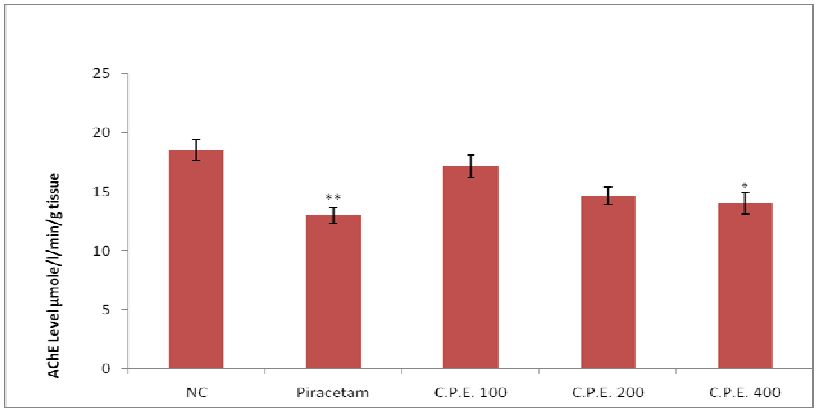

Fig. 8: Effect of hydroalcoholic extract of *C. pariera* on brain cholinesterase activities of young mice

NC – Normal control, C.P.E - Hydroalcoholic Extract of *C. pariera,* PR- Piracetam Percentage expressed in MEAN±SEM (n=6). ANOVA followed by Dunnett's Test.* p < 0.05 when compared with normal control **p< 0.01 when compared with normal control

DISCUSSION

Alzheimer's disease is a genetically heterogeneous neuro-degenerative disorder, which is slow in onset but relentless in progress. It is characterized by aphasia, apraxia and agnosia with the loss of memory as the main symptom. Despite the severity and prevalence of this disease, allopathic system of medicine is yet to provide a satisfactory drug. Therefore, we were motivated to explore the potential of medicinal plants to manage this deadly disease. In the present study CPE extract administered orally for 7 days improved learning and memory of mice significantly in both the exteroceptive behavioral models employed. The stimulus lie outside the body in exteroceptive behavior models, whereas, it lies within the body in the case of interoceptive models. In present study the higher dose 400 mg/kg significantly improved the memory of mice as reflected by diminished TL and enhanced SDL values as compared to control animals. Additionally, CPE (400 mg/kg) reduced central cholinesterase activity. Furthermore, pretreatment with CPE for 7 days protected the animals from memory deficits produced by scopolamine. These findings suggest the possible neuroprotective role for *C.pariera.*

Immunohistochemical studies suggested existence of chronic inflammation in certain regions of the brain in AD patients. Since inflammation can be damaging to host tissue, it was hypothesized that anti-inflammatory drugs might be inhibiting both the onset and the progression of Alzheimer's disease. This hypothesis is supported by the observation that indomethacin (NSAID) halted the progressive memory loss seen in AD patients. Moreover, it has also been observed that elderly patients suffering from AD showed reduction in symptoms of AD upon chronic use of anti-inflammatory drugs. Anti-inflammatory action of *C. pariera.* It might also be contributing to the observed memory enhancing activity of CPE. Oxygen free radicals are implicated in the process of age related decline in cognitive performance might be responsible for the development of Alzheimer's disease in elderly persons. *C. pariera* has been reported to possess antioxidant property as well [15].The neuroprotective effect of CPE may be attributed to its antioxidant property by the virtue of which susceptible brain cells get exposed to less oxidative stress resulting in reduced brain damage and improved neuronal function.

The symptoms of dementia are presumed to be related to impaired neurotransmission and degeneration of neuronal circuits in the affected brain areas. Cognitive deterioration occurring in patients with probably AD is associated with progressive loss of cholinergic neurons and consequent decline in levels of acetylcholine in brain. In present study, CPE significantly decreased the AChE levels in the mice whole brain homogenate, indicating its potential in the attenuations of severity of AD.

It would be interesting to indicate that quaternary nitrogen is necessary for strong activity in alkaloids possessing the benzylisoquinoline skeleton. In some species of Papaveraceae family, AChE inhibitory activity has been detected and has been traced to the benzylisoquinoline alkaloids. The roots of *C.pariera* contains benzylisoquinoline alkaloids may be responsible for memory enhancing activity.

In conclusion, based on results of experiments, we found that CPE had remarkable cognitive enhancing activity.

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