

Protective Potential of Piper Nigrum in Memory Impaired Rats

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DOI: <http://doi.org/10.5281/zenodo.2834990>

Abstract

Background: Normal aging is associated with a slow decline in brain functions such as sensory and motor performance, and at time, this decline is accompanied by progressive memory loss, dementia and cognitive dysfunctions, ultimately resulting in limited functionality. The cognitive enhancing activity of ethanolic fruit extract of *Piper longum* was investigated using two methods. Induction was carried out by Diazepam for 7 days. **Aim:** The present research was designed to evaluate the protective effect of *Piper nigrum* in memory impaired rats and compared with standard drug Piracetam. It exhibited significant protective effect in all the models. **Material and Methods:** The acetylcholine esterase activity, oxidative stress, spatial navigation task and behavioral studies were used for the screening of protective effect of *Piper nigrum*. **Result:** This study significantly demonstrated the protective effect of *Piper nigrum* in all the models. **Future Aspect:** The further research is suggested to isolate the chemical compound of the plant responsible for the activity. So, it can be used in the treatment of dementia and memory impairments.

Keywords: Diazepam, dementia, piper nigrum, piracetam

INTRODUCTION

AD is characterized by amyloid protein beta (A β) deposition in plaques and by neurofibrillary tangles (NFT), glial cells activation around plaques and region specific accumulation of oxidative damage that correlates with histopathology [1]. The cognitive enhancing activity of ethanolic fruit extract of *Piper longum* was investigated using two methods. Induction was carried out by Diazepam for 7 days. Drug extract showed significant effect when compared to control; there was significant increase in the step down latency and decrease in the transfer latency which was as effective as that of standard drug [2].

The present research was designed to evaluate the protective effect of *Piper nigrum* in memory impaired rats and compared with standard drug Piracetam. It exhibited significant protective effect in all the models.

MATERIAL AND METHODS

Extraction of *Piper nigrum* fruits

The powdered plant material (10g) was extracted with 50 ml of methanol by occasionally shaking for 72 h within 8 h interval. The extract was concentrated to remove the solvent and filtered through Whatmann filter paper (normal shaded).

Animals

Experiments were carried out on either sex rats. The animals were housed in polypropylene cages. Paddy husk was provided as bedding material, which was changed every day. They were fed with standard pellet diet and purified water. They were kept in a well-aerated room and a 12-hour light and dark cycle was maintained. The room temperature was maintained at 25 ± 2 °C. The study was conducted in the Department of Pharmacy, M.J.P. Rohilkhand University, Bareilly. Experiments were performed according to

the guidelines for care and use of laboratory animals.

Drugs and Chemicals

Aluminum chloride, 5, 5-di-thio-bis2-nitrobenzoic acid, (Ellman's reagent), acetylthiocholine iodide, trichloroacetic acid, thiobarbituric acid (TBA), nitro blue tetrazolium (NBT), hydroxylamine. All were purchased from Sigma–Aldrich and Piracetam (Nootropil) UCB India Pvt Ltd Bangalore India.

Dose Selection

Piracetam was administered at a dose of 140 mg/kg/p. o. Aluminum chloride was administered at a dose of 100 mg/kg/p. o. Dose of *Piper nigrum* was selected 50 mg/kg and 100 mg/kg [3], [4].

Experimental Design

Rats were divided into five groups; each consisting of six animals. All the groups were treated for forty two days continuously as follows-

Table 1: Experimental design.

Group	Treatment
Group I	water (Vehicle for AlCl ₃) + 1% Gum acacia (0.4 ml p. o.) vehicle for <i>Piper nigrum</i> methanolic extract
Group II	Aluminum chloride treated group 100 mg/kg/ p. o.
Group III	Aluminum chloride (100 mg/kg, p. o.) + Piracetam treated group (140 mg/kg, p. o.)
Group IV	Aluminum chloride (100 mg/kg, p. o.) + M.E.P.N. treated group (50 mg/kg, p. o.)
Group V	Aluminum chloride (100 mg/kg, p. o.) + M.E.P.N. treated group (100 mg/kg, p. o.)

Behavioral Studies

Spatial Navigation Task

Screening of spatial navigation task was carried out by Morris water maze. Training had been given rats to swim to a visible platform in a circular pool (180cm in diameter and 60cm in height) located in a test room. Principle involved was rats can escape from swimming by climbing onto the platform and over time the rats apparently learn the spatial location of the platform from any starting position at the circumference of the pool. The pool was filled with water (28±2°C) to a height of 30cm a movable platform (11 cm diameter). It was mounted on a column that was placed in a pool 2 cm beyond the water level during the acquisition phase. A platform with similar size was placed in the pool (2 cm) below the water level for the maze retention phase. During both the phases the platform was placed in the center of one of the quadrants. By adding a non-toxic dye the water was made opaque. On the edge of the pool, four equally spaced locations (Q1, Q2, Q3 and Q4) were used as starting points and this divided the pool into four equal quadrants.

Maze Acquisition Phase (Training)

Animals received a training session on day twenty consisting of four trials. In all four trials, the starting position was different. Every trial began by releasing the animal into the maze facing towards the wall of the pool. The latency to identify the escape platform was recorded to a maximum of 90 sec. Within the given time if the rat did not escape onto the platform; it was guided to the platform and was allowed to remain there for 20 sec. The time taken by rat to reach the platform within 90 sec was taken as the initial acquisition latency (IAL). At the end of the trial the rats were returned back to their home cages and a five min gap was given between the subsequent trials.

Maze Retention Phase

Following 24 h (day 21) and 21 days (day 42) after IAL, rat was released randomly at one of the edges facing the wall of the pool and tested for retention of response. Following start of Aluminum chloride administration the time taken to find the hidden platform on day 21 and day 42 was recorded and termed respectively, as first

retention latency (1st RL) and second retention latency (2nd RL)[4].

Elevated plus maze

The elevated plus maze was made of wood, consists of two opposite open arms (50cm×10cm), crossed with two enclosed walls of the same dimensions along with 50 cm height. The open arms were connected with a central square of dimensions 10cm×10cm the entire maze was placed 50cm high above the ground. After twenty days, since Aluminum chloride administered, animals were tested for Acquisition of memory. Rats were placed away from the central square individually at one end of the open arm. The time taken by the animal on maze to move from the open arm to the closed arm within specified time was recorded as the initial transfer latency (ITL). After recording the ITL animals were allowed to explore the maze for 20 sec and were then returned to the home cages. It was pushed on the back into one of the enclosed arm, if the animal did not enter the enclosed arm within 90 sec, and the ITL was recorded as 90 sec. The retention of memory and retention latency- assessed by placing the rat in an open arm was noted on day twenty and day forty two of the ITL, termed as the first retention transfer latency (1st RTL) and second retention transfer latency (2nd RTL) respectively [3].

Dissection and Homogenization

The animals were sacrificed by cervical decapitation under light anesthesia. Whole brain was carefully removed from the skull, immediately after decapitation. For preparation of homogenate, the fresh whole brain was weighed and transferred to a glass homogenizer and A 10% (w/v) tissue homogenates were prepared in 0.1 M phosphate buffer (pH 7.4). The homogenate was centrifuged at 3000 rpm for 10 min and the resultant cloudy supernatant liquid was used for biochemical estimations.

Estimation of Acetyl Cholinesterase (AChE) Activity

AChE is a marker of extensive loss of cholinergic neurons in the forebrain. By using Ellman method the AChE activity was assessed (Ellman, 1959). The assay mixture contained 0.05 ml of supernatant, 3ml of sodium phosphate buffer (pH 8), 0.1ml of acetylthiocholine iodide and 0.1ml of DTNB (Ellman reagent). The change in absorbance was measured for 2 min at 30 s interval at 412 nm using Perkin Elmer Lambda 20 UV VIS Spectrophotometer (Medico, Japan). Finally results were expressed as micromoles of acetylthiocholine iodide hydrolyzed per min per mg protein [5].

Biochemical Estimation of Markers of Oxidative Stress

Measurement of Lipid Peroxidation

In the brain, the extent of lipid peroxidation was determined quantitatively by performing the method as described by Wills. By reaction with thiobarbituric acid, the amount of malondialdehyde (MDA) was measured at 532nm using Perkin Elmer Lambda 20 UV VIS Spectrophotometer (Medico, Japan). By using the molar extinction co-efficient of chromophore (1.56×10^5 (mol/l) -1 cm -1), the values were calculated (Wills ED 1966).

Statistical Analysis

All the values were expressed as mean $\pm$ SEM. All test data were analyzed using one way analysis of variance (ANOVA) followed by parametric test. For statistical significance the criterion was $P < 0.05$. All statistical procedures were carried out using XLSTAT Software.

RESULTS AND DISCUSSION

Effect of Piper Nigrum on Behavioral Studies

Effect of Methanolic Fruit extract of Piper nigrum on aluminum chloride induced behavioral alteration (Morris water maze) in rats

Results are described as per the performance on Day 20th, 21th and 42nd

in Morris water maze. Control group showed normal initial acquisition latency and retention latency to find the platform on performance day 20th, 21th and 42nd day respectively. $AlCl_3$ was administered at the dose of 100 mg/kg, *p. o.* for 6 weeks; significantly increased

the time taken by the rats to reach platform in the target quadrant (Q3). Post treatment with Piracetam 140 mg/kg and *Piper nigrum* 50 and 100 mg/kg, *p. o.* significantly decreased the time taken by rats to reach platform (Q3) during the IAL and RL.

Experimental protocol for behavioral studies

Table 2: Experimental protocol for behavioral studies.

Randomization and grouping based on body weight	Administration of M.E.P.N., Piracetam along with Aluminum chloride and evaluation of cognitive parameters (learning and memory skills) on MWM and EPM paradigm				
	Post Treatment	Training/ acquisition Phase on MWM & EPM. (RL and TL)	Retention latency on MWM & EPM (1 st RL, RTL)	Post Treatment	Retention latency on MWM & EPM (2 nd RL,RTL)
Day 0	Day1-19	Day 20	Day 21	Day 22-41	Day 42

MWM: Morris water maze, EPM: elevated plus maze, IAL: initial acquisition latency, RL: retention latency, TL: Transfer latency.

Effect of piper nigrum on behavioral studies (Morris water maze)

Table 3: Effect of piper nigrum on behavioral studies (Morris water maze).

Groups	Treatment	Day 20 IAL	Day 21 1st RL	Day 42 2nd RL
Control	D.W+1% Gum Acacia (5mg/kg, <i>p. o.</i>)	54.67 ± 1.49	46.00 ± 0.72	42.00±0.71
Negative	$AlCl_3$ (100mg/kg, <i>p. o.</i>)+ 1% Gum acacia	77±2.09 ^a	69.67±1.09 ^a	64.17±1.18 ^a
Standard	$AlCl_3$ (100 mg/kg <i>p. o.</i>) + Piracetam (140mg/kg, <i>p. o.</i>)	66.67±0.97	41.50±0.84	39±0.72
Test 1	$AlCl_3$ (100 mg/kg, <i>p. o.</i>) + M.E.P.N. (50 mg/kg, <i>p. o.</i>)	72.50±0.84 ^b	45.66±0.83 ^b	51.17±0.93 ^b
Test 2	$AlCl_3$ (100 mg/kg, <i>p. o.</i>) + P.N.M.E (100mg/kg, <i>p. o.</i>)	69.50±0.84 ^b	45.67±0.66 ^b	44.67±0.66 ^b

On day 20 the initial acquisition latencies (IAL) and retention latencies on days 21 (1st RL) and 42 (2nd RL) following aluminum chloride treatment were observed in Morris water maze. Values are mean ± SEM.

Note: M.E.P.N. *Piper nigrum* methanolic extract;

^aP < 0.05 as compared to control group.

^bP < 0.05 as compared to $AlCl_3$ treated group. One-way ANOVA followed by XLSTAT test for multiple comparisons.

Effect of piracetam, piper nigrum on spatial navigation task in aluminum chloride treated rats

In the spatial navigation task, control group of animals quickly learned to swim

directly to the platform in the Morris water maze on day 20. Aluminum Chloride treated rats showed a significant difference in mean IAL when compared to control group on day 20, indicating that aluminum chloride on chronic administration of impaired acquisition of spatial navigation task (P < 0.05). In contrast, concomitant administration of *piper nigrum* (50 mg/kg, *p.o.*) and piracetam with aluminum chloride significantly decreased the IAL to reach the platform in the pre-trained rats as compared to aluminum chloride treated rats on day 20 (Table 4). The mean retention latencies, after following training (1stand 2nd RL) to escape onto the hidden platform was significantly decreased in

control group on days 21 and 42, respectively as compared to IAL on day 20 since the initiation of aluminum chloride treatment. However, administration of *piper nigrum* (100mg/kg,p.o) along with piracetam (100mg/kg) significantly ($P < 0.05$) potentiated their protective effects,

that is treated rats showed a significant decline in the 1st and 2nd RL as compared to aluminum chloride treated rats on days 21 and 42, respectively (Table 4) and improved the retention performance of the spatial navigation task.

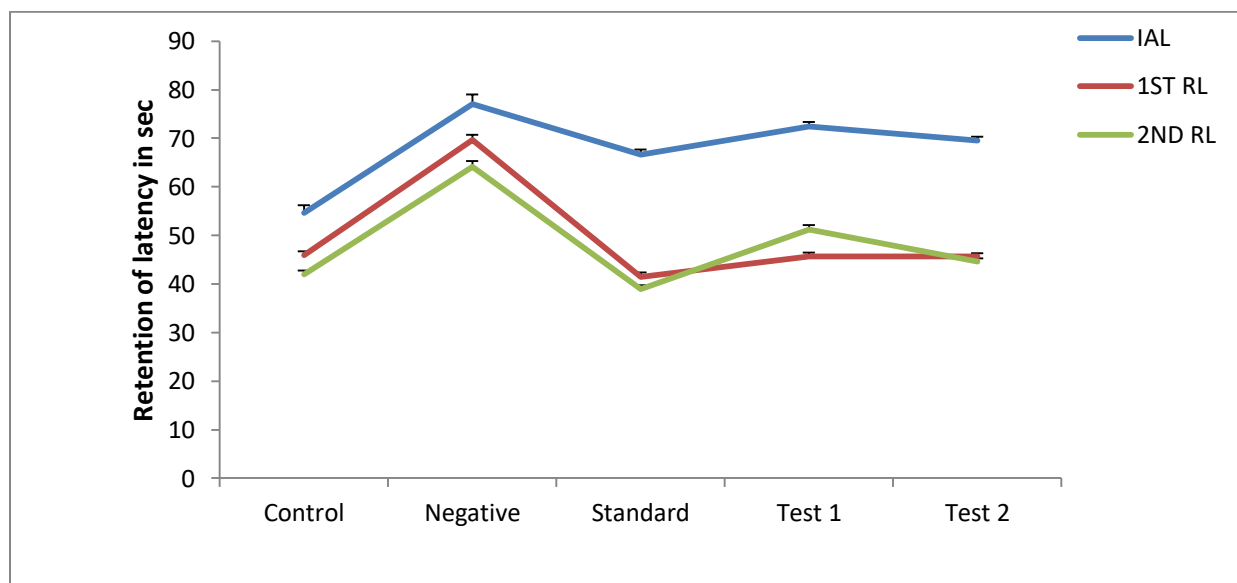


Figure 1: Effect of *piper nigrum* on behavioural studies (morris water maze).
Values are mean $\pm$ SEM.

Effect of *piper nigrum* on behavioral studies (elevated plus maze)

Table 4: Effect of *piper nigrum* on behavioral studies (elevated plus maze).

Groups	Treatment	Day 20 ITL	Day 21 1st RTL	Day 42 2nd RTL
Control	D.W+ 1% Gum Acacia (5 mg/kg, p. o.)	63.83 $\pm$ 0.60	61.00 $\pm$ 0.72	58.5 $\pm$ 0.83
Negative	AlCl ₃ (100mg/kg, p. o.) + 1% Gum acacia	69.5 $\pm$ 0.84 ^a	72.67 $\pm$ 1.20 ^a	75.67 $\pm$ 0.65 ^a
Standard	AlCl ₃ (100 mg/kg, p. o.) + Piracetam (140mg/kg, p. o.)	64.17 $\pm$ 0.60	49.50 $\pm$ 0.84	40.33 $\pm$ 0.97
Test 1	AlCl ₃ (100 mg/kg, p. o.) + P.N.M.E (50 mg/kg, p. o.)	68.50 $\pm$ 0.84 ^b	49.84 $\pm$ 1.18 ^b	56.67 $\pm$ 0.97 ^b
Test 2	AlCl ₃ (100 mg/kg, p. o.) + P.N.M.E (100mg/kg, p. o.)	67 $\pm$ 0.65 ^b	55 $\pm$ 0.72 ^b	45 $\pm$ 0.72 ^b

On day twenty, the initial transfer latencies (ITL) and retention transfer latencies on days 21 (1st RTL) and 42 (2nd RTL) following aluminum chloride treatment were observed.

Values are mean $\pm$ SEM.

Note: P.N.M.E: *Piper nigrum* methanolic extract;

^a $P < 0.05$ as compared to control group.

^b $P < 0.05$ as compared to AlCl₃ treated

group. One-way ANOVA followed by XLSTAT test for multiple comparisons.

Effect of piracetam, *piper nigrum* combination on memory performance in elevated plus maze paradigm in aluminum chloride treated rats.

In the elevated plus maze task, mean ITL on day 20 for each rat was relatively stable and showed no significant variation.

Within 90 sec. all the rats entered the closed arm. Following training, chronic aluminum chloride group performed poorly throughout the experiment and did not show any change in the retention transfer latencies (RTL) on days 21 and 42 as compared to pre-training latency on day 20, demonstrating chronic aluminum chloride treatment-induced memory impairment. Besides, piracetam

(140 mg/kg), *piper nigrum* (50mg/kg,p.o) treatment groups showed significant decrease ($P < 0.05$) in both 1st and 2nd RTL on days 21 and 28 (Table 5). However, administration of *piper nigrum* (100mg/kg) with piracetam (140 mg/kg) significantly ($P < 0.05$) potentiated their protective effects (shortened transfer latency) when compared to control group.

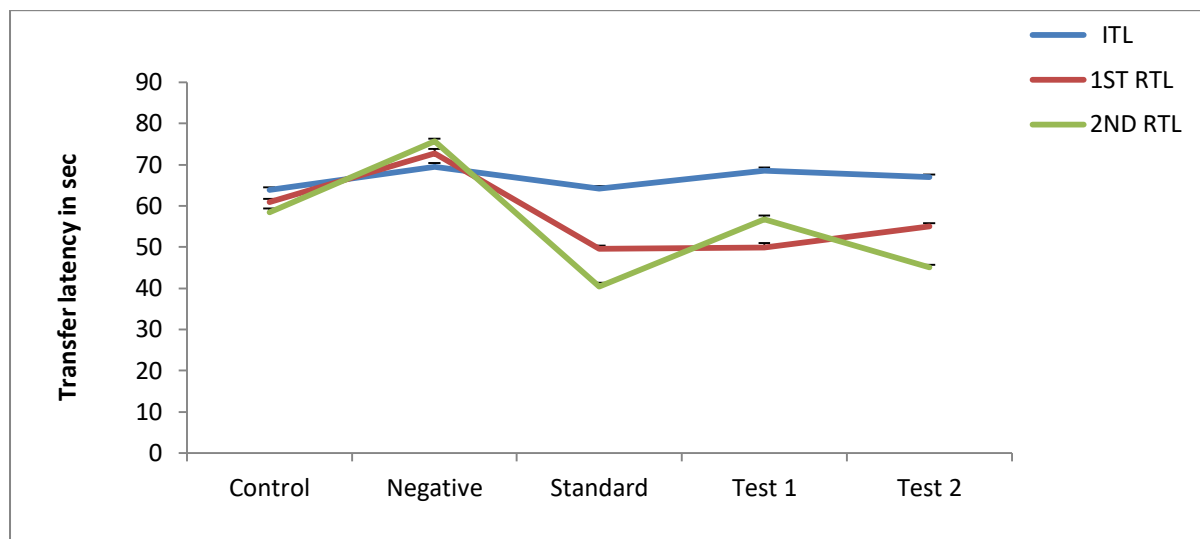


Figure 2: Effect of piper nigrum on behavioral studies (elevated plus maze).
Values are mean $\pm$ SEM.

Effect of piracetam, and *Piper nigrum* on oxidative stress parameters in Aluminum chloride treated rats

Table 5: Effect of piracetam and *Piper nigrum* on oxidative stress parameters in aluminum Chloride treated rats.

Groups	Treatment	MDA Levels nmols/mg protein
Control	D.W+ 1% Gum Acacia (5 mg/kg/p.o.)	0.324 $\pm$ 0.0116
Negative	AlCl ₃ (100 mg/kg/p.o.)+ 1%Gum acacia	0.593 $\pm$ 0.0030 ^a
Standard	AlCl ₃ (100 mg/kg p.o.) + Piracetam (140mg/kg/p.o.)	0.214 $\pm$ 0.0033 ^b
Test 1	AlCl ₃ (100 mg/kg p.o.)+P.N.M.E (50 mg/kg/p.o.)	0.315 $\pm$ 0.0030 ^{bc}
Test 2	AlCl ₃ (100 mg/kg p.o.) + P.N.M.E (100mg/kg/p.o.)	0.203 $\pm$ 0.0029 ^b

Values are mean $\pm$ SEM.

Note: P.N.M.E: *Piper nigrum* methanolic extract;

^a $P < 0.05$ as compared to control group.

^b $P < 0.05$ as compared to AlCl₃ treated group.

^c $P < 0.05$ as compared to standard piracetam group. One-way ANOVA followed by XLSTAT test for multiple comparisons.

Effect of piracetam, *piper nigrum* on oxidative stress parameters in aluminum chloride treated rats.

Chronic administration of aluminum chloride for 42 days showed significant increase in oxidative damage as evidence by increased MDA as compared to negative group (Table 6) Besides concomitant administration of *piper nigrum* (50 mg/kg, p.o.) and piracetam with aluminum chloride treated groups,

attenuated levels of oxidative stress parameters which was significant as compared to chronic aluminum chloride treatment group ($P < 0.05$). However, administration of *piper nigrum*

(100mg/kg,p.o) with piracetam (140mg/kg) significantly ($P < 0.05$) potentiated their protective effects (decreased MDA levels) as compared to their effects alone and control group Fig. 3.

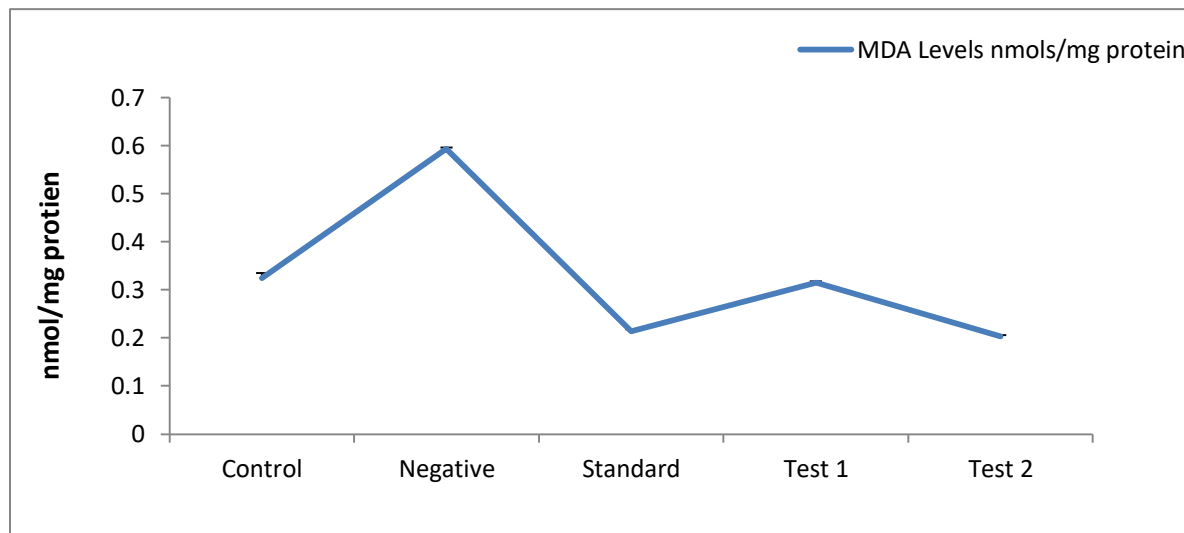


Figure 3: Effect of piracetam, and *Piper nigrum* on oxidative stress parameters in aluminum chloride treated rats.
Values are mean $\pm$ SEM.

Effect of Piracetam and *Piper nigrum* on acetylcholine esterase activity in aluminum chloride treated rats

Table 6: Effect of Piracetam and *Piper nigrum* on acetylcholine esterase activity in aluminum chloride treated rats.

Groups	Treatment	AChE Activity ($\mu\text{mol/min/mg/pr}$)
Control	D.W+ 1% Gum Acacia (5 mg/kg, p. o.)	0.0123 $\pm$ 0.000038
Negative	AlCl ₃ (100mg/kg, p. o.)+ 1%Gum acacia	0.0170 $\pm$ 0.000021 ^a
Standard	AlCl ₃ (100 mg/kg, p. o.) + Piracetam (140mg/kg, p. o.)	0.0088 $\pm$ 0.000037 ^b
Test 1	AlCl ₃ (100 mg/kg, p. o.) + M.E. P.N. (50 mg/kg, p. o.)	0.0115 $\pm$ 0.000038 ^{ab}
Test 2	AlCl ₃ (100 mg/kg, p. o.) + M.E. P.N. (100mg/kg, p. o.)	0.0096 $\pm$ 0.000148 ^{ab}

Values are mean $\pm$ SEM.

Note: P.N.M.E: *Piper nigrum* methanolic extract;

^a $P < 0.05$ as compared to control group.

^b $P < 0.05$ as compared to AlCl₃ treated group. One-way ANOVA followed by XLSTAT test for multiple comparisons.

Effect of piracetam and *piper nigrum* on acetylcholine esterase activity in aluminum chloride treated rats

Chronic administration of aluminum chloride for 42 days showed significant increase in acetylcholine esterase enzyme

activity in aluminum chloride treated group when compared to control group (Table 6). Besides concomitant administration of *piper nigrum* (50 mg/kg, p.o.) and piracetam with aluminum chloride treated groups, attenuated acetylcholine esterase enzyme activity which was significant as compared to chronic aluminum chloride treatment group ($P < 0.05$). However, administration of *piper nigrum* (100mg/kg) with piracetam (140mg/kg) attenuated acetylcholine esterase enzyme activity which was not significantly different

($P < 0.05$) when compared to piracetam treated group, which implies that the two

group treatments have equipotent therapeutic activity.

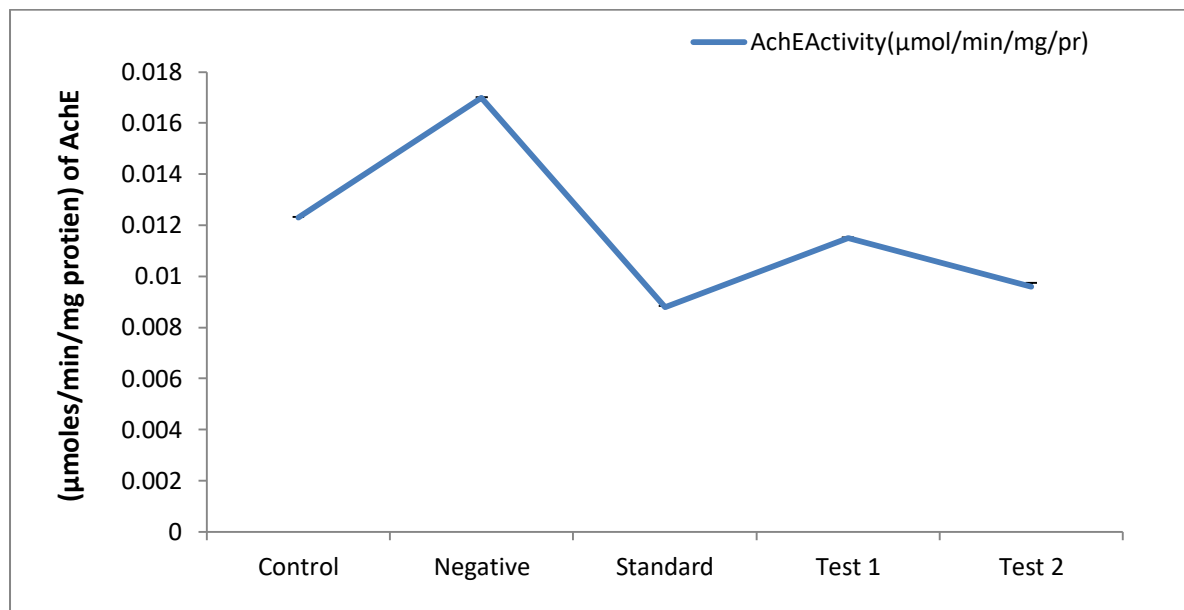


Figure 4: Effect of Piracetam and *Piper nigrum* on acetylcholine esterase activity in aluminum chloride treated rats.
Values are mean $\pm$ SEM.

The result of this study indicates that chronic administration of aluminum chloride leads in progressively increase in deterioration of spatial memory observed in both Morris water maze and elevated plus maze apparatus. Experimentally, it has been exhibited that intra-cerebral administration of aluminum chloride causes learning deficits in Morris water maze in rabbits (Rabe, Lee, Shek, & Wisniewski, 1982) that resembles to our findings. Chronic treatment with aluminum leads to impairment of glutamate- NO-cGMP pathway in the cerebellum of rats [6]. It can explain the neurobehavioral deficits and memory impairment observed. In our present study findings behavioral alterations were improved in *piper nigrum* as well as piracetam treated group. Administration of *piper nigrum* on different dose (50mg & 100mg/kg) significantly ($P < 0.05$) potentiated their protective effects (shortened transfer latency and retention performance of the spatial navigation task) when compared to control group.

Since hippocampus has abundant inputs from the basal forebrain cholinergic system and thus acetylcholine (ACh) plays a crucial role in learning and memory [7]. Acetylcholine is degraded by the enzyme acetyl cholinesterase, terminating the physiological action of the neurotransmitter. Alzheimer's disease affects cholinergic system resulting in increased activity of acetyl cholinesterase (Dai, Buijs, Kamphorst, & Swaab, 2002). Experimentally aluminum has been shown to increased acetyl cholinesterase in mouse brain (Zatta, Ibn-Lkhatay-Idrissi, Zambenedetti, Kilyen, & Kiss, 2002). In the present study, chronic Aluminum chloride treatment caused a significant increase in the acetyl cholinesterase activity leading to memory deficits, when compared to piracetam treated group, which implies that the two group treatments have equipotent therapeutic activity.

Oxidative stress is therefore implicated as one of the causes of cognitive impairment

[8]. Besides, chronic stress is said to promote oxidative stress and demolish antioxidant defense system of the brain which may form the basis for impaired memory [9]. However, administration of *piper nigrum* (50mg & 100mg/kg) significantly ($P < 0.05$) potentiated their protective effects (decreased MDA levels) as compared to their effects alone and control group. Thus the results strongly support our hypothesis that the memory deficits observed after chronic aluminum chloride treatment might have arisen as a result of mitochondria dysfunction, which is the key factor for the production of reactive oxygen species (ROS) generation and ultimately causing oxidative injury to neurons, which could therefore be prevented by antioxidant treatment. Piracetam reversed biochemical impairments (oxidative stress parameters) caused by aluminum. Piracetam was found to improve the behavioral performance in aluminum exposed animals but some side effects reported in connection with chronic use of piracetam include irritability, nervousness, agitation, anxiety and sleep disturbances. Hence *Piper nigrum* can play a role in treatment of memory deficits and A.D, which was indicated through behavioral and oxidative stress parameters.

CONCLUSION

From the epidemiologic and experimental studies reported, there is sample evidence which supports the fact that aluminum plays a pivotal role in the neuropathology of AD. It concludes the fact that chronic exposure to aluminum leads to cognitive dysfunction and related oxidative damage of brain. It clearly demonstrates that *piper nigrum* showed protective effects against aluminum-induced cognitive dysfunction and oxidative damage in rats. Further warrants the need for molecular studies to elucidate the mechanisms underlying the drug and food interaction.

COMPETING INTERESTS

None

ACKNOWLEDGEMENT

I would like to thank Mr. Mohd Ruman Khan, Ms. Huma Noor and Dr. S. Ramasamy without whom this work had never been completed. I am also highly grateful to Mr. Mohd Asif Khan who encouraged and helped me in the writing of this study.

REFERENCES

1. Retz W, Gsell W, Munch G, Rosler M, Riederer P (1998), "Free radicals in Alzheimer's disease", *J Neural Transm Suppl.*, Volume 54, pp. 221–236.
2. Bindu D Madhavi, K L. Senthil Kumar, V. Palanivel (2012), "Evaluation of Cognitive Enhancing Activity of *Piper Longum* Fruit Extract on Albino Rats", *International journal of pharmaceutical and chemical sciences*, Volume 1, Issue 3, pp. 1057–1061.
3. Ramachandran S, A.S. Sanjay, M.D. Dhanaraju (2013), "Antiamnesic effect of Piracetam potentiated with *Embllica officinalis* and *Curcuma longa* in Aluminum induced neurotoxicity of Alzheimer's disease", *International Journal of Advanced Research*, Volume 1, Issue 7, pp. 185–196.
4. Kumar A, Dogra S, Prakash A (2009), "Protective effect of curcumin (*Curcuma longa*), against Aluminum toxicity: Possible behavioral and biochemical alterations in rats", *Behavioural Brain Research*, Volume 205, pp. 384–390.
5. Ellman, G. L (1959), "Tissue sulfhydryl groups. Arch Biochem. Biophys", Volume 82, Issue 1, pp. 70–77.
6. Canales J J, Corbalan R, Montoliu C, Llansola M, Monfort P, Erceg S. Felipe V (2001), "Aluminum impairs the glutamate-nitric oxide-cGMP pathway in cultured neurons and in rat

- brain in vivo: molecular mechanisms and implications for neuropathology”, *J Inorg Biochem.*, Volume 87, Issue 1-2, pp. 63–69.
7. Prado V F, Martins-Silva C, de Castro B M, Lima R F, Barros D M, Amaral E, Prado M A (2006), “Mice deficient for the vesicular acetylcholine transporter are myasthenic and have deficits in object and social recognition Neuron”, Volume 51, Issue 5, pp. 601–612.
 8. Keller JN, Schmitt FA, Scheff SW, Ding Q, Chen Q, Butterfield DA, Markesbery WR (2005), “Evidence of increased oxidative damage in subjects with mild cognitive impairment”, *Neurology*, Volume 64, Issue 7, pp. 1152–1156.
 9. Lucca G, Comim CM, Valvassori SS, Reus GZ, Vuolo F, Petronilho F, Quevedo J (2009), “Effects of chronic mild stress on the oxidative parameters in the rat brain”, *Neurochem Int.*, Volume 54, Issue 5-6, pp. 358–362.