International Journal of Pharmacology

Research Article
Neuroprotective Effect of Protocatechuic Acid Through MAO-B Inhibition in Aluminium Chloride Induced Dementia of Alzheimer's Type in Rats

[1]Kangtao, [1]Yangqian and [2]Souravh Bais

[1]Shanxi Provincial People's Hospital, No. 256, Youyi West Road, Xi'an, 710068 Shanxi, People's Republic of China
[2]Department of Pharmacology, Rayat Institute of Pharmacy, Railmajra, SBS Nagar District, 144506 Punjab, India

Abstract

Background and Objective: Recent studies have revealed that monoamine oxidase-B (MAO-B) inhibitors effective in treating Alzheimer's disease (AD), with a promotable extension of life span. It modulates nitric oxide (NO), which contribute to cognitive function in AD. The present study investigated the potential of protocatechuic acid (PCA) as MAO-B inhibitor and its effect on release of MAO, TNF-α, acetylcholine esterase enzyme, in cognitive dysfunctions associated with experimental dementia in rats. **Materials and Methods:** Aluminium chloride (AlCl$_3$) was administered at a dose of 175 mg kg^{-1} per oral (p.o) for a period of 25 days in rats and then divided into different groups, i.e., standard group, negative control and two groups of PCA, (at a dose of 10 and 20 mg kg^{-1}, p.o.), where these groups treated and observed until the 35th day of experimental trial. Morris water mazes (MWM), photoactometer test were performed on 5th, 16th, 26th and 36th day to access learning, memory and ambulatory movements. Later, the animals were sacrificed for biochemical and histopathological studies. Extent of oxidative stress was measured by estimating the levels of Glutathion (GSH), superoxide dismutase (SOD), nitrite, catalase. Brain acetylcholine esterase activity and MAO-A, MAO-B were also estimated. The brain levels of TNF-α was measured as marker of inflammation. **Results:** AlCl$_3$ produced a marked decline in MWM performance and ambulatory movements' of animals, reflecting impairment of memory and learning. PCA treatment significantly modulates AlCl$_3$ induced memory deficits, biochemical and pathological alterations. **Conclusion:** The findings demonstrated that the memory restorative ability of PCA may be attributed due to its anti-cholinesterase, anti-oxidative and anti-inflammatory potential.

INTRODUCTION

Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disease, leads to death of neuronal cells. This manifested by deterioration in memory and cognition, impairment in performing activities of daily living and many behavioral neuropsychiatric illnesses and consequences are marked impairment of memory and abstract reasoning, with preservation of vision and movement[1].

MAO-B inhibitors might offer an alternative for AD therapy as they have a long history of clinical use and are considered safe and nontoxic[2]. This article evaluates the current role of PCA as MAO-B inhibitor in the treatment of AD and explores the concept that actions other than antioxidants, MAO inhibition and TNF suppression may also contribute to therapeutic efficacy of the drug. MAO exists in two forms, MAO-A and MAO-B, coded by two distinct gene loci[3], with different patterns of tissue distribution. MAO-A pre-ferentially deaminates serotonin (5-HT) and is more sensitive to inhibition by clorgyline, whereas, MAO-B deaminates pre-ferentially b-phenylethylamine and is inhibited by drugs such as L-deprenyl. The optical isomer D-deprenyl is biologically less active than L-deprenyl. Clinically the most important monoamine that is elevated by L-deprenyl is dopamine, which is a substrate for both MAO-A and MAO-B. L-Deprenyl (selegiline) has been used as an effective adjuvant to L-DOPA in the treatment of Parkinson's disease. It has also been reported that L-deprenyl by itself can delay the onset of disability associated with early untreated Parkinson's disease. L-Deprenyl also prevents MPTP-induced neurotoxicity and Parkinson-like symptoms in animals[2,4], so the compound with selective inhibition or non selective approach may be the symptomatic cure in AD and Parkinson's disease[5]. The more reactive oxygen species (ROS) will lead to imbalance between the formation of cellular oxidants and the anti-oxidative processes. Oxidative metabolism of acetylcholine plays an important role in AD pathogenesis[6,7]. PCA is traditionally reported as a significant antioxidant and anti inflammatory property, so the aim of present study is focused on modulation of various neurochemicals by PCA in brain tissues. PCA is reported for different pharmacological activities like antioxidant and free-radical scavenging property, cardioprotective, antihypertensive and cardiovascular remodeling[8,9], anti-hyperglycemic action[10], anti-cancer activity[11], anti-ulcer activity[12], anti-aging[13], anti-inflammatory and analgesic[14], anti-fibrotic effects[15], anti-viral activity[16] nephro-protective and hypolipidemic activity[17-22] (Fig. 1). However the diverse pharmacology of this compound is still to be unexplored. The present study investigated the potential
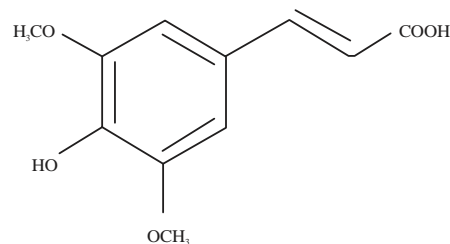

Fig. 1: Structure of PCA

of PCA as monoamine oxidase (MAO-B) inhibitor, in cognitive dysfunctions associated with experimental dementia.

MATERIALS AND METHODS

This study was conducted jointly by in RBGI, India and Shanxi Provincial People's Hospital.

Drugs and chemicals: PCA (Pubchem CID 637775) was purchased from Sigma Aldrich (S. and G. Lab Supplies). selegiline was procured as a gift sample from Sun pharmaceutical Pvt. Ltd. Baddi (H.P.) India. Chemicals like aluminium chloride, DTNB, acetylthiocholine iodide, trichloroacetic acid, thiobarbituric acid, sodium carboxy methyl cellulose was procured from Himedia Pvt. Ltd. (Mumbai) S.D. Fine Chemicals Ltd. (Mumbai). Solvents like methanol, chloroform, dichloromethane, tween 80, n-butanol and ethyl acetate were of analytical grade (AR).

Animals: Age matched young wistar albino rats of either sex, weighing 120-150 g were selected as the study. The animals were kept in the paddy husk as bedding material. Husk changed every day. The animals were housed in a group of 6 per polypropylene cages kept under controlled room temperature ($25\pm1^{\circ}$C) in 12 h light-dark cycle. The rats were allowed free access to food (Standard pallet) and water. The experiment was conducted in a noise-free environment between 9:00 AM to 2:00 PM. All procedures were approved and carried out as per the guidelines of animal experimentation (IAEC/PO/Re/S/1/5).

Acute toxicity study (LD$_{50}$): LD$_{50}$ was determined according to the guidelines of organization for economic cooperation and development (OECD) following the up and down method (OECD guideline no. 423) and fixed dose method (OECD guideline no. 420). Based on this guideline a limit test was to categorize the toxicity class (LD$_{50}$) of the compound. The limit test was performed at 2000 mg kg^{-1}, p.o. A dose range of
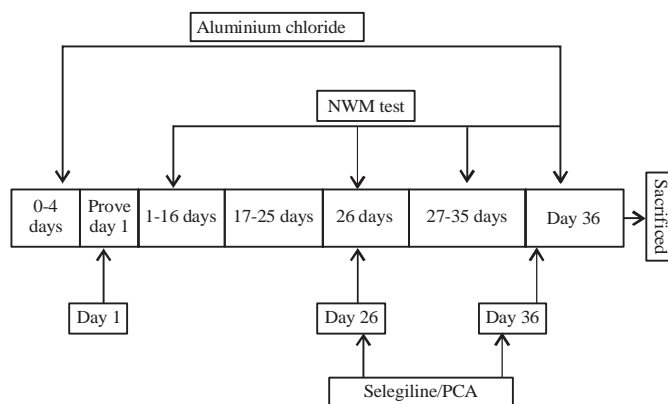
Fig. 2: Experimental protocol for aluminium chloride induced dementia

Table 1: Sequence of training trials on Morris water maze

Days	Sequence			
1	S1	S2	S3	S4
2	S2	S3	S4	S1
3	S3	S4	S1	S2
4	S4	S1	S2	S3

10, 20 mg kg^{-1} was selected for the pharmacological activity. For all the studies overnight fasted animals were used.

Experimental design: On prove day (day 5-consider as day 1), randomized animals were divided randomly into experimental groups (n = 8) (overall protocol-(5+36 = 42 days)). Control group received a normal saline (5 mL kg^{-1}, p.o.). AlCl$_3$, selegiline and PCA suspensions were made freshly at a time of dosing. Morris water mazes (MWM), photoactometer test were performed on 5th, 16th, 26th and 36th day to access learning, memory and ambulatory movements. Later, the animals were sacrificed for biochemical and histopathological studies. Extent of oxidative stress was measured by estimating the levels of glutathion (GSH), superoxide dismutase (SOD), nitrite, catalase. Brain acetylcholine esterase (Ache) activity and monoamine oxidase (MAO-A, MAO-B) were also estimated. The brain levels of TNF-α was measured as marker of inflammation (Fig. 2, Table 1).

Group I: Control group (normal saline (0.9% NaCl)-5 mL kg^{-1}, p.o., from 6th-36th day); rats were administered distilled water (10 mL kg^{-1}, p.o.) 30 min before acquisition trial was conducted from day 1-4 and 30 min before retrieval trail was conducted on day 5.

Group II: Untreated AlCl$_3$-affected rats; rats were administered with AlCl$_3$ suspension (175 mg kg^{-1}) orally from day 0 (i.e., 24 h after the completion of retention trial on day 5) to 36 days.

Group III: Selegiline-treated AlCl$_3$-affected rats; after 25 days, rats were administered standard drug suspensions (selegiline-2.5 mg kg^{-1}), (1% aqueous solution of Tween 80) orally from day 26-36.

Group IV and V: PCA-Treated AlCl$_3$-affected rats; after 25 days, rats were administered PCA suspension (at two doses 10 mg and 20 mg kg^{-1} to respective group) (1% aqueous solution of Tween 80) orally from day 26-36.

Memory assessment (water maze test): The water maze consisted of a circular tank (150 cm diameter and 40 cm height). Water pool was divided into four equally spaced quadrants North-East (NE), South-East (SE), South-West (SW) and North-West (NW) along the circumference of the pool. An escape platform (10 cm diameter) submerged 2cm below the water surface and was placed in NW quadrant. Rats were trained to locate the hidden platform at a fixed location in NW quadrant. All rats were subjected to one session of four trials per day for five consecutive days (0-5th day). During each trial, the animal was placed in each quadrant to eliminate quadrant effects. All rats were left in the platform for 30 sec and then removed and towel dried. Rats failing to find the platform within 60 sec were guided to the platform (Table 1). In day 5 (Probe day/zero day), 24 h after previous training, escape platform was removed and probe trial was conducted. The cut-off time for animal to swim was set to 60 sec before the end to the session. Similarly, the retention trials were conducted on day 5th 16th, 26th and day 36th on different groups to evaluate memory. Time elapsed in escaping to the NW quadrant, i.e. escape latency time (ELT) and total time (TT) time spent in NW quadrant, was measured during the retention trials[20] (Fig. 3).
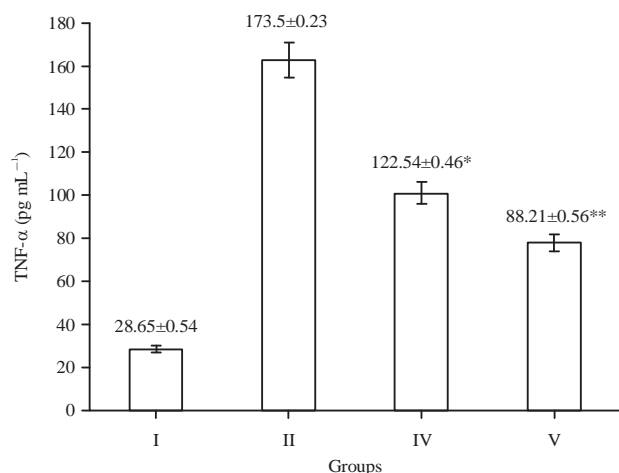
Fig. 3: Effect of PCA on TNF-α level in brain cortex. All values were represented as Mean±SEM. Data are Mean±SEM values, n = 8. Data were analyzed by one-way ANOVA followed by Tukey's-Kramer's multiple comparisons test. *p<0.05, **p<0.01, Where I: Control, II: Untreated AlCl₃-affected rats, IV: Test A-PCA (10 mg kg⁻¹, p.o.)-treated AlCl₃-affected rats and V: Test B-PCA (20 mg kg⁻¹, p.o.)-treated AlCl₃-affected rats

Photoactometer: Animals were assessed for locomotor activity using digital photoactometer. The ambulatory movements were recorded over a period of 10 min and expressed in terms of total photo beam counts for 10 min per animal. Locomotor activity (L.A.) was assessed on day 0, 5th, 11th, 26th and day 36th through Morris's water maze[20].

Evaluation
Brain tissue sampling and preparation: After 24 h of the experimental period (after 35 days), the animals were sacrificed and their brains were removed and weighed. The whole brain was washed thoroughly with ice-cold isotonic saline. A 10% issue homogenate was prepared in 0.1 M phosphate buffer (pH 8, stored 2-8°C) for various neurochemical estimations and other anti oxidative parameters.

Biochemical assessment
Acetyl choline esterase (AchE) activity: This activity was measured by Ellman *et al.*[21] method.

Estimation of glutathione (GSH): GSH level was measured by the method described by Ellman *et al.*[21] with slight modifications. The absorbance was measured spectrophoto

metrically at 412 nm (X-Rite 640B spectrophotometer). Different concentration of GSH standard was also processed similarly to prepare a standard curve (1-50 μg) simultaneously. Results were expressed as nmol of GSH mg⁻¹ of protein.

Estimation of catalase: This activity was determined by Armstrong *et al.*[22] method.

Estimation of superoxide dismutase (SOD): SOD enzyme activity was measured by the method described by Ohkawa *et al.*[23] and Yagi[24].

Estimation of TNF-α: The TNF-α level was estimated by ELISA kit.

Estimation of MAO-A and MAO-B: Estimation of MAO activity; it was assayed by the method described by Charles and McEwen[25].

Estimation of nitrite: It was measured by Nazmun method[26].

Histopathological examination: The second portion of each brain was fixed in formalin buffer (10%) for 24 h. The brains were washed in tap water and then dehydrated using serial dilutions of alcohol (methyl, ethyl and absolute ethyl). Specimens were cleared in xylene and embedded in paraffin in a hot air oven at 56°C for 24 h. Paraffin bees wax blocks were prepared for sectioning at 4 μm using a microtome. The obtained tissue sections were collected on glass slides, deparaffinized and stained with hematoxylin and eosin stains[27] for histopathological examination using a light microscope.

Statistical analysis: The data were expressed as Mean±SEM. In all the tests, the criterion for the statistical significance was set at p<0.05. The data for all studies were analyzed using one-way ANOVA followed by Tukey's-Kramer's multiple comparisons test.

RESULTS

Acute toxicity study: PCA did not show any sign and symptom of toxicity and mortality up to 2000 mg kg⁻¹, p.o.

Effect of PCA on Aluminium chloride induced behavioural parameters
Effect on ELT and time spent in target quadrant using the Morris water maze: The rats spent more time in the target

Table 2: Effect of PCA on aluminium chloride induced dementia of Alzheimer's type in rat using Morris water maze (escape latency time)

	ELT (sec)			
Groups/treatment	5th day	16th day	26th day	36th day
I (control group)	3.540±0.60	4.08±0.30	3.03±0.3	4.15±0.6b***
II (untreated AlCl₃-affected rats)	3.879±0.10	15.00±0.07	26.28±0.27	32.12±0.0a***
III (selegiline-treated AlCl₃-affected rats)	3.980±0.13	13.10±0.28	19.32±0.28	9.10±0.1(ab)***
IV (PCA (10 mg kg⁻¹, p.o.)-treated AlCl₃-affected rats)	4.070±0.07	11.00±0.45	18.83±0.22	11.12±0.1(ab)***
V (PCA (20 mg kg⁻¹, p.o.)-treated AlCl₃-affected rats)	3.970±0.04	13.14±0.10	25.17±0.06	7.13±0.23(ab)***

Data are Mean±SEM values, n = 8. Data were analyzed by one-way ANOVA followed by Tukey's-Kramer's multiple comparisons test. ***$p<0.001$. a: Compared with control, b: Compared with inducer, ELT: Escape latency time

Table 3: Effect of PCA on aluminium chloride induced dementia of Alzheimer's type in rat using Morris water maze

	T.T (sec)			
Groups/treatment	5th day	16th day	26th day	36th day
I (control group)	3.21±1.0	3.98±0.04	3.55±0.05	3.42±0.18b***
II (untreated AlCl₃-affected rats)	3.35±0.5	3.15±0.15	2.99±0.01	1.20±0.06a***
III (selegiline-treated AlCl₃-affected rats)	3.12±0.5	2.18±0.07	1.78±0.07	2.50±0.11aⁿˢb***
IV (PCA (10 mg kg⁻¹, p.o.)-treated AlCl₃-affected rats)	4.07±0.12	2.90±0.05	1.50±0.01	2.41±0.75a* b***
V (PCA (20 mg kg⁻¹, p.o.)-treated AlCl₃-affected rats)	3.64±0.11	3.12±0.05	1.70±0.06	2.70±0.27a* b***

Data are Mean±SEM values, n = 8. Data were analyzed by one-way ANOVA followed by Tukey's-Kramer's multiple comparisons test. *$p<0.05$, ***$p<0.001$ and ⁿˢ$p>0.05$. a: Compared with control, b: Compared with inducer, ⁿˢNot significant, T.T: Total time

Table 4: Effect of PCA on aluminium chloride induced dementia of Alzheimer's type in rat using photoactometer

	L.A (count/10 min)			
Groups/treatment	5th day	16th day	26th day	36th day
I (Control group)	148.32±1.40	149.01±0.09	150.09±0.70	149.80±0.63b***
II (untreated AlCl₃-affected rats)	147.17±1.30	96.09±0.20	79.08±1.29	95.20±1.02a***
III (selegiline-treated AlCl₃-affected rats)	147.25±1.71	89.95±0.05	72.13±2.02	134.40±2.5aⁿˢ b***
IV (PCA (10 mg kg⁻¹, p.o.)-treated AlCl₃-affected rats)	149.16±0.20	86.07±0.09	84.10±0.09	120.02±1.05a** b***
V (PCA (20 mg kg⁻¹, p.o.)-treated AlCl₃-affected rats)	147.85±3.50	89.20±0.18	83.23±2.50	138.20±3.58aⁿˢ b***

Data are Mean±SEM values, n = 8. Data were analyzed by one-way ANOVA followed by Tukey's-Kramer's multiple comparisons test. **$p<0.01$, ***$p<0.001$ and ⁿˢ$p>0.05$. a: Compared with control, b: Compared with inducer, ⁿˢNot significant, LA: Loco motor activity

quadrant (NW) during retention trial on Probe day (Day 5). Control group showed the normal retrieval of memory on 36th day. AlCl₃ treated rats (Group II) significantly raised ELT and reduced, time spent in the target quadrant (NW). Untreated AlCl₃-affected rats (Group III) significantly raised the ELT and reduced the TT during the retention trial on 16th and 26th day, but the selegiline-treated AlCl₃-affected group resulted, a decrease in ELT and increased in TT during retention trial on 36th day. PCA-treated AlCl₃-affected (10 and 20 mg kg⁻¹) rats showed, decrease in ELT and increase in TT during retention trial on 36th day Table 2 and 3.

Effect on locomotor activity using photoactometer: Locomotor activities (ambulatory movements) of rats were recorded for a period of 10 min and expressed in terms of total photo beam counts for 10 min/animal. Control group rats (Group I) showed the normal locomotor activity on 36th day (149.8±0.63 counts/10 min). Aluminium chloride treated rats (Group II) resulted in a significant decreased in locomotor activity on 16th, 26th and 36th day ($p<0.001$) as

compared to control group rats (Group, I). Untreated AlCl₃-affected rats (Group III) resulted in a significant decreased in locomotor activity on 16th and 26th day, but after selegiline treatment results in a significant increased in locomotor activity on 36th day ($p<0.001$) when compared to AlCl₃ treated rats (Group II). PCA-treated AlCl₃-affected (10/20 mg kg⁻¹) resulted, in a significant increase in locomotor activity ($p<0.001$) when compared to AlCl₃ treated rats (Group II) Table 4.

Effect on biochemical parameters: The levels of AchE, TBARS and nitrite were found elevated in Untreated AlCl₃-affected rats as compared to control group. The animals treated with selegiline showed a significant reduction in the levels of AchE, TBARS and nitrite on 36th day of trial as compared with untreated AlCl₃-affected group. The elevated levels of AchE, TBARS and nitrite were declined on treatment with PCA in a dose-dependent manner, as compared with untreated AlCl₃-affected group. The levels GSH, catalase and SOD were significantly decreased in untreated AlCl₃-affected rats as

Table 5: Effect of PCA on various biochemical parameters in rats' brain

Groups/treatment	AchE (nM L^{-1} min^{-1} g^{-1} of tissue)	GSH (Nm mg^{-1} of protein)	TBARS (Nm mg^{-1} of protein)	Nitrite (Nm mg^{-1} of protein)	SOD (units mg^{-1} of protein)	Catalase (μM of H_2O_2 decomposed min^{-1} mg^{-1} of protein)
I (Control)	4.80 ± 0.19 b***	9.15 ± 0.43b***	1.91 ± 0.15b***	1.70 ± 0.25b***	48.80 ± 3.5b***	1.04 ± 0.023b***
II (Inducer) $AlCl_3$	8.81 ± 0.19 a***	3.20 ± 0.27a***	4.36 ± 0.04a***	4.53 ± 0.23a***	11.76 ± 2.31a***	0.23 ± 0.045a***
III (Standard)	5.78 ± 0.28 a[ns] b***	7.93 ± 0.22a* b***	2.64 ± 0.04(ab)***	2.39 ± 0.14a[ns] b***	43.34 ± 1.54 a[ns] b***	0.85 ± 0.015a** b***
IV	6.70 ± 0.39a* b***	5.16 ± 0.19(ab)***	3.29 ± 0.037(ab)***	3.10 ± 0.27(ab)**	32.80 ± 2.81a** b***	0.69 ± 0.050(ab)***
V	6.44 ± 0.38a** b***	6.94 ± 0.12(ab)***	2.90 ± 0.052(ab)***	2.84 ± 0.12a* b***	39.07 ± 3.77a[ns] b***	0.80 ± 0.028(ab)***

Group I: Control Group, Group II: Untreated $AlCl_3$-affected rats, Group III: Selegiline-treated $AlCl_3$-affected rats, Group IV: PCA (10 mg kg^{-1}, p.o.)-treated $AlCl_3$-affected rats, Group V: 20 mg kg^{-1}, p.o.-treated $AlCl_3$-affected rats. Data are Mean$\pm$SEM values, n = 8. Data were analyzed by one-way ANOVA followed by Tukey's-Kramer's multiple comparisons test. *p<0.05, **p<0.01, ***p<0.001 as compared with inducer, [ns]Not significant

Table 6: Effect of PCA on $AlCl_3$ induced toxicity with reference to change in body and brain weight

Groups/treatment	Change in body weight (initial day b.wt.-final day b.wt.) (g)		Brain weight (g)		Brain/body weight ratio/100 g of rat	
	Male	Female	Male	Female	Male	Female
I (control group)	0.54 ± 0.21	-0.51 ± 0.34	1.24 ± 0.40	1.32 ± 0.76	1.12	1.09
II (untreated $AlCl_3$-affected rats)	1.90 ± 0.25	0.29 ± 0.10	0.89 ± 0.25	0.78 ± 0.23	0.74	0.51
III (selegiline-treated $AlCl_3$-affected rats)	-2.89 ± 0.56*	-2.58 ± 0.78*	0.98 ± 0.32*	1.05 ± 0.56**	0.94	0.98
IV (PCA (10 mg kg^{-1}, p.o.)-treated $AlCl_3$-affected rats)	-1.54 ± 0.56*	-2.89 ± 0.87	1.08 ± 0.54*	1.20 ± 0.21	0.89	0.85
V (PCA (20 mg kg^{-1}, p.o.)-treated $AlCl_3$-affected rats)	-3.02 ± 1.03**	-2.54 ± 0.89*	1.30 ± 0.52**	1.05 ± 0.35**	0.79	0.98

Data are Mean$\pm$SEM values, n = 8. (male-4, Female-4). Data were analyzed by one-way ANOVA followed by Tukey's-Kramer's multiple comparisons test. *p<0.05, **p<0.01, as compared with inducer

Table 7: Effect of PCA on MAO level in brain tissues

Groups/treatment	MAO-A (nmol mg^{-1} protein h)	MAO-A inhibition (%)	MAO-B (nmol mg^{-1} protein h)	MAO-B inhibition (%)
I (control group)	31.10 ± 0.12		24.6 ± 1.06	
II (untreated $AlCl_3$-affected rats)	38.10 ± 0.34	00.00	33.7 ± 0.65	00
III (selegiline-treated $AlCl_3$-affected rats)	21.47 ± 0.14*	34.80	25.1 ± 0.14*	50
IV (PCA (10 mg kg^{-1}, p.o.)-treated $AlCl_3$-affected rats)	26.80 ± 0.32*	18.00	29.2 ± 0.75	28
V (PCA (20 mg kg^{-1}, p.o.)-treated $AlCl_3$-affected rats)	21.90 ± 0.88**	32.00	26.2 ± 0.20*	45

Data are Mean$\pm$SEM values, n = 8. Data were analyzed by one-way ANOVA followed by Tukey's-Kramer's multiple comparisons test. *p<0.05, **p<0.01, as compared with inducer

compared to a control group. The animals treated with selegiline showed a significant increase in the levels of GSH, catalase and SOD on 36th day of trial as compared with untreated $AlCl_3$-affected group. The reduced levels of GSH, catalase and SOD were increased significantly with a dose of 20 mg kg^{-1} of PCA as compared with untreated $AlCl_3$-affected group (Table 5).

Effect on brain TNF-α level: All $AlCl_3$ treated rats showed an increase in TNF-α level (Fig. 3) in the brain. PCA treated rats showed a significant reduction in TNF-α levels as compared to control.

Effect on body weight and brain's weight: Untreated $AlCl_3$-affected rats showed a decrease in body weight and brain weight while other groups did not show any marked deference in body and brain weight of rats (Table 6).

Effect of PCA on MAO-A and MAO-B levels: The effects of PCA on MAO concentration were shown in Table 7. The MAO-A and MAO-B activities in a control group were 31.1 ± 0.12 and 24.6 ± 1.06 nmol mg^{-1} of protein, respectively. PCA at a dose of 10 mg kg^{-1}, p.o. showed 18% (MAO-A) and 28% (MAO-B) inhibition. PCA at a dose of 20 mg kg^{-1}, p.o. showed a marked inhibition in both type, MAO-A (32%) and MAO-B (45%).

Histopathological changes in brain: As shown in Fig. 4, the control animals (Fig. 4a), had neuronal cells were visualised normally under microscope; cells are active and relatively packed with prominent nuclei. $AlCl_3$ treated rats showed (Fig. 4b) necrosis of cells and more neuronal vacuolation. The selegiline treated rats (Fig. 4c), showed a mild necrosis and deformed nuclei. The PCA treated rats (10 mg kg^{-1}, p.o) showed slight neuronal decongestion and the rats treated with high dose of PCA (20 mg kg^{-1}, p.o) showed, no cell decongestion cell deformity, Test A (Fig. 4d).
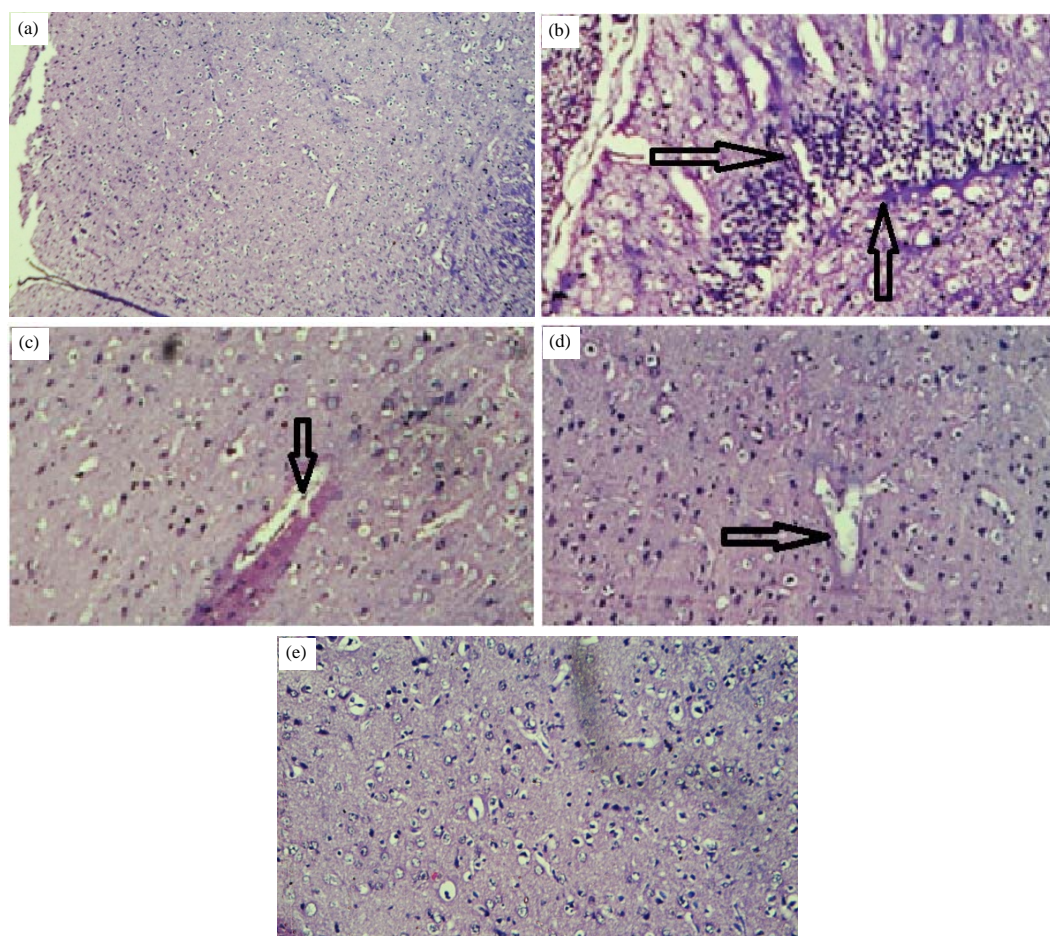
Fig. 4(a-e): Histopathology of brain (cerebral cortex) showing neuronal degeneration and inflammation. a: Control, (group I), b: Negative (group II), c: Standard (group III), d: Test A (group IV) and e: Test B (group V)

DISCUSSION

Administration of chronic AlCl₃ causes severe memory deficits in rats as predicted by this study. Followed by PCA treatment, showed an improvement in memory deficits. Aluminium as metal ions commonly present in drinking water, soil and tooth paste, moreover, it is used to manufacture cooking utensils[28]. Literature reports showed that its chronic use leads to oxidative deterioration of cellular lipids, proteins and DNA[29]. Lipid peroxidation is one, can cause a tissue damage under its chronic use[30,31] therefore, aluminium can be considered as a contributing factor in AD. After chronic administration, it accumulates in all brain regions with greater quantity in cortex and hippocampus[32,33] and damaged it. These regions play an important role in learning and memory[34].

In this study, a significant oxidative stress in aluminium treated rats was observed, as indicated by an increase in lipid peroxidation, nitrite levels, decrease in reduced glutathione levels and catalase and superoxide dismutase activity. This could be due to the reduced axonal mitochondria turnover, disruption of Golgi and reduction of synaptic vesicles induced by aluminium treatment and release of oxidative products like malondialdehyde TBARS, carbonyls, peroxynitrites and enzymes like superoxide dismutase within the neurons[35]. Moreover the groups treated with PCA showed significant antioxidant activity[8] against the neuronal damage caused by aluminium.

Impaired cholinergic transmission is one of the complications seen in the etiopathogenesis of memory deficit in AD. The neurodegeneration in frontal cortex and hippocampus areas within the brain[36] resulting in impaired cholinergic transmission by two ways. Firstly, (i) decline in Ach release, (ii) decreased choline acetyltransferase activity (ChAT), which results in the scarcity of acetylcholine[37,38]. Secondly, elevated acetyl cholinesterase (AchE) enzyme further adds to
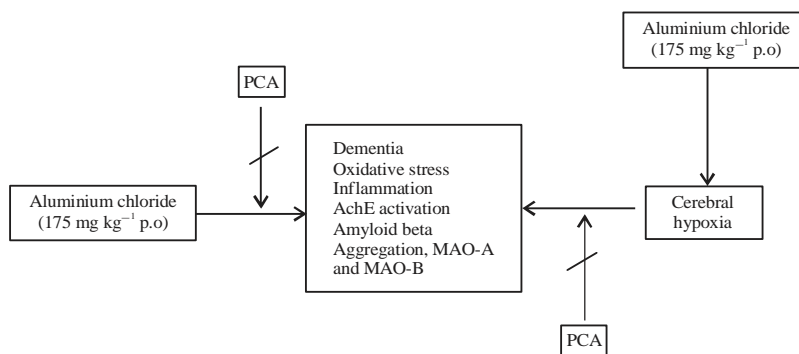
Fig. 5: Proposed mechanism for therapeutic effect of PCA

scarcity of Ach at the synapse by degrading the available Ach[36]. This degradation of Ach is abolished by PCA (AchE inhibitor) so it's effective in AD through improvement in cholinergic transmission.

PCA (hydroxycinnamic acid) belongs to phenolic acids with bioactive carboxylic acids; this class mainly includes caffeic acid, ferulic acid and PCA[39,40]. It is a frequent phytochemical in the human diet[12]. It's a potent inhibitor of an enzyme AchE[41,42]. This could be a possible mechanism by it inhibits cerebral hypoxia and improved memory disturbance by activating cholinergic function acetylcholine (Ach) and choline acetyltransferase (ChAT)[43].

Chronic aluminium administration has been reported to result in behavioural changes such as cognitive[44] and locomotor impairment[45]. This study also showed an impaired behaviour of rats treated with aluminium. The cognitive deficit is evident to declined performance in Morris water maze test[46] and radial arm maze test[47]. In current study, aluminium treatment resulted in behavioral changes such as a spatial memory deficit. Selegiline and PCA antagonized the spatial memory deficit caused by aluminium. This suggested that the neuro protective role of PCA in correcting cognitive dysfunction was associated with aluminium exposure.

Assessment of locomotor activity is a requirement for evaluating any possible CNS depressant/stimulant effect of interventions on animals. Similar to previous reports[43], a decline in locomotor activity in aluminium treated rats was observed, indicating the CNS depressant effect of chronic aluminium exposure. Treatment with Selegiline and PCA corrected the locomotor in-coordination caused by aluminium chloride.

MAO inhibitors are unique in that they exert their protective effect on both vascular and neuronal tissue by increasing nitric oxide (NO) production in brain tissues and cerebral blood vessels. Which is evidentially observed less in

AD brain[44]. So this study also indicated that PCA may play a significant role as MAO inhibitor and increasing NO release and decreasing nitrite in brain tissues.

Histopathological examination of aluminium chloride induced AD brain showed spongy cell, neuronal necrosis. Cells degenerated with small nuclei leading to eosinophilic deposition. However, besides these pathological hallmarks, AD brain exhibited a clear evidence of chronic inflammation and oxidative damage[1,44-47]. These were also thought to play a significant role at the onset and progression of AD. Present study also supported an evidence of inflammation with high concentration of TNF-α in brain tissues as compared with control rats. Administration of PCA in AD rats improved the pathogenesis of AD as demonstrated by an improvement in the behavioral (levels of activity and motor coordination), Inflammatory (TNF-α) and biochemical parameters in the brains, which was further confirmed by an improvement in brain tissue characteristics on histopathological analysis.

Accumulation of free radicals may cause degenerative events of aging such as AD and because PCA showed its potential against this neurotoxic (AlCl₃) agent so it should be used in treatment of dementia or AD (Fig. 5).

CONCLUSION

From the above pharmacological, biochemical and histopathological studies, it has been concluded that PCA showed a significant anti inflammatory activity, antioxidants and neuromodulatory effects against aluminium chloride induced dementia of Alzheimer's type in rats.

SIGNIFICANCE STATEMENT

This study was designed to evaluate the potential role of PCA in memory deficits produced by AlCl₃ and findings of this

study suggested the significant MAO inhibition by PCA which creates further interest among scientific researchers to explore its molecular interactions with this enzyme. This study also mentions the therapeutic benefits of PCA as nutraceuticals in the treatment of neurodegenerative disease.

ACKNOWLEDGMENT

Not disclosed

REFERENCES

1. Thomas, T., 2000. Monoamine oxidase-B inhibitors in the treatment of Alzheimers disease. Neurobiol. Aging, 21: 343-348.
2. Heikkila, R.E., A. Hess and R.C. Duvoisin, 1984. Dopaminergic neurotoxicity of 1-Methyl-4-phenyl-1,2,5,6-tetrahydropyridine in mice. Science, 224: 1451-1453.
3. Shih, J.C., 1991. Molecular basis of human MAO-A and MAO-B. Neuropsychopharmacology, 4: 1-7.
4. Kimura, R. and M. Ohno, 2009. Impairments in remote memory stabilization precede hippocampal synaptic and cognitive failures in 5XFAD Alzheimer mouse model. Neurobiol. Dis., 33: 229-235.
5. Terry, A.V.Jr. and J.J. Buccafusco, 2003. The cholinergic hypothesis of age and Alzheimer's disease-related cognitive deficits: Recent challenges and their implications for novel drug development. J. Pharmacol. Exp. Ther., 306: 821-827.
6. Daiello, L.A., E.K. Festa, B.R. Ott and W.C. Heindel, 2008. Cholinesterase inhibitors improve visual attention in drivers with Alzheimer's disease. Alzheimer's Dementia: J. Alzheimer's Assoc., 4: T498-T498.
7. Liu, Y., G. Fiskum and D. Schubert. 2002. Generation of reactive oxygen species by the mitochondrial electron transport chain. J. Neurochem., 80: 780-787.
8. Sherer, T.B., J.R. Richardson, C.M. Testa, B.B. Seo and A.V. Panov *et al.*, 2007. Mechanism of toxicity of pesticides acting at complex I: Relevance to environmental etiologies of Parkinson's disease. J. Neurochem., 100: 1469-1479.
9. Kakkar, S. and S. Bais, 2014. A review on protocatechuic acid and its pharmacological potential. ISRN Pharmacol. 10.1155/2014/952943.
10. Ciftci, O., O.M. Disli and N. Timurkaan, 2013. Protective effects of protocatechuic acid on TCDD-induced oxidative and histopathological damage in the heart tissue of rats. Toxicol. Ind. Health, 29: 806-811.
11. Scazzocchio, B., R. Vari, C. Filesi, M. D'Archivio and C. Santangelo *et al.*, 2011. Cyanidin-3-O-β-glucoside and protocatechuic acid exert insulin-like effects by upregulating PPARγ activity in human omental adipocytes. Diabetes, 60: 2234-2244.
12. Tanaka, T., T. Tanaka and M. Tanaka, 2011. Potential cancer chemopreventive activity of protocatechuic acid. J. Exp. Clin. Med., 3: 27-33.
13. Kore, K.J., P.P. Bramhakule, R.M. Rachhadiya and R.V. Shete, 2011. Evaluation of antiulcer activity of protocatechuic acid ethyl ester in rats. Int. J. Pharm. Life Sci., 2: 909-915.
14. Shi, G.F., L.J. An, B. Jiang, S. Guan and Y.M. Bao, 2006. *Alpinia* protocatechuic acid protects against oxidative damage *in vitro* and reduces oxidative stress *in vivo*. Neurosci. Lett., 403: 206-210.
15. Li, C., W. Jiang, H. Zhu and J. Hou, 2012. Antifibrotic effects of protocatechuic aldehyde on experimental liver fibrosis. Pharm. Biol., 50: 413-419.
16. Zhou, Z., Y. Zhang, X.R. Ding, S.H. Chen and J. Yang *et al.*, 2007. Protocatechuic aldehyde inhibits hepatitis B virus replication both *in vitro* and *in vivo*. Antiviral Res., 74: 59-64.
17. Lende, A.B., A.D. Kshirsagar, A.D. Deshpande, M.M. Muley, R.R. Patil, P.A. Bafna and S.R. Naik, 2011. Anti-inflammatory and analgesic activity of protocatechuic acid in rats and mice. Inflammopharmacology, 19: 255-263.
18. Borate, A.R., A.A. Suralkar, S.S. Birje, P.V. Malusare and P.A. Bangale, 2011. Antihyperlipidemic effect of protocatechuic acid in fructose induced hyperlipidemia in rats. Int. J. Pharm. Bio Sci., 2: 456-460.
19. Lee, J.H., H.J. Lee, W.C. Choi, S.W. Yoon and S.G. Ko *et al.*, 2009. *Rhus verniciflua* stokes prevents cisplatin-induced cytotoxicity and reactive oxygen species production in mdck-i renal cells and intact mice. Phytomedicine, 16: 188-197.
20. Bais, S., R. Kumari and Y. Prashar, 2017. Therapeutic effect of Sinapic acid in aluminium chloride induced dementia of Alzheimer's type in rats. J. Acute Dis., 6: 154-162.
21. Ellman, G.L., K.D. Courtney, V. Andres Jr. and R.M. Featherstone, 1961. A new and rapid colorimetric determination of acetylcholinesterase activity. Biochem. Pharmacol., 7: 88-95.
22. Armstrong, D.M., M.D. Ikonomovic, R. Sheffield and R.J. Wenthold, 1994. AMPA-selective glutamate receptor subtype immunoreactivity in the entorhinal cortex of non-demented elderly and patients with Alzheimer's disease. Brain Res., 639: 207-216.
23. Ohkawa, H., N. Ohishi and K. Yagi, 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem., 95: 351-358.
24. Yagi, K., 1998. Simple procedure for specific assay of lipid hydroperoxides in serum or plasma. Free. Radic. Antioxid. Protocol., 108: 107-110.
25. Charles, M. and J. McEwen, 1977. Methods in Enzymology. Vol. 17, Part B. Academic Press, New York and London, pp: 692-698.
26. Lyle, N., D. Bhattacharyya, T.K. Sur, S. Munsi, S. Paul, S. Chatterjee and A. Gomes, 2009. Stress modulating antioxidant effect of *Nardostachys jatamansi*. Indian J. Biochem. Biophys., 46: 93-98.

27. Bancroft, J.D., A. Steven and D.R. Turner, 1996. Theory and Practice of Histological Technique. 4th Edn., Churchill Livingstone, New York, Pages: 766.

28. Hansson, O., H. Zetterberg, P. Buchhave, E. Londos, K. Blennow and L. Minthon, 2006. Association between CSF biomarkers and incipient Alzheimer's disease in patients with mild cognitive impairment: A follow-up study. Lancet Neurol., 5: 228-234.

29. Maccioni, R.B., J.P. Munoz and L. Barbeito, 2001. The molecular bases of Alzheimer's disease and other neurodegenerative disorders. Arch. Med. Res., 32: 367-381.

30. Bjertness, E., J.M. Candy, A. Torvik, P. Ince and F. McArthur *et al.*, 1996. Content of brain aluminum is not elevated in Alzheimer disease. Alzheimer Dis. Assoc. Disord., 10: 171-174.

31. Sargazi, M., A. Shenkin and N.B. Roberts, 2006. Aluminium-induced injury to kidney proximal tubular cells: Effects on markers of oxidative damage. J. Trace Elem. Med. Biol., 19: 267-273.

32. Nourooz-Zadeh, J., A. Rahimi, J. Tajaddini-Sarmadi, H. Tritschler, P. Rosen, B. Halliwel and D.J. Betteridge, 1997. Relationships between plasma measures of oxidative stress and metabolic control in NIDDM. Diabetologica, 40: 647-653.

33. Campbell, A., 2002. The potential role of aluminium in Alzheimer's disease. Nephrol. Dial. Transplant, 17: 17-20.

34. Deloncle, R. and O. Guillard, 1990. Mechanism of Alzheimer's disease: Arguments for a neurotransmitter-aluminium complex implication. Neurochem. Res., 15: 1239-1245.

35. Rodriguez-Puertas, R., A. Pazos, J.J. Zarranz and J. Pascual, 1994. Selective cortical decrease of high-affinity choline uptake carrier in Alzheimer's disease: An autoradiographic study using ^{3}H-hemicholinium-3. J. Neural Transm.-Parkinson's Dis. Dementia Sect., 8: 161-169.

36. Hammond, P. and S. Brimijoin, 1988. Acetylcholinesterase in Huntington's and Alzheimer's diseases: Simultaneous enzyme assay and immunoassay of multiple brain regions. J. Neurochem., 50: 1111-1116.

37. Fishman, E.B., G.C. Siek, R.D. MacCallum, E.D. Bird, L. Volicer and J.K. Marquis, 1986. Distribution of the molecular forms of acetylcholinesterase in human brain: Alterations in dementia of the Alzheimer type. Ann. Neurol., 19: 246-252.

38. Niciforovic, N. and H. Abramovic, 2014. Sinapic acid and its derivatives: Natural sources and bioactivity. Compr. Rev. Food Sci. Food Saf., 13: 34-51.

39. Bunzel, M., J. Ralph, H. Kim, F. Lu and S.A. Ralph *et al.*, 2003. Sinapate dehydrodimers and sinapate-ferulate heterodimers in cereal dietary fiber. J. Agric. Food Chem., 51: 1427-1434.

40. He, L., H.T. Li, S.W. Guo, L.F. Liu, J.B. Qiu, F. Li and B.C. Cai, 2008. Inhibitory effects of sinapine on activity of acetylcholinesterase in cerebral homogenate and blood serum of rats. China J. Chin. Mater. Med., 33: 813-815.

41. Ferreres, F., F. Fernandes, C. Sousa, P. Valentao, J.A. Pereira and P.B. Andrade, 2009. Metabolic and bioactivity insights into *Brassica oleracea* var. *acephala*. J. Agric. Food Chem., 57: 8884-8892.

42. Karakida, F., Y. Ikeya, M. Tsunakawa, T. Yamaguchi, Y. Ikarashi, S. Takeda and M. Aburada, 2007. Cerebral protective and cognition-improving effects of sinapic acid in rodents. Biol. Pharm. Bull., 30: 514-519.

43. Erazi, H., W. Sansar, S. Ahboucha and H. Gamrani, 2010. Aluminum affects glial system and behavior of rats. Comptes Rendus Biologies, 333: 23-27.

44. Khan, K.A., N. Kumar, P.G. Nayak, M. Nampoothiri and R.R. Shenoy *et al.*, 2013. Impact of caffeic acid on aluminium chloride induced dementia in rats. J. Pharm. Pharmacol., 65: 1745-1752.

45. Abdel-Aal, R.A., A.A.A. Assi and B.B. Kostandy, 2011. Rivastigmine reverses aluminum-induced behavioral changes in rats. Eur. J. Pharmacol., 659: 169-176.

46. Pratico, D. and J.Q. Trojanowski, 2000. Inflammatory hypotheses: Novel mechanisms of Alzheimer's neurodegeneration and new therapeutic targets? Neurobiol. Aging, 21: 441-445.

47. Pratico, D. and N. Delanty, 2000. Oxidative injury in diseases of the central nervous system: Focus on Alzheimer's disease. Am. J. Med., 109: 577-585.