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RESEARCH ARTICLE

Antiamnesic effect of Piracetam potentiated with *Emblica officinalis* and *Curcuma longa* in aluminium induced neurotoxicity of Alzheimer's disease

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Abstract

Potent Neurotoxic effect of aluminium has been associated with Alzheimer's disease (AD) for decades. Chronic exposure to aluminium induces oxidative stress and increases amyloid beta levels in vivo. Only symptomatic relief has been modulated by Current treatment for AD provide thus necessitating the development of new drugs with fewer side effects. Hence present study was designed to investigate the potentiation effect of piracetam when used along with chronic treatment of *curcuma longa* and *emblica officianalis* against aluminium-induced cognitive dysfunction and oxidative damage in rats. Aluminium chloride (100 mg/kg, p.o.) was administered to rats daily for 6weeks. Rats were concomitantly treated with piracetam (200mg/kg, i.p.), *curcumin* (100 mg/kg, p.o.) and *emblica* (100 mg/kg, p.o.) daily for a period of 6weeks. On the 21st and 42nd day of the study behavioural studies to evaluate memory (Morris water maze and elevated plus maze task paradigms) were done. The rats were sacrificed on 43rd day following the last behavioural test and various biochemical tests were performed to assess the extent of oxidative damage. Chronic administration of aluminium chloride resulted in poor retention of memory in elevated plus maze, Morris water maze task paradigms and caused marked oxidative damage. There was also a significant increase in the acetyl cholinesterase activity and aluminium concentration in aluminium treated rats. Chronic treatment with *curcumin* and *emblica* (100 mg/kg, p.o.) significantly improved these behavioural and biochemical alterations and attenuated increased acetyl cholinesterase. In addition, co-administration of *curcumin* and *emblica* (100 mg/kg, p.o.) along with piracetam (100 mg/kg; i.p.) significantly elevated the protective effects (oxidative damage) as compared to their effects alone. The results clearly suggest that *curcumin* and *emblica* potentiated protective effects of piracetam against aluminium-induced cognitive dysfunction and oxidative damage in rats.

Introduction

Alzheimer's disease (AD) is the progressive neurodegenerative disease of aging, and characterized by amyloid protein beta (Aβ) deposition in plaques and by neurofibrillary tangles (NFT), glial activation around plaques, and region-specific accumulation of oxidative damage that correlates with histopathology. Various mechanisms of neuronal degeneration in AD have been proposed, which include formation of oxidative stress, mitochondrial dysfunction, free radicals, inflammatory processes, environmental impact factors, genetic factors, and apoptosis and so on. All these factors may interact and amplify each other in a vicious cycle of toxicity leading to neuronal dysfunction proceeding to cell dysfunction, and finally cell death. Among various environmental impact factors, metal toxicity plays a vital role in development of Alzheimer's disease mostly through aluminium toxicity. Aluminium, the third most abundant element in the earth's crust, is a constituent of cooking utensils, medicines and drinking water. It

is not an essential element and can gain easy access into the body via air, food and water (Ochmanski & Barabasz, 2000). Aluminium has long been known to be a neurotoxin in mammals(Andrasi, Pali, Molnar, & Kosel, 2005). Aluminium exposure is proposed to be involved in the development of Alzheimer's disease(Gauthier et al., 2000). It produced clinical and pathological features which were strikingly similar to those seen in Alzheimer's disease (Huang et al., 1997). Aluminium was detected in both senile plaques and neurofibrillary tangle bearing neurons in the brains of patients with Alzheimer's disease (McLachlan, Bergeron, Smith, Boomer, & Rifat, 1996). Animal studies showed that aluminium exposure caused neuropathological and neurobehavioral changes resulting in impaired learning ability(Alleva, Rankin, & Santucci, 1998) and so aluminium can be used as an experimental model for Alzheimer's disease (Winnicka, Tomasiak, & Bielawska, 2005).

Cholinergic system in the brain is responsible for memory and learning(Cain, 1998). In the cholinergic system, especially the basal forebrain projections to hippocampus and cortex, is known to be particularly affected in Alzheimer's disease(Whitehouse, Price, Clark, Coyle, & DeLong, 1981). Aluminium exposure was associated with impairment in the cholinergic system by altering cholinergic projection function and structure, hence representing the way by which aluminium could contribute to the pathological process in AD (Bilkei,Gorzo, 1993).

Cholinesterase inhibition represents an important therapeutic strategy in the management of Alzheimer's disease. Cholinesterase inhibitors increase the availability of acetylcholine through inhibition of its destruction, hence it causes an enhancement of cholinergic transmission in the brain and improvement in the symptoms of Alzheimer's disease (Blennow, de Leon, & Zetterberg, 2006). Neurotransmitter acetylcholine function has been improved by piracetam via muscarinic cholinergic (ACh) receptors, which are implicated in memory processes(Winnicka et al., 2005). Furthermore, piracetam may have an effect on NMDA glutamate receptors, which are involved with memory and learning processes. Piracetam is thought to increase of permeability cell membrane (Muller, Eckert, & Eckert, 1999; Winnicka et al., 2005). Piracetam may exert its global effect on brain neurotransmission via modulation of ion channels (i.e., Na+, K+). In brain piracetam has been found to increase oxygen consumption, apparently in connection to ATP metabolism, and adenylate kinase activity has been increased in rat brains (Grau, Montero, & Balasch, 1987; Nickolson &Wolthuis, 1976). The side effects reported in connection with piracetam include nervousness, irritability, agitation, sleep disturbances and anxiety. The incidence of these during clinical trials was 5% or less and they were more often noted in the older patients taking more than 2.4 g daily. Some patients may complain of fatigue or drowsiness. Gastro-intestinal (GI) problems such as nausea, diarrhoea, vomiting and stomach ache have also been reported. Other symptoms, such as vertigo, headaches, trembling have occasionally been reported.

Hence to overcome these difficulties antioxidants and plant phenolics are being tried as chemo protective agents in epidemiological and experimental studies to regulate the progression of oxidative stress related diseases. *Curcumin* is a hydrophobic polyphenol derived from the rhizome of herb *Curcuma longa* belonging to family zingiberaceae. It has been shown to exhibit wide variety of biological and pharmacological activities namely antioxidant, anti-inflammatory(Ruby, Kuttan, Babu, Rajasekharan, & Kuttan, 1995; Srimal & Dhawan, 1973), antimicrobial and anticarcinogenic(Kim, Choi, & Lee, 2003; Kuttan, Bhanumathy, Nirmala, & George, 1985)activities. *Curcumin* can suppress inflammation, cognitive, oxidative damage deficits and amyloid accumulation which are the characteristic features of AD. Further, *curcumin* has been reported to inhibit the formation of amyloid oligomers, fibrils, bind plaques and reduce amyloid in vivo (Aggarwal & Sung, 2009). Apart from AD, therapeutic benefits of *curcumin* have also been demonstrated in ethanol induced oxidative injury in brain , CCl_4- induced hepatic injury(Fu, Zheng, Lin, Ryerse, & Chen, 2008), cadmium-induced oxidative damage (Eybl, Kotyzova, & Koutensky, 2006) and cyclosporine-induced renal dysfunction (Tirkey, Kaur, Vij, & Chopra, 2005). Apart from *curcumin* the fruits of *Emblica officinal is* (Gaertn.) are used in Ayurveda, the classical Indian system of medicine, as potent rasayanas, a class of plant-derived drugs reputed to promote health and longevity by increasing defence against disease and revitalizing the body in debilitated conditions. Low molecular weight (<1000) hydrolysable gallotannins (EOT) comprising punigluconin, pedunculagin emblicanin A and emblicanin B, isolated from the fresh juice or solvent extracts of *Emblica* fruits , were shown to have significant antioxidant effects in vitro and in vivo (Bhattacharya, Chatterjee, Ghosal, & Bhattacharya, 1999). EOT was shown to exert antioxidant effects against iron-overload hepatotoxicity (Bhattacharya, Kumar, Ghosal, & Bhattacharya, 2000) and to elevate rat frontal cortical and striatal concentrations of superoxide dismutase (SOD), catalase (CAT) and

glutathione peroxidase (GPX), and reduce lipid peroxidation in these brain areas (Bhattacharya et al., 1999). EOT also prevented the adverse effects of chronic foot shock- induced stress on SOD, CAT, GPX and lipid peroxidation in these brain areas (Bhattacharya, Ghosal, & Bhattacharya, 2000). Based on above background it clearly suggests that *Curcuma longa* and *Emblica officinalis* have potent antioxidant properties and can useful in Alzheimer's treatment. Hence present study was designed to investigation the potentiation effect of piracetam when used along with chronic treatment of *Curcuma longa* and *Emblica officinalis* against aluminium-induced cognitive dysfunction and oxidative damage in rats.

Materials and Methods:

1. Animals

All experiments were conducted using male Albino Wistar rats (150–200 g), at about 6–8 weeks of age and performed at Dept. Of Pharmacology laboratory, GIET School of Pharmacy, CPCSEA Reg No-1069/PO/ac/07/CPCSEA; all animals were procured from, National institute of nutrition (NIN), Hyderabad. The animals were maintained with free access to water and food and kept at 25 ± 2 ˚C under a controlled 12 h light/dark cycle. Current study was approved by the Institutional Animal Ethics Committee (IAEC) of Indian Institute of Toxicology Research, Lucknow with a research project approval no GSP/IAEC/2013/04/02, and all experiments were carried out in accordance with the guidelines laid down by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Ministry of Environment and Forests, Government of India, New Delhi, India.

2. Drugs and chemicals

Aluminium chloride, 5,5-dithio-bis2-nitrobenzoicacid, (Ellman's reagent), acetylthiocholine iodide, trichloroacetic acid, thiobarbituric acid (TBA),nitro blue tetrazolium (NBT), hydroxylamine all were purchased from Sigma–Aldrich (Bangalore, India), Piracetam (Nootropil®).

3. Grouping and treatment protocol

Aluminium chloride, curcumin solutions are made freshly at the beginning of each experiment. Commercially available ayurvedic preparation *Emblica officinal is* was obtained from authorized ayurvedic shop. Piracetam was give through ip routie obtained from commercially available NOOTROPIL injection. For oral administration, aluminium chloride was dissolved in drinking water, *curcumin* and *emblica* was dissolved in 0.5% carboxymethyl cellulose. Animals were randomized into five groups based on their body weight. Each group having minimum six numbers of animals. The groups were as follows:

Group 1: Control (receive vehicle for aluminium chloride) (n=6)

Group 2: Aluminium chloride (AlCl3) treated (100 mg/kg; p.o.) + vehicle for curcumin (n=6)

Group 3: Piracetam (200mg/kg; ip.) + aluminium chloride (100 mg/kg; p.o.) (n=6)

Group 4: *Emblica* (100 mg/kg; p.o.) + *curcumin* (100 mg/kg: p.o.) +aluminium chloride (100 mg/kg; p.o.) (n=6)

Group5; Piracetam (100mg/kg; ip.)+*Emblica* (100mg/kg; p.o.)+ Curcumin (100mg/kg: p.o.)+ Aluminium chloride (100 mg/kg; p.o.) (n=6)

The doses of *curcumin*, aluminium chloride, and piracetam and *emblica officinalis* were selected based on those reported in previous literatures. The study was carried out for a period of 42 days (6 weeks). The drug was administered orally 1 h after aluminium chloride administration.

4. Behavioural studies:

4.1. Assessment of cognitive performance:

4.1.1. Spatial navigation task:

Evaluation of the acquisition and retention of a spatial navigation task was performed by using 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 40cm a movable circular platform (9cm diameter), mounted on a column was placed in a pool 2 cm above 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 centre 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 (N, S, E, and W) were used as starting points and this divided the pool into four equal quadrants.

A. Maze acquisition phase (training).

Animals received a training session on day 20 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 5 min gap was given between the subsequent trials.

B. Maze retention phase (testing for retention of the learned task).

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 aluminium chloride administration the time taken to find the hidden platform on day 21 and day 42 was recorded and termed as first retention latency (1st RL) and second retention latency (2nd RL), respectively.

4.1.2. Elevated plus maze:

The elevated plus maze consisted of two opposite open arms (50cm×10 cm), crossed with two closed walls of the same dimensions with 40cmhighwalls. The open arms were connected with a central square of dimensions 10cm×10cm the entire maze was placed 50cmhigh above the ground. Animals are tested for Acquisition of memory on day 20 from the start of aluminium chloride administration. 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. By placing the rat in an open arm, retention of memory was assessed and the retention latency was noted on day 21 and day 42 of the ITL and was termed as the first retention transfer latency (1st RTL) and second retention transfer latency (2nd RTL) respectively.

5. Dissection and homogenization

The animals were sacrificed by cervical decapitation under light anaesthesia. 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.

6. 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 (Norwalk, CT, USA). Finally results were expressed as micromoles of acetylthiocholine iodide hydrolyzed per min per mg protein.

7. Biochemical estimation of markers of oxidative stress

7.1 Measurement of lipid peroxidation.

In the brain the extent of lipid peroxidation was determined quantitatively by performing the method as described by Wills(Wills, 1966). By reaction with thiobarbituric acid, the amount of malondialdehyde (MDA) was measured at 532nm using Perkin Elmer Lambda 20 UV VIS Spectrophotometer (Norwalk, CT, USA). By using the molar extinction co-efficient of chromophore (1.56×105 (mol/l) −1 cm−1) the values were calculated.

7.2 Superoxide dismutase activity.

SOD activity was assayed by the method of Kono (Kono, 1978). The assay system consisted of EDTA 0.1mM, sodium carbonate50mM and 96mM of nitro blue tetrazolium (NBT). In the cuvette, 2 ml of the above mixture,0.05 ml of the supernatant and0.05 ml of hydroxylamine were added and the auto-oxidation of hydroxylamine was measured for 2min at 30s interval by measuring the absorbance at 560nm using Perkin Elmer Lambda 20 UVVIS Spectrophotometer (Norwalk, CT, USA).

7.3 Catalase activity.

Catalase activity was assessed by the method of Luck, wherein the breakdown of hydrogen peroxide is measured. The assay mixture consisted of 0.05 ml of the supernatant of the tissue homogenate3 ml of H_2O_2, phosphate buffer. The change in absorbance was recorded for 2min at 30 s interval at 240 nm using Perkin Elmer Lambda 20 UV VIS Spectrophotometer (Norwalk, CT, USA). The results

were expressed as micromoles of hydrogen peroxide decomposed per min per mg protein.

7.4 Protein estimation.

The protein content was estimated by Biuret method(Gornall, Bardawill, & David, 1949) using bovine serum albumin as a standard.

8. Statistical analysis:

All the values were expressed as mean ±SEM. All test data were analysed using One way analysis of variance (ANOVA) followed by post hoc Tukey's test. For statistical significance the criterion was P<0.05. All statistical procedures were carried out using sigma stat Graph Pad Prism (Graph Pad Software, CA)

9. Results:

9.1 Effect of piracetam, *emblica* and *curcumin* combination on memory performance in elevated plus maze paradigm in aluminium 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 aluminium 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 aluminium chloride treatment-induced memory impairment. Besides, piracetam (200 mg/kg), *curcumin* and *emblica*(100mg+100mg/kg) treatment groups showed significant decrease ($P < 0.05$)in both 1st and 2nd RTL on days 21 and 28 (Table 1). However, co-administration of *curcumin* and *emblica* (100mg+100mg/kg) along with piracetam (100mg/kg) significantly (P <0.05) potentiated their protective effects (shortened transfer latency) when compared to control group. (Table 1) and (Figure 1).

Table 1

Effect of piracetam, *emblica* and *curcumin* combination on memory performance in elevated plus maze paradigm in aluminium chloride treated rats.

Treatment (mg/kg)	Mean transfer latency (sec)		
	IAL	1st RTL	2nd RTL
Control	64.30±1.54	17.33±2.25	12.16±2.24
$Alcl_3$ (A) (100)	67.33±1.73[a]	75.6±1.40 [a]	72.50±0.84[a]
P (200)+A(100)	66.30±2.76	26.66±0.98	20.58±1.17
E(100)+C(100)+A(100)	68.16±2.38	34.60±1.22 [b]	28.03±1.06[b]
P(100)+ E(100)+C(100)+ A(100)	66.16±1.64	25.83±01.51[b NS]	18.66±0.58 [b NS]

On day 20 the initial transfer latencies (ITL) and retention transfer latencies on days 21 (1st RTL) and 42 (2nd RTL) following aluminium chloride treatment were observed.

Values are **mean ± SEM**. **Note**: $Alcl_3$ (A): aluminium chloride; P: piracetam; E: *Emblica officinalis*; C: *Curcuma longa*; NS: Not significant.

For statistical significance,

[NS]P < 0.05 as compared to control group

[a] P < 0.05 as compared to control group.

[b] P < 0.05 as compared to $Alcl_3$ treated group. One-way ANOVA followed by Tukey's test for multiple comparisons.

Figure 1 Effect of piracetam, *emblica* and *curcumin* combination on memory performance in elevated plus maze.

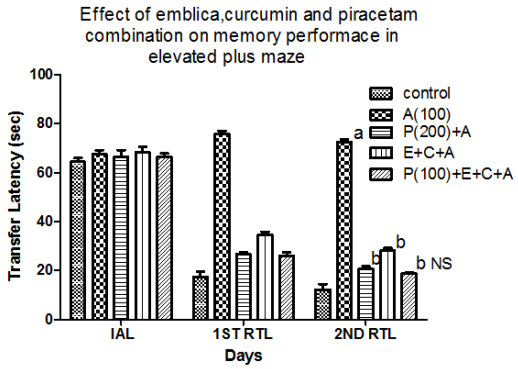

Fig 1:On day 20 the initial acquisition latencies (IAL) and retention latencies on days 21 (1st RTL) and 42 (2nd RTL) following aluminium chloride treatment were observed in elevated plus maze.
Values are mean ± SEM. **Note**: Alcl3 (A): Aluminium chloride; P: piracetam; E: *Emblica officinalis*; C: *Curcuma longa*; NS: Not significant.
For statistical significance,
$^{NS}P < 0.05$ as compared to control group
^{a} $P < 0.05$ as compared to control group.
^{b} $P < 0.05$ as compared to Alcl3 treated group. One-way ANOVA followed by Tukey's test for multiple comparisons

9.2 Effect of piracetam, *emblica* and *curcumin* combination on spatial navigation task in aluminium 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.Aluminium Chloride treated rats showed a significant difference in mean IAL when compared to control group on day 20, indicating that aluminium chloride on chronic administration of impaired acquisition of spatial navigation task ($P < 0.05$).In contrast, concomitant administration of *curcumin*, *emblica*(100 mg/kg, p.o.) and piracetam with aluminium chloride significantly decreased the IAL to reach the platform in the pre-trained rats as compared to aluminium chloride treated rats on day 20 (Table 2). The mean retention latencies, after following training ($1^{st}$and 2^{nd} 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 aluminium chloride treatment. However, co-administration of *curcumin* and *emblica*(100mg+100mg/kg) 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 aluminium chloride treated rats on days 21 and 42, respectively (Table 2) and improved the retention performance of the spatial navigation task.

Table 2: Effect of piracetam, *emblica* and *curcumin* combination on spatial navigation task in aluminium chloride treated rats.

Treatment(mg/kg)	Mean Latency (sec)		
	IAL	**1st RL**	**2nd RL**
Control	50.16±2.16	21.16±1.87	9.30±1.73
$Alcl_3$ (A) (100)	65.16±2.02[a]	61.60±2.08[a]	59.90±2.82[a]
P (200)+A(100)	51.62±1.98	24.66±2.67	11.88±1.99
E(100)+C(100)+A(100)	53.66±1.76	31.00±0.73[b]	22.83±1.33[b]
P(100)+E(100)+C(100)+ A(100)	52.66±1.25[b]	22.66±0.884[b NS]	10.60±1.31[b NS]

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

Values are **mean ± SEM**.

Note: Alcl3 (A): Aluminium chloride; P: Piracetam; E: *Emblica officinal is*; C: *Curcuma longa*; NS: Not significant.

For statistical significance,

$^{NS}P < 0.05$ as compared to control group

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

^{b} $P < 0.05$ as compared to Alcl3 treated group. One-way ANOVA followed by Tukey's test for multiple comparisons.

Figure 2. Effect of piracetam, *emblica* and *curcumin* combination on spatial navigation task in Morris water maze.

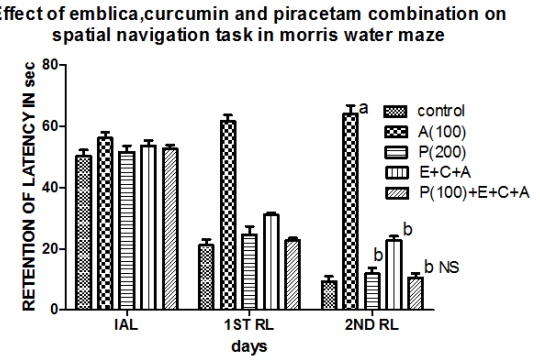

Fig 2: Values are **mean ± SEM**. **Note**: Alcl3 (A): aluminium chloride; P: piracetam; E: *Emblica officinal is*; C: *Curcuma longa*; NS: Not significant.

For statistical significance,

$^{NS}P < 0.05$ as compared to control group

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

$^{b}P < 0.05$ as compared to Alcl3 treated group. One-way ANOVA followed by Tukey's test for multiple comparisons

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9.3 Effect of piracetam, *emblica* and *curcumin* combination on oxidative stress parameters in aluminium chloride treated rats.

Chronic administration of aluminium chloride for 42 days showed significant increase in oxidative damage as evidence by increased MDA and depleted catalase and SOD enzyme activity as compared to naive group (Table 3). Besides concomitant administration of *curcumin*, *emblica*(100 mg/kg, p.o.) and piracetam with aluminium chloride treated groups, attenuated levels of oxidative stress parameters which was significant as compared to chronic aluminium chloride treatment group ($P < 0.05$). However, co-administration of *curcumin* and *emblica*(100mg+100mg/kg) along with piracetam(100mg/kg) significantly ($P < 0.05$) potentiated their protective effects (decreased MDA levels and restored SOD and catalase levels) as compared to their effects alone and control group(Figure's 3,4,5).

Table 3: Effect of piracetam, *emblica* and *curcumin* combination on oxidative stress parameters in aluminium chloride treated rats.

Treatment (mg/kg)	MDA levels, nmol/mg protein	Catalase levels, µmol of H_2O_2 decomposed/min/mg protein	Superoxide dismutase, Units/mg protein
Control	0.1985±0.006	1.0411±0.024	51.09±3.61
$Alcl_3$ (A) (100)	0.5935±0.020^{a}	0.2591±0.0530^{a}	12.01±2.14^{a}
P (200)+A(100)	0.2176±0.012^{b}	0.8285±0.017$^{a\ b}$	45.66±3.51^{b}
E(100)+C(100)+A(100)	0.3175±0.0115$^{b\ c}$	0.7290±0.0521$^{a\ b}$	39.66±2.63$^{a\ b}$
P(100)+E(100)+C(100)+ A(100)	0.2046±0.019$^{b\ NS}$	0.8845±0.031$^{a\ b\ NS}$	47.33±1.203$^{b\ NS}$

Values are **mean ± SEM**. **Note**: Alcl3 (A): aluminium chloride; P: piracetam; E: *Emblica officinal is*; C: *Curcuma longa*; NS: Not significant.
For statistical significance,

$^{NS}P < 0.05$ as compared to control group.
^{a} $P < 0.05$ as compared to control group.
^{b} $P < 0.05$ as compared to $Alcl_3$ treated group.
^{c} $P < 0.05$ as compared to standard piracetam P (200) group. One-way ANOVA followed by Tukey's test for multiple comparisons.

Figure 3 Effect of piracetam, *emblica* and *curcumin* combination on Lipid peroxidation in aluminium chloride treated rats.

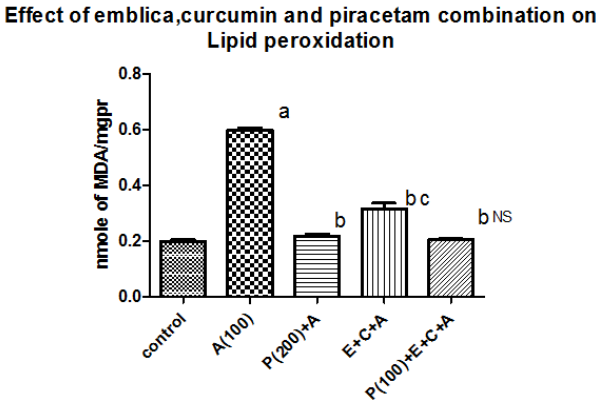

Fig 3: Values are **mean ± SEM**. **Note**: Alcl3 (A): aluminium chloride; P: piracetam; E: *Emblica officinalis*; C: *Curcuma longa*; NS: Not significant.
For statistical significance,
$^{NS}P < 0.05$ as compared to control group.
^{a} P < 0.05 as compared to control group.
^{b} P < 0.05 as compared to $Alcl_3$ treated group.
$^{c}P < 0.05$ as compared to standard piracetam P (200) group. One-way ANOVA followed by Tukey's test for multiple comparisons.

Figure 4. Effect of piracetam, *emblica* and *curcumin* combination on superoxide dismutase activity in aluminium chloride treated rats.

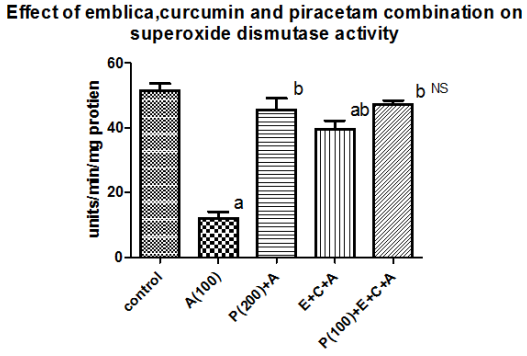

Fig 4: Values are **mean ± SEM**. **Note**: Alcl3 (A): aluminium chloride; P: piracetam; E: *Emblica officinalis*; C: *Curcuma longa*; NS: Not significant.
For statistical significance,
$^{NS}P < 0.05$ as compared to control group.
^{a} P < 0.05 as compared to control group.
^{b} P < 0.05 as compared to $Alcl_3$ treated group.
$^{c}P < 0.05$ as compared to standard piracetam P (200) group. (One-way ANOVA followed by Tukey's test for multiple comparisons.

Figure 5. Effect of piracetam, *emblica* and *curcumin* combination on catalase activity in aluminium chloride treated rats.

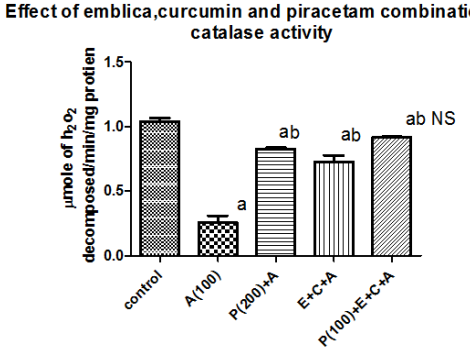

Fig 5: Values are **mean ± SEM**. **Note**: Alcl3 (A): aluminium chloride; P: piracetam; E: *Emblica officinalis*; C: *Curcuma longa*; NS: Not significant.
For statistical significance,
$^{NS}P < 0.05$ as compared to control group.
^{a} P < 0.05 as compared to control group.
^{b} P < 0.05 as compared to $Alcl_3$ treated group.
$^{c}P < 0.05$ as compared to standard piracetam P (200) group. One-way ANOVA followed by Tukey's test for multiple comparisons.

9.4 Effect of piracetam,*emblica* and *curcumin*combination on acetylcholine esterase activity in aluminium chloride treated rats.

Chronic administration of aluminium chloride for 42 days showed significant increase in acetylcholine esterase enzyme activity in aluminium chloride treated group when compared to control group (Table 4). Besides concomitant administration of *curcumin*, *emblica*(100 mg/kg, p.o.) and piracetam with aluminium chloride treated groups, attenuated acetylcholine esterase enzyme activity which was significant as compared to chronic aluminium chloride treatment group ($P < 0.05$).However, co-administration of *curcumin* and *emblica* (100mg+100mg/kg) along with piracetam (100mg/kg) attenuated acetylcholine esterase enzyme activity which was not significantly different ($P < 0.05$)when compaired to piracetam treated group, which implies that the two group treatments have equipotent therapeutic activity.

Table 4:
Effect of piracetam, *emblica* and *curcumin* combination on acetylcholine esterase activity in aluminium chloride treated rats.

Treatment (mg/kg)	AchE Activity (µmol/min/mg pr)
Control	0.005535±0.00287
$Alcl_3$ (A) (100)	0.016837±0.00796 [a]
P (200)+A(100)	0.008852±0.00212 [b]
E(100)+C(100)+A(100)	0.011520±0.00263 [a b]
P(100)+ E(100)+C(100)+A(100)	0.009648±0.00202 [a b NS]

Values are **mean ± SEM**. **Note**: Alcl3 (A): aluminium chloride; P: piracetam; E: *Emblica officinalis*; C: *Curcuma longa*; NS: Not significant.
For statistical significance,
[NS]P < 0.05 as compared to standard piracetam P (200) group.
[a] P < 0.05 as compared to control group.
[b] P < 0.05 as compared to Alcl3 treated group. One-way ANOVA followed by Tukey's test for multiple comparisons.

Figure 6. Effect of piracetam, emblica and curcumin combination on acetylcholineesterase activity in aluminium chloride treated rats.

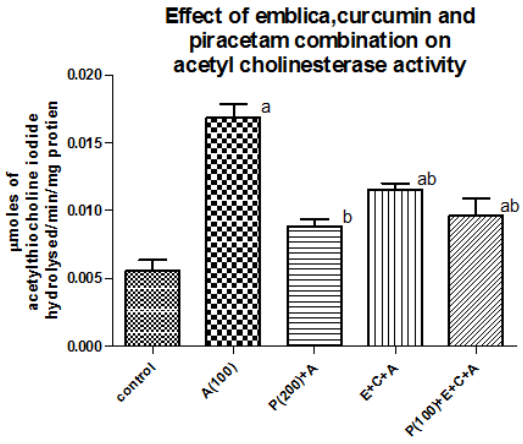

Fig 6: Values are **mean ± SEM**. **Note**: Alcl3 (A): aluminium chloride; P: piracetam; E: *Emblica officinalis*; C: *Curcuma longa*; NS: Not significant.
For statistical significance,
[NS]P < 0.05 as compared to standard piracetam P (200) group.
[a] P < 0.05 as compared to control group.
[b] P < 0.05 as compared to Alcl3 treated group. One-way ANOVA followed by Tukey's test for multiple comparisons.

10. Discussion:

Aluminium is a ubiquitous metal and has been implicated in the aetiology of Alzheimer's disease where it exacerbates brain oxidative damage (Becaria, Bondy, & Campbell, 2003; Nehru, Bhalla, & Garg, 2007), and induces A deposition. AD is characterized by impairment in working memory (Germano & Kinsella, 2005), visuoperception, attention and semantic memory. In present study, chronic exposure of aluminium increased aluminium concentration in hippocampus and cerebral cortex as compared to the control animals. It has been observed that high aluminium level in brain is associated with decline in visual memory and attention concentration in hemodialysis patients (Bolla et al., 1992). The results of our study indicate that chronic administration of aluminium chloride results in progressive deterioration of spatial memory in both Morris water maze and elevated plus maze task paradigms. Experimentally, it has been shown that intracerebral administration of aluminium chloride causes learning deficits in Morris water maze task in rabbits (Rabe, Lee, Shek, & Wisniewski, 1982)which is in concordance with our findings. Chronic treatment with aluminium leads to impairment of glutamate- NO-cGMP pathway in the cerebellum of rats (Canales et al., 2001) which can explain the neurobehavioral deficits and memory impairment observed. In our present findings, coadministration of *curcumin* and *emblica* (100mg+100mg/kg) along with piracetam (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.

Hippocampus is reported to play a key role in spatial learning and memory (Bai et al., 2009). Since hippocampus has abundant inputs from the basal forebrain cholinergic system and thus acetylcholine (ACh) plays a crucial role in learning and memory (Prado et al., 2006). Acetylcholine is degraded by the enzyme acetyl cholinesterase, terminating the physiological action of the neurotransmitter. Alzheimer's disease affects cholinergic system resulting in decreased activity of acetyl cholinesterase (Dai, Buijs, Kamphorst, & Swaab, 2002). Experimentally aluminium has been shown to decrease acetyl cholinesterase in mouse brain (Zatta, Ibn-Lkhayat-Idrissi, Zambenedetti, Kilyen, & Kiss, 2002). In the present study, chronic aluminium chloride treatment caused a significant decrease in the acetyl cholinesterase activity leading to memory deficits, but later was significantly restored by chronic co-administration of *curcumin* and *emblica* (100mg+100mg/kg) along with

piracetam (100mg/kg). However chronic co-administration of *curcumin* and *emblica* (100mg+100mg/kg) along with piracetam (100mg/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.

The role of oxygen free radicals in neurodegeneration and cognitive decline has been well reviewed (Serrano & Klann, 2004). A number of findings suggest that reactive oxygen species (ROS) can accumulate excessively in the brain and can severely attenuate the neuronal function (Massaad & Klann, 2011). Oxidative stress is therefore implicated as one of the causes of cognitive impairment (Keller et al., 2005). Besides, chronic stress is said to promote oxidative stress and demolish antioxidant defence system of the brain (Lucca et al., 2009), which may form the basis for impaired memory. In the present investigation, chronic aluminium chloride treatment resulted in significant oxidative damage as indicated by increase lipid peroxidation and depletion of catalase and superoxide dismutase activity, thus strengthening the oxidative theory of cognitive deficits and its complications. However, co-administration of curcumin and *emblica* (100mg+100mg/kg) along with piracetam (100mg/kg) significantly ($P < 0.05$) potentiated their protective effects (decreased MDA levels and restored SOD and catalase levels) as compared to their effects alone and control group. Thus the results strongly support our hypothesis that the memory deficits observed after chronic aluminium chloride treatment might have arisen as a result of mitochondria dysfunction, which is the key factor for the production of ROS generation and ultimately causing oxidative injury to neurons, which could therefore be prevented by antioxidant treatment.

The use of piracetam is expected to compensate cholinergic deficits indirectly by inhibiting the destruction of acetylcholine and directly by increasing the expression of choline acetyltransferase. Piracetam reversed histopathological and biochemical impairments (oxidative stress parameters) caused by aluminium. The corrected biochemical parameters, in addition to enhancement of cholinergic activity, might explain the ability of piracetam to improve the behavioural performance in aluminium exposed animals. But some side effects reported in connection with chronic use of piracetam include irritability, nervousness, agitation, anxiety and sleep disturbances. Hence co-administration of *curcumin* and *emblica* along with piracetam study plan was designed to decrease the toxic profile of piracetam and help to achieve better therapeutic efficiency. However, results also clearly indicates that chronic co administration of *curcumin* and *emblica* (100mg+100mg/kg) along with piracetam (100mg/kg) combination have potent therapeutic activity, which was indicated through behavioural and oxidative stress parameters.

Conclusion:

From the epidemiologic and experimental studies reported, there is ample evidence which supports the fact that aluminium plays a pivotal role in the neuropathology of AD. This study validates the fact that chronic exposure to aluminium causes cognitive dysfunction and related oxidative damage. It clearly demonstrates that *curcumin* and *emblica* potentiated protective effects of piracetam against aluminium-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.

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