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Therapeutic effect of Sinapic acid in aluminium chloride induced dementia of Alzheimer's type in rats

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ABSTRACT

Objective: To evaluate the effect of sinapic acid against Aluminium chloride-induced dementia of Alzheimer's disease (AD) type in rat. **Methods**: The study was designed to induce dementia by chronic exposure of aluminium chloride at a dose of 175 mg/kg, p.o. for a period of 25 days in rats and then divided into different groups, *i.e.* Treatment group, negative control and two groups of sinapic acid, (at a dose of 20 and 40mg/kg, p.o.), where these groups treated and observed till the 35th day of experimental trial. The behavioural, neuronal and biochemical parameters were determined during or end of experiment. Histological changes in the brain were also observed. **Results**: Aluminium chloride at a dose of 175 mg/kg, o.p. had significantly induced the dementia and sinapic acid, at a dose of 40 mg/kg, p.o., possessed therapeutic effect against Aluminium chloride induced-dementia of AD type in rats. **Conclusions**: Sinapic acid is a class of compound wide spread in plant kingdom and could be a better source of neutraceuticals in brain disorders. The compound showed an *in vivo* MAO-A and MAO-B inhibiting activity and their role in Alzheimer's disease type of dementia was unexplored. The article also provides information on acute toxicity of sinapic acid with no toxicological sign on brain with chronic dose of $AlCl_3$.

1. Introduction

Dementia is a chronic or progressive disease affecting 24.3 million people worldwide in nature. There are disturbances and often gradual decrease in multiple higher cortical functions, including memory, thinking, remembering, orientation, comprehension, calculation, learning capacity, language, judgement, mood, confusion, and change in personality and other behavioral changes[1,2]. It is more common in people over 65 of age. Therefore, the main risk factor for dementia is age[3]. The terms senile and pre-senile dementia had been used to differentiate between patients under or over 65 years, but these are no longer in common use because the two types share some etiological features[4].

There is no cure for dementia. Cholinesterase inhibitors such as donepezil are often used and may be beneficial in mild to moderate disease[5]. The sinapic acid is already reported as cholinesterase inhibitors (*in vitro*)[24] and the present study were aimed to evaluate the therapeutic potential of sinapic acid in $AlCl_3$ induced dementia with special reference to its anticholine esterase inhibition activity (*in vivo*) and modulation of monoamine oxidase (MAO) in dementia.

Alzheimer's disease (AD) is a progressive and irreversible neurodegenerative disease, which damages and kills brain cells, manifested by deterioration in memory and cognition, impairment in performing activities of daily living, and many behavioral neuropsychiatric illnesses[6].

The pathological indications of AD are senile plaques, which are spherical accumulations of the protein –amyloid accompanied by degenerating neuronal processes, and neurofibrillary tangles, composed of paired helical filaments and other proteins. This corresponds to the clinical features of marked impairment of memory and abstract reasoning, with preservation of vision and movement[7].

The selective deficiency of acetylcholine in AD, has given rise

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to the "cholinergic hypothesis," which proposes that a deficiency of acetylcholine is critical in the genesis of symptoms of AD[8]. Therefore, a major approach for the treatment of AD has involved attempts to augment the cholinergic function within the brain. This involves the use of acetyl cholinesterase inhibitors such as rivastigmine, donepezil, tacrine and galantamine[9]. 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[10,11]. Sinapic acid is also possessed a significant antioxidant and anti inflammatory property, so the aim of present study is focused on modulation of various neurochemicals by sinapic acid in brain tissues.

Sinapic acid is a small naturally-occurring hydroxycinnamic acid and a member of the phenylpropanoid family. It has been proposed as a potent antioxidant by many researchers[12,13]. Sinapic acid is widespread in the plant kingdom (fruits, vegetables, cereal grains, oilseed crops, and some spices and medicinal plants) and as such is common in the human diet.

The previous reported pharmacological activities of sinapic acid are antioxidant and free-radical scavenging property, Antioxidant and cardioprotective, Antihypertensive and cardiovascular remodeling[14], Antihyperglycemic action[15], Anti-anxiety activity[16], Anticancer activity[17], Neuroprotective activity[18], Anti-inflammatory activity[19], Hepatoprotective and Anti fibrotic effects[20], Antimicrobial activity[21] and Hypolipidemic activity[22,23].

Sinapic acid is 3, 5-dimethoxy-4-hydroxycinnamic acid. It may be found in the free form, but like other hydroxycinnamic acids, it is also found in the form of esters (Figure 1).

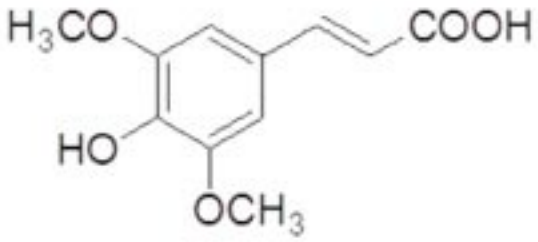

Figure 1. Structure of sinapic acid.

2. Materials and methods

2.1. Drugs and chemicals

Sinapic acid[SLBK4211V] (Pubchem CID 637775) was purchased from Sigma Aldrich (S & G Lab Supplies). Rivastigmine tartrate 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).

2.2. Animals

Age matched young wistar albino rats of either sex, weighing 120-150 g were selected for 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 (2 male, 4 Female) per polypropylene cages kept under controlled room temperature (25±1 ℃) in 12 hours 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 Committee for purpose Control and Supervision of Experiments on Animals (CPCSEA) [CPCSEA Reg. No. 874/PO/Re/S/2005/CPCSEA].

2.3. Acute toxicity study

LD_{50} was determined according to the guidelines of organization for economic cooperation & 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 2 000 mg/kg, *p.o.* A dose range of 20 mg/kg, 40 mg/kg was selected for the pharmacological activity. For all the studies overnight fasted animal of either sex were used.

2.4. Experimental design

On prove day (day 5), Randomized animals are divided into experimental groups randomly (n=6). $AlCl_3$, Rivastigmine and Sinapic acid suspensions were made freshly each day for administration. Rats were administered with $AlCl_3$ (175 mg/kg) orally from day 6 (*i.e.*, 24 h after the completion of retention trial on day 5) to 25 days. $AlCl_3$ was dissolved in distilled water and administered orally at a dose of 0.5 mL/100 g body weight. Standard drug (Rivastigmine-2.5 mg/kg), was suspended in 1% aqueous solution of Tween 80 was given orally from day 26 to 36 day. Test drug Sinapic acid (20 and 40 mg/kg), suspended in a 1% aqueous solution of Tween 80 was given orally from day 26 to 36 day. Control group receive normal saline by respective route. The estimations of various neuro chemicals were done by various methods available in literature. The TNF α levels were estimated by ELISA kit. The groups were as follows.

Group I control Group (Normal saline (0.9% NaCl)-5mL/kg, *p.o.*, from 6th- 36th day).

Group II Untreated $AlCl_3$-affected rats.

Group III Rivastigmine-treated $AlCl_3$- affected rats.

Group IV Sinapic acid (20 mg/kg, *p.o.*)-treated $AlCl_3$- affected rats.

Group V Sinapic acid (40 mg/kg, *p.o.*)-treated $AlCl_3$- affected rats

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, south-east, south-west and north-west (NW)) along the circumference of the pool. An escape platform (10 cm diameter) submerged 2 cm below the water surface 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 four consecutive days (0-4th day). During each trial, the animal was placed in each quadrant to eliminate quadrant effects. All rats were left in the platform for 30 s and then removed and towel dried. Rats failing to find the platform within 60 s were guided to the platform. On 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 s before the end of session. Similarly, the retention trials were conducted on day 5, 16, 26 and day 36 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[24].

Using a digital photoactometer, locomotor activity was assessed in animals. The ambulatory movements were recorded over a period of 10 minutes and expressed in terms of total photo beam counts for 10 minutes per animal. Locomotor activity (L.A.)was assessed on day 5, 16, 26 and day 36 before probe trial in Morris's water maze[25].

Figure 2. Proposed mechanism for therpeutic effect of Sinapic acid.

2.5. Evaluation

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

Acetyl choline esterase (AchE) activity: it was measured by Ellman method[26]. 0.4 mL supernatant of brain homogenate, 2.6 mL of 0.05 M phosphate buffer and 0.1 mL of 0.1 M DTNB[5,5-dithiobis-(2-nitrobenzoic acid)] were processed and Measured the absorbance at 412 nm through U.V. spectrophotometry, until the reading was constant and then added 0.02 mL acetylthiocholine iodide (substrate). Immediately took absorbance at 412 nm for continue 7 minutes of the interval of one minute. The results were expressed as nM/L/min/gm of tissue.

GSH was measured by the method described by Ellman[27-30]. Supernatant of brain homogenate, 10% TCA (1:1 ratio) were added and centrifuge at 1 000 rpm for 10 minutes. 0.05 mL supernatant, two mL of 0.3 M dihyrogen phosphate, 0.25 mL freshly prepared DTNB were added and took absorbance at 412 nm. The results were expressed as nM/mg of protein.

Estimation of Catalase: Catalase activity was measured by Luck method[31], wherein the breakdown of H_2O_2 was measured. 0.05 mL supernatant of brain homogenate, three mL H_2O_2 phosphate buffers were added and recorded change in absorbance for 2 min. at the 30s intervals at 240 nm. The results were expressed as micromoles of H_2O_2 decomposed/min./mg/of protein.

Estimation of Superoxide dismutase (SOD): SOD activity was measured by Kono method[32]. The assay system consisted of EDTA 0.1 Mm, sodium carbonate 50 mM and 96 mM of nitro blue tetrazolium (NBT). In the cuvette, 2 mL of the above mixture, 0.05 mL supernatant of brain homogenate and 0.05 mL of hydroxylamine were added, and the auto-oxidation of hydroxylamine was measured for 2 min. at the 30s intervals by measuring the absorbance at 560 nm. The results were expressed as Units/mg protein.

Estimation of Nitrite: it was measured by Najmun method[33]. 0.2 mL of the supernatant of brain homogenate was mixed with freshly prepared Griess reagent solution and immediately took absorbance at 546 nm. The results were expressed as nM/mg of protein.

Histopathological study: 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 ℃ for 24 h. Paraffin beeswax 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[34] for histopathological examination using a light microscope.

2.6. Statistical analysis

The data was 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 was analyzed using one-way ANOVA followed by Tukey-Kramer Multiple Comparisons test.

3. Results

3.1. Acute toxicity study

Sinapic acid did not show any sign and symptom of toxicity and mortality up to 2 000 mg/kg, *p.o.*

3.2. Effect of Sinapic acid on Aluminium chloride induced behavioural parameters

3.2.1. Effect of Sinapic acid on Aluminium chloride induced dementia of Alzheimer's type in rat using the Morris Water Maze

The rat spends significantly more time in the target quadrant (NW) in search of the missing platform as compared to the time spent in other quadrants (SE, SW and NE) during the retention trial conducted on the 5th day (probe day). Control group showed the normal retrieval of memory on 36th day[ELT (5.15±0.08 P<0.001) and TT (3.42±0.18 P<0.001)]. $AlCl_3$ treated rats (Group II) significantly raised ELT and reduced the time spent in the target quadrant (NW) *i.e.* TT markedly in search of the missing platform during the retention trial on 16th, 26th and 36th day[ELT (P<0.001) and TT (P<0.001)] as compared to control group rats (Group, I). Untreated $AlCl_3$-affected rats (Group III) significantly raised the ELT and reduced the TT during the retention trial on 16th and 26th day, but the rivistigmine-treated $AlCl_3$- affectedresulted in decreased in ELT and increased in TT during retention trial on 36th day, when compared to $AlCl_3$ treated animals (Group II). Untreated $AlCl_3$-affected rats(Group IV and V) significantly raised the ELT and reduced in TT during the retention trial 16th and 26th day but the sinapic acid-treated $AlCl_3$-affected (20 and 40 mg/kg) rats resulted in decreased in ELT and increased in TT during retention trial on 36th day, ELT and TT when compared to $AlCl_3$ treated animals (Group II) (Table 1 and Table 2).

3.2.2. Effect of Sinapic acid on Aluminium chloride induced dementia of Alzheimer's type in rat 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 (149.2±1.63 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). $AlCl_3$ pre-treated rats (Group III) resulted in a significant decreased in locomotor activity on 16th and 26th day, but the rivistigmine-treated $AlCl_3$- affectedresulted in a significant increased in locomotor activity on 36th day (P<0.001) when compared to $AlCl_3$ treated rats (Group II). $AlCl_3$ pre-treated rats (Group IV and V) resulted in a significant decreased in locomotor activity on 16th and 26th day but sinapic acid-treated $AlCl_3$-affected(20/40 mg/kg) resulted in a significant increased in locomotor activity 130.13±1.65 and 138.2±5.58 (P<0.001) when compared to $AlCl_3$ treated rats (Group II)) (Table 3).

3.2.3. Effect of Sinapic acid on biochemical Parameters

The figures explicate the levels of AchE (Figure 3A), TBARS

Table 1
Effect of Sinapic acid on Aluminium chloride induced dementia of Alzheimer's type in rat using Morris Water Maze (Escape latency Time).

Groups/ Treatment	ELT (sec)			
	5th day	16th day	26th day	36th day
I (Control Group)	4.98±0.1	5.04±0.1	5.03±0.1	5.15±0.1^{b***}
II (Untreated AlCl3-affected rats)	4.879±0.1	14.0±0.05	26.28±0.27	23.12±0.1^{a***}
III(Rivastigmine-treated AlCl3- affected rats)	4.98±0.13	11.1±0.08	19.32±0.28	6.1±0.1$^{a*** b***}$
IV (Sinapic acid (20mg/kg, p.o.)-treated AlCl3- affected rats)	4.87±0.07	11.0±0.12	18.83±0.22	9.12±0.1$^{a*** b***}$
V (Sinapic acid (40mg/kg, p.o.)-treated AlCl3- affected rats)	4.97±0.04	12.94±0.04	21.87±0.07	8.13±0.03$^{a*** b***}$

Data are mean±SEM values, n=6. Data were analyzed by One way ANOVA followed by Tukey-Kramer Multiple Comparisons test. *P<0.05, **P<0.01, ***P<0.001 and nsp>0.05. a- compared with control, b-compared with inducer, NS Not significant, ELT- Escape latency Time.

Table 2
Effect of Sinapic acid on Aluminium chloride induced dementia of Alzheimer's type in rat using Morris Water Maze.

Groups/ Treatment	T.T (sec)			
	5th day	16th day	26th day	36th day
I (Control Group)	3.53±1.01	3.98±0.04	3.55±0.05	3.42±0.18^{b***}
II (Untreated AlCl3-affected rats)	3.65±0.10	3.35±0.13	1.99±0.04	1.20±0.06^{a***}
III(Rivastigmine-treated AlCl3- affected rats)	3.62±0.09	2.98±0.04	1.78±0.02	2.8±0.15^{b***}
IV (Sinapic acid (20mg/kg, p.o.)-treated AlCl3- affected rats)	3.57±0.12	2.90±0.05	1.50±0.01	2.48±0.25$^{a* b***}$
V (Sinapic acid (40mg/kg, p.o.)-treated AlCl3- affected rats)	3.64±0.11	3.12±0.05	1.90±0.02	2.6±0.23$^{a* b***}$

Data are mean±SEM values, n=6. Data were analyzed by One way ANOVA followed by Tukey-Kramer Multiple Comparisons test. *P<0.05, **P<0.01, ***P<0.001 and nsp>0.05. a- compared with control, b-compared with inducer, NS Not significant, T.T- Total Time.

Table 3
Effect of Sinapic acid on Aluminium chloride induced dementia of Alzheimer's type in rat using Photoactometer.

Groups/ Treatment	L.A (count/10min)			
	5th day	16th day	26th day	36th day
I (Control Group)	150.27±1.71	155.01±0.09	151.99±0.64	149.2±1.63^{b***}
II (Untreated AlCl3-affected rats)	146.11±2.3	83.13±0.116	83.08±1.29	95.2±1.02^{a***}
III(Rivastigmine-treated AlCl3- affected rats)	149.95±2.71	81.95±0.07	79.13±2.07	144.4±2.53^{b***}
IV (Sinapic acid (20 mg/kg, p.o.)-treated AlCl3- affected rats)	151.76±1.20	86.07±0.09	84.941±0.09	130.122±1.65$^{a** b***}$
V (Sinapic acid (40 mg/kg, p.o.)-treated AlCl3- affected rats)	148.85±3.023	89.20±0.18	85.23±2.50	138.2±5.58^{b***}

Data are mean±SEM values, n=6. Data were analyzed by One way ANOVA followed by Tukey- Kramer Multiple Comparisons test. *P<0.05, **P<0.01, ***P<0.001 and nsp>0.05.a-compared with control, b-compared with inducer, NS Not significant, LA.-loco motor activity.

(Figure 3B), Nitrite (Figure 3C), GSH (Figure 4D), Catalase (Figure 4E), and SOD (Figure 4F) level in brain homogenate of control and experimental groups of rats. The levels of AchE, TBARS, and Nitrite were significantly elevated in Untreated $AlCl_3$-affected rats as compared to a control group. The animals treated with revastigmine showed a significant reduction in the levels of AchE, TBARS, and Nitrite on 36th day of trial as compared with Untreated $AlCl_3$-affected group. The elevated levels of AchE, TBARS, and Nitrite were declined up on treatment with sinapic acid in a dose dependent manner during the experimental trial as compared with Untreated $AlCl_3$-affected group.

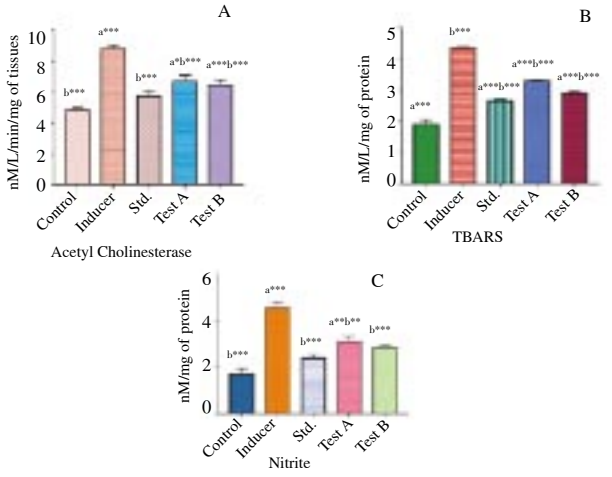

Figure 3. (A)Effect of Sinapic acid on Acetyl Cholinesterase level in brain. (B) Effect of Sinapic acid on TBARS level in brain (C) Effect of Sinapic acid on Nitrite level in brain. All values were represented as mean±SEM. Data are mean±SEM values, n=6. Data were analyzed by One way ANOVA followed by Tukey- Kramer Multiple Comparisons test. *p<0.05, **p<0.01, ***p<0.001 and ns p>0.05.a-compared with control, b-compared with inducer, NS Not significant. (where Inducer- Untreated $AlCl_3$-affected rats, Std.- Rivastigmine-treated $AlCl_3$- affected rats, Test A- Sinapic acid (20mg/kg, *p.o.*)-treated $AlCl_3$- affected rats, and Test B- Sinapic acid (40mg/kg, *p.o.*)-treated $AlCl_3$- affected rats).

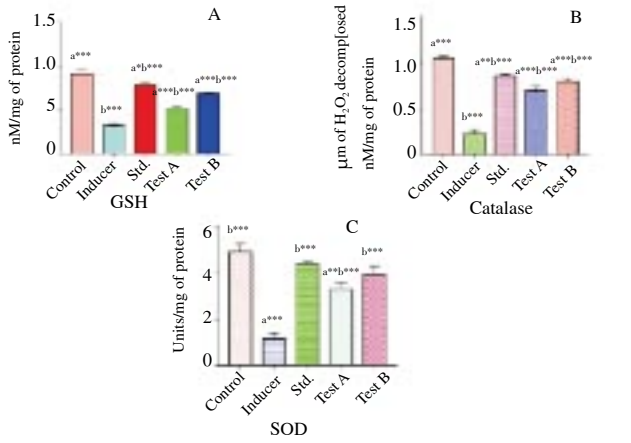

Figure 4. (D)Effect of Sinapic acid on GSH level in brain. (E) Effect of Sinapic acid on Catalase level in brain. (F) Effect of sinapic acid on SOD level in brain. All values were represented as mean±SEM. Data are mean±SEM values, n=6. Data were analyzed by One way ANOVA followed by Tukey- Kramer Multiple Comparisons test. *p<0.05, **p<0.01, ***p<0.001 and nsp>0.05.a-compared with control, b-compared with inducer, NS Not significant. (where Inducer- Untreated $AlCl_3$-affected rats, Std.- Rivastigmine-treated $AlCl_3$- affected rats, Test A- Sinapic acid (20mg/kg, *p.o.*)-treated $AlCl_3$- affected rats, and Test B- Sinapic acid (40mg/kg, *p.o.*)-treated $AlCl_3$- affected rats).

The levels GSH, Catalase and SOD were significantly decreased in Untreated $AlCl_3$-affected rats as compared to a control group. The animals treated with revastigmine showed a significant increase in the levels of GSH, Catalase and SOD on 36th day of trial as

Table 4
Effect of sinapic acid on MAO level in Brain tissues.

Groups/ Treatment	MAO-A (nmol/mg protein•h)	MAO- A Inhibition (%)	MAO-B (nmol/mg protein•h)	MAO- B Inhibition (%)
I (Control Group)	33.3±0.72		27.6±1.02	
II (Untreated AlCl3-affected rats)	33.1±0.43	00	32.7±0.97	00
III(Rivastigmine-treated AlCl3- affected rats)	32.7±0.54	4.80	27.2±0.29	00
IV (Sinapic acid (20mg/kg, p.o.)-treated AlCl3- affected rats)	26.8±0.32	18	20.2±0.73	28
V (Sinapic acid (40mg/kg, p.o.)-treated AlCl3- affected rats)	21.9±0.88	35	15.5±1.2	45

Data are mean±SEM values, *n*=6.

Table 5
Effect of sinapic acid on AlCl3 induced toxicity with reference to change in Body and Brain weight.

Groups/ Treatment	Change in Body Weight (gm)		Brain weight (gm)		Brain/Body weight Ratio/100 gm of Rat	
	Male	Female	Male	Female	Male	Female
I (Control Group)	+2.54±0.21	3.54±0.34	1.24±0.4	1.32±0.76	1.12	1.09
II (Untreated AlCl3-affected rats)	-1.24±0.25	0.29±0.1	0.89±0.25	0.78±0.23	0.74	0.51
III(Rivastigmine-treated AlCl3- affected rats)	-2.89±0.56	-2.58±0.78	0.98±0.32	1.05±0.56	0.94	0.98
IV (Sinapic acid (20mg/kg, p.o.)-treated AlCl3- affected rats)	-3.54±0.56	-2.89±0.87	1.08±0.54	1.2±0.21	0.89	0.85
V (Sinapic acid (40mg/kg, p.o.)-treated AlCl3- affected rats)	-3.02±1.03	-2.54±0.89	1.3±0.52	1.05±0.35	0.79	0.98

Data are mean±SEM values, *n*=6. (male-2, Female-4).

compared with Untreated $AlCl_3$-affected group. The reduced levels of GSH, Catalase and SOD were increased significantly with a dose of 40 mg/kg of sinapic acid as compared with Untreated $AlCl_3$-affected group.

3.2.4. Effect of sinapic acid on brain TNF-αLevel in $AlCl_3$ induced dementia

All the treated animals showed an increase in brain TNF-α level (Fig 5). Sinapic acid treatment produced a significant and dose dependent reduction in TNF- α levels as compared to control.

Untreated $AlCl_3$-affected rats showed decrease in body weight and brain weight while other groups did not show any marked deference in body and brain weight of rats.

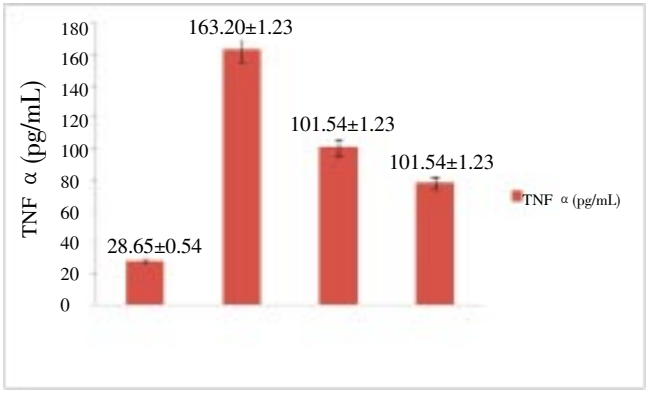

Figure 5. Effect of Sinapic acid on TNF level in brain cortex. All values were represented as mean±SEM. Data are mean±SEM values, n=6. Data were analyzed by One way ANOVA followed by Tukey- Kramer Multiple Comparisons test. *p<0.05, **p<0.01, ***p<0.001 and nsp>0.05.a-compared with control, b-compared with inducer, NS Not significant. (where I- control, II-Untreated $AlCl_3$-affected rats, IV- Test A- Sinapic acid (20mg/kg, *p.o.*)-treated $AlCl_3$- affected rats, and V-Test B- Sinapic acid (40mg/kg, *p.o.*)-treated AlCl3- affected rats).

3.3. Histopathological study of the rat brain in Aluminium chloride-induced dementia of Alzheimer's type

In normal control animals (A), neuronal cells were active and relatively packed with prominent nuclei.

In aluminium chloride treated group (B), spongy cell, neuronal necrosis. Cells degenerated with small nuclei leading to eosinophilic deposition.

In standard drug treated group (rivastigmine 2.5 mg/kg) (C) treated group, the cells undergone slight neuronal necrosis and packed nuclei.

In Test A (sinapic acid 20 mg/kg) (D) few cells undergo neuronal decongestion whereas Test B (sinapic acid 40 mg/kg) (E), showed significant improvement in the group there is no cell degeneration with prominent nuclei (Figure 6).

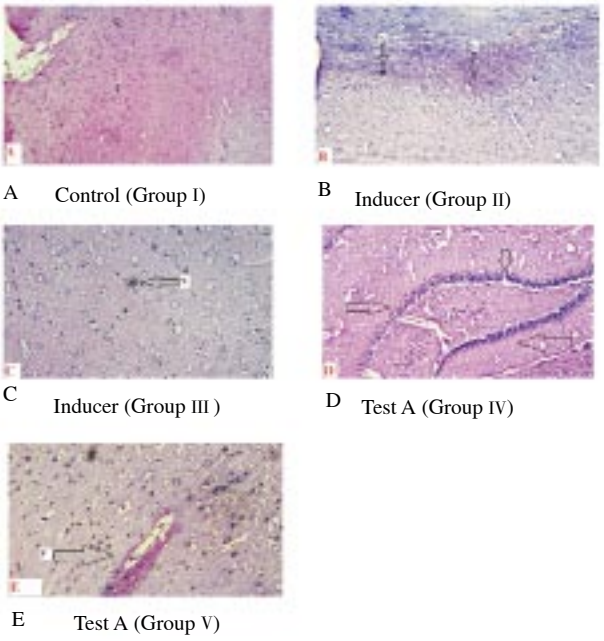

Figure 6. Histopathology of brain (cerebral cortex) showing Neuronal degeneration and inflammation. (D: Cellular degeneration, V: Neuronal vacuolation, N: Necrosis). (where Inducer- Untreated $AlCl_3$-affected rats, Std.- Rivastigmine-treated $AlCl_3$- affected rats, Test A- Sinapic acid (20mg/kg, *p.o.*)-treated $AlCl_3$- affected rats, and Test B- Sinapic acid (40mg/kg, *p.o.*)-treated $AlCl_3$- affected rats).

4. Dicussion

AD is now the most common cause of dementia[35]. The incidence of AD increases with age[36]. Impairment of short-term memory usually is the first clinical feature. When the disease progresses, additional cognitive abilities are impaired, as the ability to calculate, and use common objects and tools[7].

Aluminium found in our daily life as in drinking water, soil and tooth paste, moreover, it is used to manufacture cooking utensils. Aluminium is a non-redox active metal which is capable of increasing the cellular oxidative milieu by potentiation of pro-oxidant properties of transition metals such as iron and copper[37]. It leads to oxidative deterioration of cellular lipids, proteins and DNA[38]. Lipid peroxidation can cause tissue damage under chronic condition[39,40] therefore, Aluminium can be considered as a contributing factor in AD. After chronic exposure, aluminium accumulates in all brain regions with greater accumulation in cortex and hippocampus[41-43]. Hippocampus and frontal cortex play an important role in learning and memory[44], which is severely affected in neurodegenerative disorders such as AD and Parkinson disease (PD).

It is a potent cholinotoxin and causes apoptotic neuronal loss which is a characteristic symptom of neurodegeneration associated with AD[49].

Chronic administration of aluminium chloride results in progressive deterioration of spatial memory in Morris water maze task

paradigms. It leads to impairment of glutamate-NO-cGMP pathway to the cerebellum of rats[50]. In our study, chronic administration of aluminium chloride resulted in marked oxidative stress as indicated by an increase in lipid peroxidation, nitrite levels, decrease in reduced glutathione levels, 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, which results in release of oxidative products like malondialdehyde[TBARS], carbonyls, peroxynitrites and enzymes like superoxide dismutase within the neurons[51].

Acetyl cholinesterase inhibitors are the only agents approved by the Food and Drug Administration (FDA) for the treatment of AD. Rivastigmine was used as standardized drug as it is the only proven pharmacological therapy for the symptomatic treatment of AD[52]. 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[53] resulting in impaired cholinergic transmission by two ways. Firstly, in AD patients, it occurs either due to (I) decline in Ach release (ii) decreased choline acetyltransferase activity (ChAT), which results in the scarcity of Acetylcholine[54,55]. Secondly, elevated acetyl cholinesterase (AchE) enzyme further adds to scarcity of Ach at the synapse by degrading the available Ach. This degradation of Ach is abolished by rivastigmine (AchE inhibitor) so it's effective in AD through improvement in cholinergic transmission.

Sinapic acid (Hydroxycinnamic acid) belongs to the class of phenolic acids with bioactive carboxylic acids; the class mainly includes caffeic acid, ferulic acid, and sinapic acid[56,57]. It is a frequent phytochemical in the human diet. It's a potent inhibitor of an enzyme AchE[58,59]. The possible mechanism by it inhibits cerebral hypoxia[60] and improved memory disturbance by activating cholinergic function[Acetylcholine (Ach) and choline acetyltransferase (ChAT)].

Chronic aluminium exposures have been reported to result in cognitive[58] and locomotor impairment[59]. The cognitive deficit is evident to declined performance in Morris water maze test[60] and radial arm maze test[61]. In our study, aluminium treatment resulted in behavioral changes such as a spatial memory deficit, indicated by increased escape latency and decreased in time spent in target quadrant[NW]. Rivastigmine and sinapic acid antagonized the spatial memory deficit caused by aluminium. This suggests the neuro protective role of sinapic acid in correcting cognitive dysfunction 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[60], a decline in locomotor activity in aluminium treated rats was observed, indicating the CNS depressant effect of chronic aluminium exposure. Treatment with rivastigmine and sinapic acid corrected the locomotor incoordination caused by aluminium chloride.

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[60,61]. These are also thought to play a significant role at the onset and progression of AD. Present study also supports an evidence of inflammation with high concentration of TNFα in brain tissues as compared with control rats. Administration of rivastigmine and sinapic acid 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.

It is well established that aluminium is a neurotoxic agent that induces the production of free radicals and various inflammatory cascade in the brain[62,63]. Accumulation of free radicals may cause degenerative events of aging such as AD and because sinapic acid showed its potential against this neurotoxic agent so it should be used in dementia or AD.

From the above pharmacological, biochemical and histopathological studies, it has been concluded that at the doses of 40 mg/kg, *p.o.* of sinapic acid possesses the potentially protective effect against aluminium chloride induced dementia of Alzheimer's type in rats.

Sinapic acid provides a significant effect against neuroinflammatory disorders because of its TNF-α modulatory activity.

Sinapic acid could be used as choline esterase inhibitors with an adjuant therapy in AD disease.

The compound is also proved to be as MAO modulator so it may be beneficial against other neurodegenerative disease.

Conflict of intrest statement

The authors declare that there is no conflict of interests regarding the publication of this paper.

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Authors contribution

Souravh Bais –ES-supervision of Practical works and participated in the design to the study and performed the statistical analysis. Yash Prashar- FG-conceived of the study, and participated in its design and coordination and helped to draft the manuscript. Renu

Kumari- conceived of the study, and participated in its design and coordination and helped to draft the manuscript.

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