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Research Article

EVALUATION OF NEUROPROTECTIVE EFFECT OF
MANILKARA ZAPOTA LEAF EXTRACT AGAINST
ALUMINIUM CHLORIDE INDUCED MEMORY
IMPAIRMENT ON WISTAR ALBINO RATSSheshadrigowda T V^{*1}, T Balasubramanian², Suresha B S³¹2nd year M pharma, Student of department of Pharmacology, Bharathi College of
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Bharathinagara, Mandya, Karnataka, India -571422**Abstract:**

Alzheimer's disease is a progressive neurodegenerative disorder associated with cognitive and memory impairment, in which oxidative stress plays an important role in neuronal damage and disease progression. Medicinal plants containing antioxidant phytoconstituents may provide potential neuroprotective agents. The present study was undertaken to evaluate the antioxidant and neuroprotective potential of methanolic extract of Manilkara zapota leaves (MEMZL) against aluminium chloride-induced Alzheimer's disease in rats. The leaves were extracted with methanol and subjected to preliminary phytochemical screening, while in-vitro antioxidant activity was evaluated using DPPH, phosphomolybdenum and ferric-reducing assays. In-vivo neuroprotective activity was evaluated in Wistar albino rats divided into five groups (n=6), with Alzheimer's-like cognitive impairment induced using aluminium chloride (175 mg/kg, orally). Rivastigmine (2.5 mg/kg) was used as the standard drug, while MEMZL was administered at doses of 200 and 400 mg/kg. Memory function was assessed using Elevated Plus Maze, Y-maze and Cook's Pole tests, along with evaluation of brain catalase activity, total protein concentration and histopathological changes in the cerebral cortex and hippocampus. Methanolic extraction yielded 14.06% w/w extract, and phytochemical screening revealed the presence of alkaloids, flavonoids, carbohydrates, steroids, saponins, glycosides, proteins, phenolic compounds and tannins. MEMZL exhibited concentration-dependent antioxidant activity with a DPPH IC₅₀ of 70.07 µg/ml. In aluminium chloride-treated rats, MEMZL improved behavioural parameters, restored brain catalase activity and total protein concentration, and reduced neuronal degeneration and pathological alterations in the cerebral cortex and hippocampus. The 400 mg/kg dose generally produced greater improvement than the 200 mg/kg dose. Overall, the methanolic extract of Manilkara zapota leaves demonstrated significant antioxidant and neuroprotective activity in the aluminium chloride-induced Alzheimer's disease model, indicating its potential as a source of neuroprotective agents for further investigation.

Keywords: Manilkara zapota, Alzheimer's disease, Antioxidant, Neuroprotection, Aluminium chloride, Cognitive impairment.

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INTRODUCTION:

Alzheimer's disease (AD) is a chronic, progressive, irreversible, and incurable neurodegenerative disorder and the most common cause of dementia worldwide. It is characterized by a gradual decline in cognitive function and memory, impaired daily activities, and behavioural changes. Pathological changes may begin many years before clinical symptoms appear. Due to its increasing prevalence, prolonged course, and significant healthcare and socioeconomic burden, Alzheimer's disease has become a major public health challenge¹. Alzheimer's disease selectively damages neurons in specific regions and neural networks of the brain, including the cerebral cortex, hippocampus, amygdala, anterior thalamus, basal forebrain, and several monoaminergic nuclei of the brainstem. A hallmark of the affected neurons is the presence of intracellular neurofibrillary tangles (NFTs)². Neurofibrillary tangles (NFTs) are mainly composed of abnormally hyperphosphorylated tau protein. Normally, tau stabilizes microtubules and supports neuronal structure and axonal transport. In Alzheimer's disease, excessive tau phosphorylation causes it to detach from microtubules, leading to microtubule destabilization and impaired axonal transport. Hyperphosphorylated tau forms oligomers and subsequently aggregates into paired helical filaments, which accumulate as NFTs. These tangles disrupt neuronal and synaptic function, ultimately contributing to neuronal degeneration and apoptosis.³.

Manilkara zapota is a highly nutritious fruit belonging to the Sapotaceae family and is commonly grown in regions ranging from tropical wet climates to subtropical cool and dry areas. It is known by several names, including chiko, sapota, naseberry, and sapodilla, across American, Asian, and European countries⁴. The family comprises approximately 70 genera and around 800 species of trees. Within the genus *Manilkara*, about 85 species have been identified⁵.

A wide range of phytochemicals have been isolated from different parts of the plant, and various components of the tree have traditionally been used to treat ailments such as dysentery, fever, and diarrhoea⁶. Phytochemical investigations have shown that the plant primarily contains phenolic compounds such as protocatechuic acid, quercitrin, myricitrin, catechin, gallic acid, vanillic acid, caffeic acid, syringic acid, coumaric acid, and ferulic acid⁷.

The leaves of sapodilla contain major constituents such as lupeol acetate, oleanolic acid, apigenin- 7-O- α -L-rhamnoside, myricetin-3-O- α -L-rhamnoside, and caffeic acid. Studies by Ganguly and Rahman have shown that *Manilkara zapota* leaves exhibit cytotoxic, antioxidant, antimicrobial, and mild central nervous system depressant activities, indicating their potential therapeutic use against cancer, tumors, infectious diseases, and oxidative stress⁸.



Figure 1: Leaves of *Manilkara zapota*.

Taxonomical Nomenclature:⁹**Table 1: Taxonomical Nomenclature of *Manilkara zapota*.**

Kingdom	Plantae (plants)
Sub kingdom	Tracheobionta (vascular plants)
Super division	Spermatophyta (seed plants)
Division	Magnoliophyta (flowering plants)
Class	Magnoliopsida (dicotyledanae)
Sub class	Dilleniidae
Order	Ebenales
Family	Sapotaceae
Genus	<i>Manilkara</i> . (Manilkara)
Species	<i>M. zapota</i> (L.)

MATERIAL AND METHODS:**Collection and Authentication of plant material:**

For the present study fresh leaves of the plant *Manilkara Zapota* was collected from Bharathinagara, Maddur taluk, Mandya district, Karnataka. The leaves were identified, confirmed and authenticated by Dr. Thejesh Kumar M.P. M.Sc., PhD. Professor and Head, Department of Botany (PG), Bharathi College (PG & RC), Bharathinagara. The collected plant leaves were washed, shade dried, subjected for size reduction into a fine powder, and stored in an airtight container at room temperature for further use.

Preparation of the plant leaf extract:

The leaves were cleaned with running tap water and rinsed with distilled water to remove debris and contaminants. The leaves were shade dried at room temperature, and powdered. Dried powder was taken and extracted with methanol for about 72 hours by a Soxhlet extractor. The obtained extract was evaporated and then concentrated extract was stored for further use.

IN VITRO ANTIOXIDANT ASSAY:

The free radical scavenging activity of various concentrations of plant extract was measured in terms of DPPH scavenging assay.

DPPH assay of *Manilkara zapota*:

The different concentration of (20-100µg) extract and ascorbic acid were used to carry out the DPPH assay. To each test tube of standard ascorbic acid and extract, 1 mL of methanolic solution of DPPH was added. The reaction mixture was incubated in the dark at room temperature for 20 minutes. After 20 minutes, the absorbance of the mixture was recorded at 517 nm using a UV-Visible spectrophotometer. 1mL of DPPH was taken as a control. % DPPH radical scavenging activity was calculated using the following equation.

$$\% \text{ RSA} = \{A_0 - A_1 / A_0\} * 100$$

where A_0 is the absorbance of the control, and A_1 is the absorbance of the extract/standard.

Then % radical scavenging activity (RSA) was plotted against concentration, and from the graph, the IC_{50} value was calculated¹⁰.

Phosphomolybdate assay of *Manilkara zapota*:^{11,12}

To the aliquots of standard and test samples added 3ml of premixed phosphomolybdenum reagent solution. The test tubes were covered and incubated in a water bath at 95°C for 90 minutes. After the samples were cooled, the absorbance of the mixture was measured at 695 nm. Ascorbic acid was used as reference standard.

Ferric Reducing antioxidant power of *Manilkara zapota*:^{13,14,15}

The aliquots of standard and extracts in 1 ml of DMSO were mixed with 2.5 ml of (pH 6.6) phosphate buffer and 2.5 ml of (1%) potassium ferricyanide. The mixture was incubated at 50°C in water bath for 20 minutes. After cooling, 2.5 ml of (10%) trichloroacetic acid was added to the mixture, which was then centrifuged at 3000 rpm for 10 minutes. The upper layer of solution 5 ml was mixed with mixture of 5 ml distilled water and a freshly prepared 1 ml (0.1%) ferric chloride solution. The absorbance was measured at 700 nm in UV-visible spectrometer.

Evaluation of anti-Alzheimer's potential of *Manilkara zapota* leaf extract on aluminium chloride-induced Alzheimer's rat model:**Experimental animals:**

Healthy Wistar albino male rats of approximately same age (10 to 12 weeks), weighing between 120-170 g were taken for evaluating anti-Alzheimer's activity. The animals were procured from

Vertebrates, Magadi Road, Bangalore. The animals were acclimatized by keeping them in animal house facility of Bharathi College of Pharmacy, Bharathinagara. They were housed in polypropylene cages containing bedding material as husk and maintained under standard husbandry conditions and 12 h light and 12 h dark cycle. They were fed with commercial pelleted rat chow with water *ad libitum*. The animals were maintained in accordance with the CCSEA guidelines. The anti-Alzheimer's activity was conducted after obtaining the approval from Institutional Animal Ethic Committee (IAEC) with a reference no. BCP/IAEC/2025/08 dated 12/07/2025.

Experimental design:¹⁶

A total of 30 Wistar albino rats weighing around 120- 160 g will be divided into five groups, each group consisting of six animals.

Group 1: Normal control

Group 2: Alzheimer's control

Group 3: Alzheimer's+ Rivastigmine (2.5 mg/kg)

Group 4: Alzheimer's + Extract (200 mg/kg)

Group 5: Alzheimer's + Extract (400 mg/kg)

Experimental procedure:

Alzheimer's was induced using aluminium chloride at 175 mg/kg body weight. The study was carried out for up to 36 days. As mentioned above, animals were weighed, recorded, numbered and divided into five groups of six animals each. Group 1: Animals received a standard pellet diet and purified water for 24 hrs over a period of 36 days and were considered the normal control group. Group 2: Animals received aluminium chloride (175 mg/kg) each day over a period of 20 days, and they were considered the Alzheimer's control group. Group 3, Group 4 and Group 5 animals received aluminium chloride (175 mg/kg body weight) each day over a period of 20 days. After that, standard dose (Rivastigmine 2.5 mg/kg) and leaf extract of *Manilkara zapota* at low dose (200 mg/kg) and high dose (400 mg/kg) were administered to Group 3, Group 4 and Group 5, respectively, for 11 days. After 24 hrs of the last treatment, all the animals were weighed, sacrificed, and the brain was collected.

Behavioural studies:

Behavioural tests were performed to functionally validate cognitive dysfunction amnesic models and to assess treatments. Methods to assess hippocampus-dependent memory functions were used.

Elevated Plus Maze:

The Elevated Plus Maze (EPM) is a widely used behavioural test for evaluating anxiety-like behaviour and learning and memory in rodents. The apparatus consists of two open and two closed arms, elevated 50 cm above the floor. Each arm measures

35 × 5 cm, with 15 cm high walls in the closed arms and a central area of 5 × 5 cm. The EPM test was conducted according to the methods described by Walf and Frye (2007) and Auti and Kulkarni (2019). The test was performed in two phases: acquisition phase, in which initial transfer latency (ITL) was recorded, and retention phase, in which memory was assessed the following day. Transfer latency was recorded after Alzheimer's induction to confirm cognitive impairment and after treatment to evaluate the effect of the extract. Each animal was placed in the open arm facing away from the central area, and the transfer latency was recorded as the time taken to enter the closed arm¹⁷.

Cook's Pole Method:

The Cook's Pole Climbing test was used to assess conditioned avoidance response and long-term memory in experimental rats. The apparatus consisted of a wooden/Plexiglas box (27 × 27 × 27 cm) with a stainless-steel grid floor and a wooden pole (10 × 7 × 1.7 cm) positioned at the centre. The animals were acclimatised to the apparatus for 1 minute. A buzzer was sounded, followed 5 seconds later by a mild foot shock through the grid floor. The escape latency, defined as the time taken by the rat to climb onto the wooden pole, was recorded in seconds. The buzzer and shock were immediately stopped once the animal climbed the pole. A single trial was conducted as described by Sakurai et al. (2008), and escape latency was used as the endpoint for memory assessment¹⁸.

Y Maze test:

The Y-maze test was used to evaluate short-term spatial working memory in experimental rats. The apparatus consisted of three arms arranged at 120° angles and designated as A, B, and C. Rats naturally tend to explore a new arm rather than immediately returning to a previously visited arm. This behaviour was assessed by measuring spontaneous alternation. Before testing, the apparatus was cleaned, and the animals were familiarised with the maze. Each rat was placed at the centre of the Y-maze and allowed to explore freely for 8 minutes. The sequence of arm entries was recorded, with an entry counted when all four paws entered the arm. The apparatus was cleaned, disinfected, and dried between trials to prevent olfactory cues. The percentage of spontaneous alternation was calculated using the formula:

$$\% \text{ alternation} = \left\{ \frac{\text{Number of alternation}}{(\text{Total arm entries} - 2)} \right\} \times 100$$

The % alternation was used as an indicator of spatial working memory and cognitive performance in the aluminium chloride-induced Alzheimer's disease rat model¹⁹.

Estimation of brain enzyme:

Catalase assay:

The activity of catalase in supernatants of brain tissue homogenates was measured. Catalase decomposes H_2O_2 to give water (H_2O) and oxygen (O_2) and oxidation of hydrogen donors. H_2O_2 shows a continuous increase in absorption with reducing wavelength in ultraviolet range. H_2O_2 decomposition can be directly followed by the decrease in absorbance at the wavelength of 240 nm. Absorbance difference per unit time is a measure of catalase enzyme activity using the molar extinction coefficient of $H_2O_2 = 43.6 \text{ M cm}^{-1}$. One mmol of H_2O_2 consumed / min/ mg of protein is equal to one unit of activity and this is expressed as U/ml protein. To 0.1 ml supernatant was added to cuvette containing 1.9 ml of phosphate buffer (50 mM, pH 7.0), and the reaction was initiated by adding 1 ml of H_2O_2 (10 mM in phosphate buffer). The change in absorbance was recorded for 1 min at 240 nm using spectrophotometer (Model UV-1650PC). Phosphate buffer was used as a blank. The catalase activity was calculated based on the rate of decomposition of H_2O_2 and was expressed as mmole/min/mg protein²⁰.

Determination of total protein by Lowry's method:²¹

The Lowry protein assay is a sensitive colorimetric method used to estimate protein concentration in biological samples. It is based on the reaction of proteins with alkaline copper reagent followed by the Folin–Ciocalteu reagent, producing a bluish colour proportional to the protein concentration. The absorbance is measured at 750 nm using a spectrophotometer.

Reagents Required:

1. Reagent A: 2% sodium carbonate in 0.1 N sodium hydroxide.
2. Reagent B: 0.5% copper sulphate ($CuSO_4 \cdot 5H_2O$) in 1% potassium sodium tartrate.
3. Alkaline copper reagent (Reagent C): Prepared freshly by mixing 50 mL of Reagent A with 1 mL of Reagent B.

4. Diluted Folin–Ciocalteu reagent (Reagent D): Folin–Ciocalteu reagent diluted with an equal volume of 0.1 N NaOH.
5. Standard: Bovine serum albumin (BSA) working standard containing 200 $\mu\text{g/mL}$ protein.

BSA standards (0.2–1.0 mL) and 1 mL of sample were taken in separate tubes and made up to 1 mL with distilled water. 5 mL of alkaline copper reagent was added, mixed, and incubated for 10 minutes. Then, 0.5 mL of diluted Folin–Ciocalteu reagent was added, mixed, and incubated in the dark for 30 minutes. Absorbance was measured at 750 nm against the blank. Protein concentration was determined from the BSA standard curve.

Statistical analysis:

All the values were expressed as mean $\pm$ S.E.M and statistical significance was analysed using one-way ANOVA followed by Dunnett's multiple comparison test. p-values <0.0001 were considered very highly significant, <0.001 considered highly significant, <0.01 were considered moderately significant and <0.05 considered significant.

RESULT AND DISCUSSION:**DPPH Assay:**

In the DPPH assay, both ascorbic acid and *M. zapota* extract demonstrated concentration-dependent increases in percentage inhibition. The % reactive scavenging activity (RSA) produced by *M. zapota* increased from 18.9% at 20 $\mu\text{g/mL}$ to 67.45% at 100 $\mu\text{g/mL}$, while ascorbic acid increased from 43.25% to 82.1% over the same concentration range. The calculated IC_{50} values were 70.07 $\mu\text{g/mL}$ for *M. zapota* and 33.77 $\mu\text{g/mL}$ for ascorbic acid. Thus, the DPPH findings provide evidence for the antioxidant potential of the methanolic extract of *M. zapota* leaves (MEMZL). DPPH radical scavenging potential of Ascorbic acid and *M. zapota* leaf extract shown in table 2 and figure 2.

Table 2: Percentage RSA and IC_{50} value of DPPH radical by ascorbic acid and MEMZL.

Sample	Concentration ($\mu\text{g/mL}$)	% RSA	IC_{50} value ($\mu\text{g/mL}$)
Standard (Ascorbic acid)	20	43.25	33.77 $\mu\text{g/mL}$
	40	52.8	
	60	63.15	
	80	72.65	
	100	82.1	
MEMZL	20	18.9	70.07 $\mu\text{g/mL}$
	40	34.75	
	60	44.2	
	80	55.1	
	100	67.45	

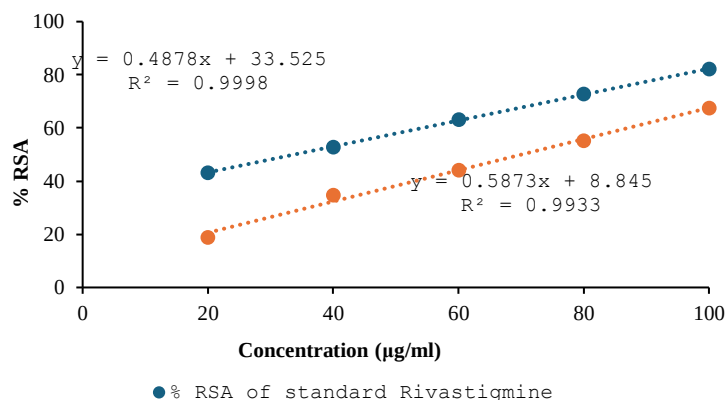


Figure 2: DPPH radical scavenging potential of ascorbic acid and MEMZL.

Phosphomolybdenum Assay:

This assay is based on the reduction of phosphomolybdate ion in the presence of an antioxidant resulting in the formation of a green phosphate/MoV complex which is measured spectrophotometrically. The phosphomolybdate ion reducing power potentials of the MEMZL in

comparison with the standard ascorbic acid at 765 nm is explained in table 3 figure 3. The result indicates that phosphomolybdate ion reducing activity of standard ascorbic acid increased with the increase in their concentration. 100 µg/ml of plant extract shown antioxidant activity which is equivalent to ascorbic acid at the concentration of 8.490 µg/ml.

Table 3: Concentration and absorbance values of standard ascorbic acid and MEMZL of Phosphomolybdenum Assay.

Sample	Concentration (µg/ml)	Absorbance (765nm)
Standard (Ascorbic acid)	6	0.482
	12	0.587
	18	0.659
	24	0.748
	30	0.871
MEMZL	100	0.521

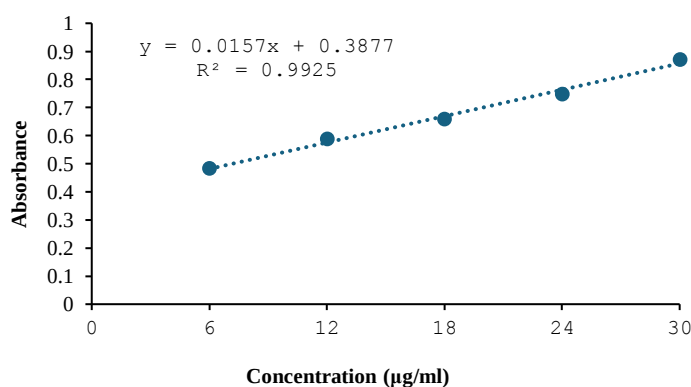


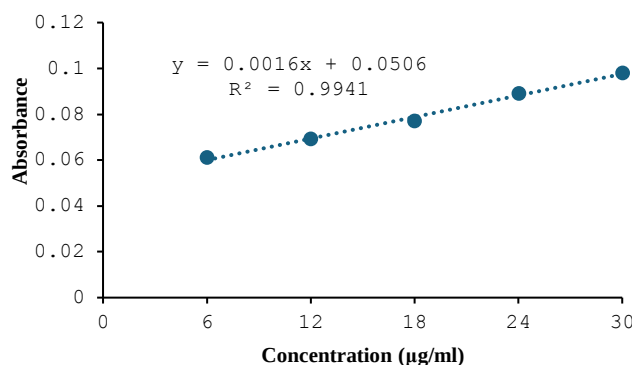
Figure 3: Phosphomolybdenum assay of standard ascorbic acid.

Ferric reducing antioxidant power assay:

The ferric ion reducing power potentials of the MEMZL in comparison with the standard ascorbic acid at 700 nm is explained in table 4 figure 4. The result indicates that ferric reducing activity of standard ascorbic acid increased with the increase in their concentration. 60 µg/ml of plant extract antioxidant activity equivalent to ascorbic acid at the concentration of 15.25 µg/ml.

Table 4: Concentration and absorbance values of standard ascorbic acid and MEMZL of Ferric reducing antioxidant power assay.

Sample	Concentration (µg/ml)	Absorbance (765nm)
Standard (Ascorbic acid)	6	0.061
	12	0.069
	18	0.077
	24	0.089
	30	0.098
Sample (MEMZL)	60	0.075

**Figure 4: Ferric reducing assay of standard ascorbic acid.**

Aluminium chloride administration produced marked cognitive impairment in experimental rats. In the Elevated Plus Maze (EPM), the Alzheimer's control group showed increased transfer latency compared with the normal control, indicating impaired learning and memory. Treatment with *Manilkara zapota* methanolic leaf extract at 200 and 400 mg/kg reduced transfer latency, with a more pronounced improvement at the higher dose, suggesting enhanced memory retention (Table 5 and Figure 5). Similarly, in the Cook's pole climbing test, aluminium chloride increased escape latency,

indicating impaired conditioned avoidance learning. Treatment with *M. zapota* extract reduced escape latency compared with the Alzheimer's control group, demonstrating improvement in learning and memory, particularly at the higher dose (Table 5 and Figure 6). In the Y-maze test, aluminium chloride-induced rats exhibited reduced spontaneous alternation behaviour, indicating impaired spatial working memory. Administration of *M. zapota* extract increased spontaneous alternation compared with the disease control, with greater improvement observed at 400 mg/kg (Table 5 and Figure 7).

Table 5: Behaviour assessment data.

Animal groups (n=6)	TL before treatment (sec)	TL after treatment (sec)	EL before treatment (sec)	EL after treatment (sec)	% Alternation
Normal control	17.33 ± 1.14	13.33 ± 0.49	12.16 ± 0.87	11.83 ± 0.87	82.46 ± 2.01
Alzheimer's control	39.5 ± 3.72####	36.83 ± 2.32####	38.83 ± 3.8####	39.66 ± 2.94####	39.51 ± 1.05####
Alzheimer's + Standard Rivastigmine	38 ± 0.93####	28.5 ± 0.42***	35.83 ± 2.3####	29.5 ± 0.76***	47.85 ± 0.48***
Alzheimer's + Extract (200 mg/ kg)	41.5 ± 3.34####	32 ± 0.73*	40.1 ± 3.1####	33.5 ± 0.88*	44.30 ± 0.81**
Alzheimer's + Extract (400 mg/kg)	39.16 ± 0.70####	30.33 ± 0.88**	38 ± 2.24####	31 ± 0.57**	46.69 ± 0.8***

Data are presented as mean ± SEM values, n=6. Data were analyzed by one-way ANOVA followed by the Dunnet's multiple comparison test and the significance of results showed as ####p<0.0001 when compared with normal control and ***p<0.0001, **p<0.001, *p<0.01, *p<0.05 when compared with the Alzheimer's control.

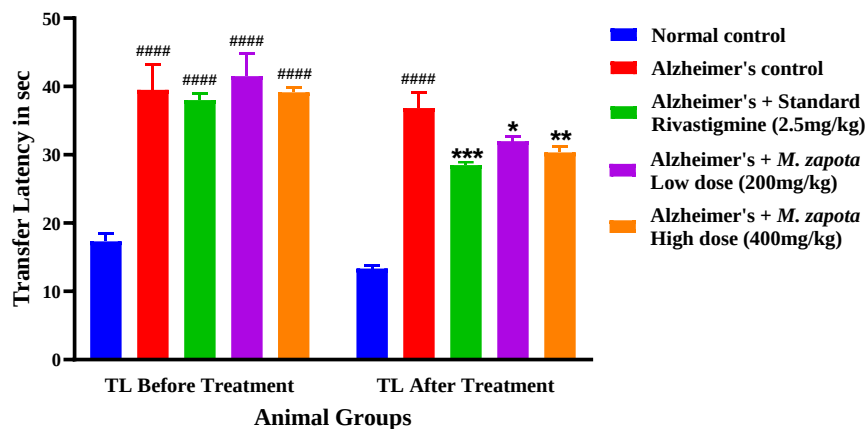


Figure 5: Effect of MEMZL on Transfer Latency in Elevated Plus Maze test in aluminium chloride-induced Alzheimer's rats.

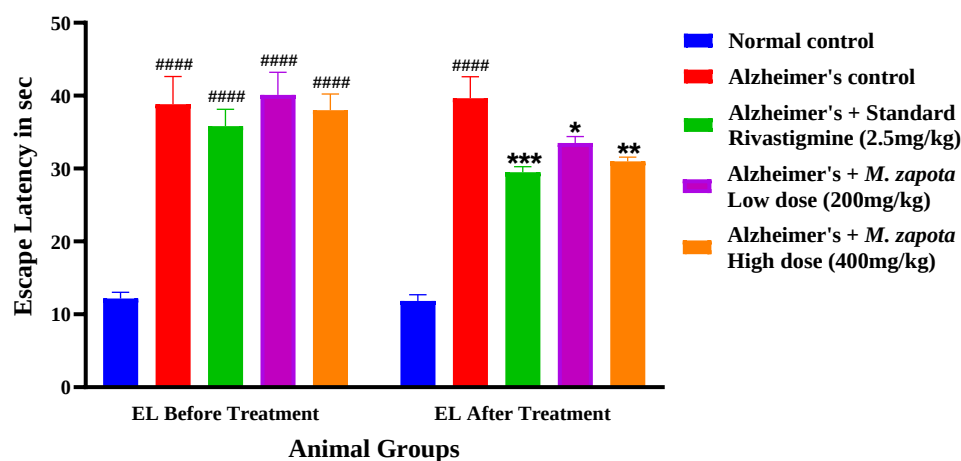


Figure 6: Effect of MEMZL on Escape Latency in Cook's Pole test in aluminium chloride-induced Alzheimer's rats.

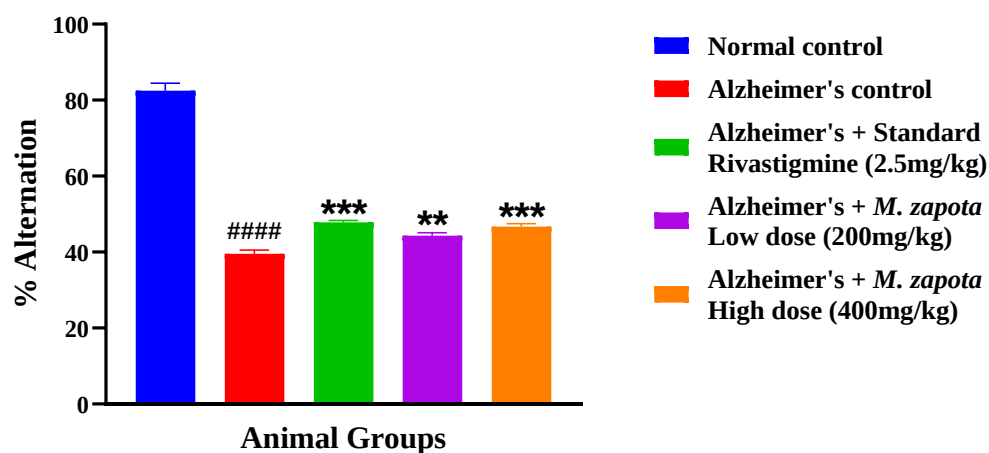


Figure 7: Effect of MEMZL on % Alternation in Y maze.

Oxidative stress was evaluated by measuring catalase activity. Catalase levels in normal control rats (Group 1) were found to be 290.36 ± 0.65 U/ml of protein. Alzheimer's control rats (Group 2)

showed a very highly significant decrease ($p < 0.0001$) in catalase levels in the brain as compared to the normal control group. Rats treated with the standard drug rivastigmine (Group 3)

showed a very highly significant increase ($p < 0.0001$) in catalase levels in the brain when compared to Group 2. Treatment of the rats with *M. zapota* (Groups 4 and 5) resulted in a moderate significant ($p < 0.01$) and highly significant ($p < 0.001$) increase in catalase levels in the brain when compared to Alzheimer's control rats (Group 2) table 6 and figure 8. The reduction in catalase activity in the Alzheimer's control group indicates impairment of endogenous antioxidant defence under the experimental disease condition. Since catalase contributes to the detoxification of hydrogen peroxide, reduced activity may reflect increased oxidative burden. The progressive restoration of catalase activity following *M. zapota* treatment, particularly at 400 mg/kg, suggests

improvement of the endogenous antioxidant defence system. This finding is also consistent with the *in-vitro* antioxidant results, indicating that the extract may exert neuroprotective effects partly through decrease of oxidative stress (Table 6 and Figure 8).

Brain protein concentration was reduced in the Alzheimer's control group ($p < 0.0001$) compared with the normal control. Treatment with Rivastigmine and *M. zapota* extract (200 and 400 mg/kg) increased protein levels towards normal, with greater improvement at 400 mg/kg, suggesting a protective effect against aluminium chloride-induced neuronal protein alterations (Table 6 and Figure 9).

Table 6: Effect of *M. zapota* leaf extract on catalase activity and protein concentration

Animal groups (n=6)	Catalase activity (U/ml)	Protein concentration (mg/dL)
Normal control	290.36 $\pm$ 0.65	1.62 $\pm$ 0.02
Alzheimer's control	162.38 $\pm$ 0.85 ####	0.99 $\pm$ 0.06 ####
Alzheimer's + Standard Rivastigmine	250.45 $\pm$ 0.91 ****	1.49 $\pm$ 0.01 ***
Alzheimer's + Extract (100 mg/kg)	216.05 $\pm$ 0.70 **	1.27 $\pm$ 0.02 **
Alzheimer's + Extract (200 mg/kg)	242.20 $\pm$ 0.70 ***	1.45 $\pm$ 0.02 ***

Data are expressed in mean $\pm$ SEM values, n=6. Data were analyzed by one-way ANOVA followed by the Dunnett's multiple comparison test and the significance of results showed as #### $p < 0.0001$ when

compared with normal control group and **** $p < 0.0001$, *** $p < 0.001$, ** $p < 0.01$, * $p < 0.05$ when compared with the Alzheimer's control group.

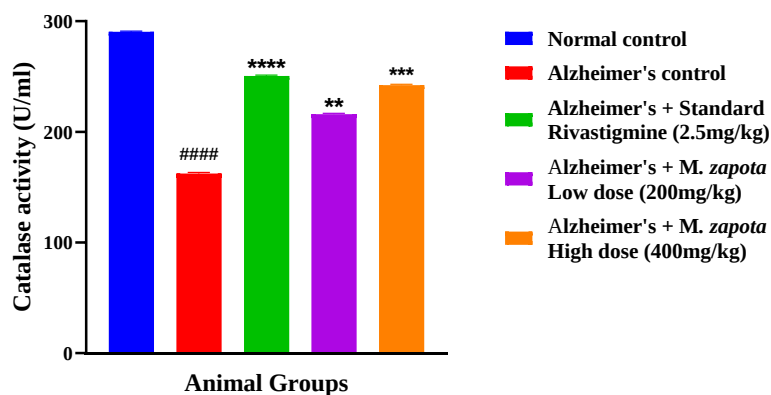


Figure 8: Effect of MEMZL on catalase activity in rat brain.

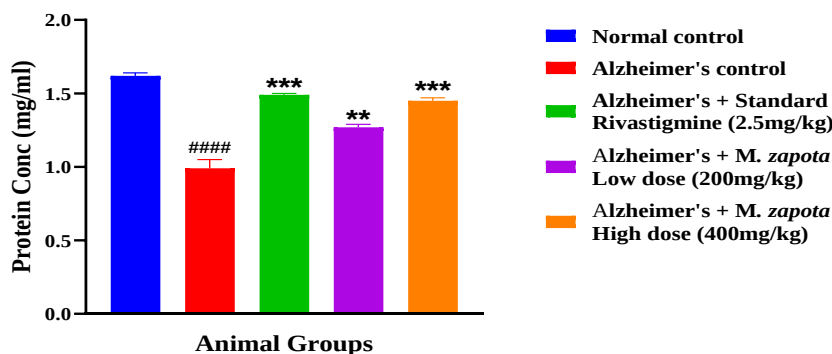


Figure 9: Effect of MEMZL on protein concentration.

CONCLUSION:

The present study demonstrated that methanolic *Manilkara zapota* leaf extract possesses promising neuroprotective and anti-Alzheimer's potential against aluminium chloride-induced cognitive impairment in rats. Treatment with *M. zapota* at 200 and 400 mg/kg improved learning and memory, as evidenced by reduced transfer and escape latencies in the Elevated Plus Maze and Cook's pole tests and increased spontaneous alternation in the Y-maze. The extract also improved catalase activity and brain protein concentration compared with the Alzheimer's control group. Overall, the findings suggest that *M. zapota* may provide neuroprotection through its antioxidant potential, with the 400 mg/kg dose showing greater improvement in the assessed parameters. Further studies are necessary to establish its precise mechanism of action and therapeutic potential.

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