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Cassia tora Mitigates Aluminium Chloride Induced Alterations in Pro-inflammatory Cytokines, Neurotransmitters, and Beta-amyloid and Tau Protein Markers in Wistar Rats

Vikas Pankaj Bhargava[1], Aashish Kumar Netam[1], Rambir Singh[2] and Poonam Sharma[1]*

[1]Department of Zoology, Indira Gandhi National Tribal University, Amarkantak - 484887, Madhya Pradesh, India; pnm245@yahoo.com

[2]Department of Horticulture, Aromatic and Medicinal Plants, Mizoram University, Aizawl - 796004, Mizoram, India

Abstract

Background and Aim: Exposure to Aluminium (Al) has been reported to cause neurotoxicity in laboratory animals. Amyloid-β (Aβ) plaque formation, tau protein hyperphosphorylation, and neuroinflammation have been indicated as the possible mechanism of Al-induced neurodegeneration. The present study aimed to understand the mechanism of aluminium chloride ($AlCl_3$)-induced neurotoxicity in Wistar rats and to assess the neuroprotective effect of methanolic extract of *Cassia tora* leaves (MECT). **Material and Methods:** Seventy-two male Wistar rats were randomly divided into nine groups. $AlCl_3$ (100 mg/kg bw) and MECT (300 mg/kg bw) were given orally by gavage and memantine (MEM) was administered intraperitoneally (20 mg/kg bw) to rats, daily for 60 days. The spatial learning memory and recognition memory were evaluated using the Morris Water Maze (MWM) test. The levels of oxidative stress, neurotransmitter markers, pro-inflammatory markers, Aβ proteins plaques formation and tau protein hyperphosphorylation were evaluated. Histopathology of brain tissue was performed to assess the extent of tissue damage on $AlCl_3$ exposure. **Results:** MECT significantly improved cognitive behaviours in $AlCl_3$-exposed rats during the MWM test. Treatment with MECT resulted in a significant recovery of antioxidant enzyme function, the activity of neurotransmitter markers and pro-inflammatory cytokine levels. MECT prevented the aggregation of Aβ proteins and tau protein phosphorylation. Also, it inhibited the loss of neuronal integrity in the cortex and hippocampus regions of the brain in $AlCl_3$-exposed rats. **Conclusion:** The findings demonstrate that a methanolic extract of *Cassia tora* leaves ameliorated $AlCl_3$-induced neurodegeneration in Wistar rats.

Keywords: Aluminium Chloride, Beta-amyloid, *Cassia tora*, Neurotoxicity, Tau Protein

1. Introduction

Aluminium (Al) is a common element found in large amounts in the Earth's crust. It is used extensively in daily life. Humans are exposed to Al through food products, drinking water, toothpaste, aluminium foil, utensils, cosmetics and pharmaceuticals[1]. In addition, acid rain and bauxite mines exploration result in the discharge of

*Author for correspondence

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Al salts from insoluble minerals, raising the risk of human exposure[2]. Al induces a broad spectrum of toxicity directly linked to its bioavailability[3,4]. Several studies reported that high Al concentration is toxic for all living organisms, including humans. Al can enter biological systems through several channels, but the gastrointestinal and respiratory tracts are the main routes[5]. It accumulates in various organs such as the liver, kidney, lungs, spleen, heart, blood, bones, testis, muscles and brain, causing multiple system toxicity[4,6,7]. The brain is considered to be most vulnerable to the toxic manifestation of Al[8]. Previous studies reported that neuropathological, neurobehavioral, neurophysical and neurochemical changes occur after Al exposure[9,10]. It leads to cognitive impairment and neurodegeneration.

Al binds to transferrin (Tf) in the blood, enters the brain by crossing the Blood-Brain Barrier (BBB) via Transferrin-Receptor Mediated Endocytosis (TfR-ME), and accumulates in various brain parts. In some studies, it has been reported that Al binds to citrate in the blood and generates Al citrate. Al citrate can cross the BBB and facilitate the entry of Al into the brain, independent of the TfR-ME pathway[11]. In addition, Al exposure increases the permeability of the BBB[7]. Continuous exposure to Al increases the generation of free radicals, lipid peroxidation, and oxidative damage to proteins[12] and diminishes antioxidant status such as glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST) in the brain. As a result, levels of oxidative stress (mitochondrial or endoplasmic reticulum) and inflammation rise[10,13]. Various studies have stated that oxidative events are the first step in the onset of neurodegenerative disorders[8]. Oxidative stress has a critical role in altering the levels of pro-inflammatory cytokines and cholinergic neurotransmission[1]. Also, it elevates the production and self-aggregation of amyloid-β (Aβ) proteins which further form plaques on the outside of neurons[10]. Excess production and deposition of Aβ proteins lead to hyper-phosphorylation of tau proteins, lipid peroxidation, disturbance of cell functions, inflammation and apoptosis. The hyper-phosphorylation of tau proteins leads to the formation and accumulation of neurofibrillary tangles (NFTs) inside the neurons leading to neuronal death[11].

Most of the research for neurological diseases in the last few decades has been directed towards disease-modifying therapies[14]. The Food and Drug Administration (FDA) has approved two types of drugs specifically for the treatment of neurodegenerative disease symptoms. The first type is cholinesterase inhibitors drugs such as donepezil, galantamine and rivastigmine. The second type is N-methyl-D-aspartate (NMDA) receptor antagonist drugs like memantine. Both types of drugs prevent the breakdown of chemical messengers in different ways and regulate their activity in the brain, which is important for learning and memory. In addition, previous studies reported several side effects, such as nausea, vomiting, diarrhoea, dizziness, headache, loss of appetite, weight loss, fatigue, muscle weakness, muscle cramps, constipation, etc., that are associated with these types of drugs[15]. Besides, the risk of somnolence, hypertension, confusion and abnormal gait or falling increases with the use of memantine medicine. Briggs *et al*., 2016[14] reported that most of the patients discontinued their treatment because of the side effects of cholinesterase inhibitors. In this context, developing new and safe therapeutics, especially those of herbal origin, is an important research endeavour.

Medicinal plant-based drugs have been used in the treatment of diseases since ancient times[7]. *Cassia tora* Linn. (*C. tora*) (Family: Leguminosae/Fabaceae; Subfamily: Caesalpinioideae) is a well-known medicinal plant commonly found in India and other tropical countries. It is eaten as a vegetable and has numerous biological activities[16]. Various medicinal properties of this plant have been mentioned in the Indian traditional system of medicine as laxatives, antiseptics, antioxidants and anti-inflammatory activities, etc. *C. tora* is also beneficial in treating leprosy, ringworm, bronchitis, cardiac diseases, hepatic disorder, liver tonic, anthelmintic, dyspepsia, haemorrhoids, ophthalmic and skin infections[17]. *C. tora* is widely consumed as a vegetable (Chakoda bhaji) in the Amarkantak region of central India. The region is known for its bauxite mines and local people claim that consumption of *C. tora* helps in the amelioration of Al-induced toxicity. Hence, the present study aimed to investigate the possible protective effect of *C. tora* on $AlCl_3$-induced neurotoxicity in Wistar rats and explore its mechanisms of neuroprotection.

2. Materials and Methods

2.1 Chemicals

$AlCl_3$ anhydrous was purchased from Loba Chemie Pvt. Ltd., India and memantine hydrochloride (MEM) was bought from Sigma-Aldrich, USA. Methanol and Dimethylsulphoxide (DMSO) were procured from Himedia,

India. All other chemicals and reagents used in the study were of analytical grade and had the highest purity.

2.2 Collection of *Cassia tora* Leaves

Fresh leaves of *C. tora* were collected during August-October (2018) from the surroundings of the hills of Indira Gandhi National Tribal University, Amarkantak, District Anuppur, Madhya Pradesh, India. The plant was authenticated by the Department of Botany, Indira Gandhi National Tribal University, Amarkantak, Madhya Pradesh, and a voucher specimen (IGNTU/2018/PS/01) was retained.

2.3 Preparation of *Cassia tora* Leaf Extract

The methanolic extract of *C. tora* leaves (MECT) was prepared in our laboratory as per the previously standardized method[18]. Briefly, 150g of *C. tora* leaves powder was extracted in the soxhlet extractor for 72 hours with 1000 ml of methanol. It was then filtered and concentrated using a rotary evaporator and then stored at 4°C until used.

2.4 Animals

Male Wistar rats (200±20 g) were obtained from the CSIR-Central Drug Research Institute (CDRI), Lucknow, Uttar Pradesh, India. Animals were acclimatised to the laboratory environment two weeks before experimentation and were maintained in standard laboratory conditions (23±2°C, a humidity of 50±5%, and a 12-h light/dark cycle). The rats were housed in sanitised polypropylene cages (4 animals per cage) with sterile paddy husk as bedding and *ad libitum* access to food and water. The experimental protocol was approved by the Institutional Animal Ethics Committee (Protocol Code IGNTU/IAEC/2018/11) of IGNTU, Amarkantak, Madhya Pradesh, and all experiments were carried out according to the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

2.5 Experimental Design

A total of 72 rats were divided into 9 groups of 8 animals each. $AlCl_3$ and MECT were administered orally by gavage, whereas MEM was given by intra-peritoneal injection for 60 days. Normal saline (500 µl/kg BW) was used as a vehicle for $AlCl_3$ and MEM and DMSO (1 ml/kg BW) for the MECT. Doses were selected according to the previous studies; $AlCl_3$[19], MECT[16] and MEM[20]. The experimental schedule is given in Table 1.

2.6 Morris Water Maze Test

The Morris Water Maze (MWM) test was carried out to estimate cognition performance. The MWM test was initiated on the 15th day for each group. The MWM test

Table 1. Treatment schedule

Group	Description
Control (C)	Normal diet and water *ad libitum* for 60 days
Exposure of aluminium chloride ($AlCl_3$)	$AlCl_3$ (100 mg/kg bw) for 60 days
Pre-treatment of methanolic extract of *Cassia tora* (Pre-CT)	MECT (300 mg/kg bw for 60 days) followed by $AlCl_3$ (100 mg/kg bw) for 60 days
Post-treatment of methanolic extract of *Cassia tora* (Post-CT)	$AlCl_3$ (100 mg/kg bw) for 60 days followed by MECT (300 mg/kg bw) for 60 days
Combination of aluminium chloride and methanolic extract of *Cassia tora* ($AlCl_3$+CT)	$AlCl_3$ (100 mg/kg bw) + MECT (300 mg/kg bw) for 60 days
Treatment of methanolic extract of *Cassia tora* (CT)	MECT (300 mg/kg bw) for 60 days
Post-treatment of memantine (Post-MEM)	$AlCl_3$ (100 mg/kg bw) for 60 days followed by MEM (20 mg/kg bw) for 60 days
Combination of aluminium chloride and memantine ($AlCl_3$+MEM)	$AlCl_3$ (100 mg/kg bw) + MEM (20 mg/kg bw) for 60 days
Treatment of memantine (MEM)	MEM (20 mg/kg bw) for 60 days

was performed as described previously[21]. The rats were allowed to swim to a platform in a circular pool (150 cm diameter×60 cm height) filled with water (24±2°C). The pool was divided into four equal quadrants: north, south, east, and west. A coloured platform (10 cm in diameter) was flooded 1 cm below the water surface in the centre of one of the four quadrants throughout the task. The water was made opaque by adding starch powder to hide the platform. A digital camera recorded the motion of animals throughout the MWM test.

In the spatial navigation task, each rat was given four trials per day for four consecutive days to locate and climb on the hidden platform. The rats were given a maximum time of 90 seconds (s) to find the hidden platform. If the rats were found on the platform successfully within 90 s, the rat was allowed a 5-8 s rest period on the platform. If the rats failed to find the hidden platform during 90 s, the animal was physically placed on the platform and allowed a 10-15 s rest period. Then the next trial was performed. The latency time to reach the coloured platform was assessed in each trial. The data for each day were averaged over the four trials before being used for statistical analysis.

One day after the spatial navigation trials, a single 60 s probe task was carried out in which the platform was removed from the pool. The time spent in the target quadrant was recorded. It was carried out in order to assess the extent of memory consolidation.

2.7 Sample Collection and Tissue Preparation

The behavioural and body weight changes were monitored throughout the experiment. After the MWM task, the rats were sacrificed by halothane inhalation, and the brain samples were rapidly removed and cut into two hemispheres (left and right) on an ice-cooled board. The hippocampal region was dissected from the hemispheres and stored at -80°C for biochemical analysis. Blood samples were collected immediately from the heart. Then the serum was separated by centrifugation at 3000 rpm for 15 min (at 4°C) and stored at -20°C for the biochemical analysis. The brain tissues were homogenised in 9 vol of cold phosphate buffer (0.1 M; pH 7.4) using a glass-Teflon homogenizer. To separate the nuclear debris, the tissue homogenates were centrifuged at 9000 rpm for 20 min at 4°C. The brain extract was used for the assay of AChE, MAO, LPO, GSH, CAT, SOD, GPx, GR, GST, Aβ-40 and Aβ-42. Serum was used for IL-1β, IL-6, TNF-α, IFN-γ, and tau protein estimation.

2.8 Aluminium Estimation

The Al level was analysed by Inductively Coupled Plasma-Optical Emission Spectrometry (ICP-OES) in the rat blood according to the method described by Ang and Lee, 2005[22] with some modifications. Samples were analysed at Aakaar Biotechnologies Private Ltd., Lucknow. Briefly, in 0.2 ml of the blood sample, 10 ml of a freshly prepared acid mixture of 1:3 mixture of 65% HNO_3 and 37% HCl was added (2.5 ml nitric acid and 7.5 ml hydrochloric acid). The mixture was boiled gently over a water bath (95°C) for 4-5 h or until the sample had completely dissolved. The final volume of the sample was made with water, then ICP-OES analysis was performed on the PerkinElmer® Optima™ 8000 and the results were expressed as mg/l.

2.9 Antioxidant Enzymes

The lipid peroxidation (LPO) level was estimated by the method described by Ohkawa e*t al.*, 1979[23]. Reduced glutathione (GSH) was measured using Ellman's, 1959[24] methods. The activity of superoxide dismutase (SOD) was determined using the method described by Kakkar e*t al.*, 1984[25]. Catalase (CAT) activity was assessed by the method of Luck, 1971[26]. The activity of glutathione-S-transferase (GST) was assayed by the method of Habig and Jakoby, 1981[27] and Kumar e*t al.*, 2011[28]. Glutathione peroxidase (GPx) and glutathione reductase (GR) activities were assayed by the procedure adopted by Tair e*t al.*, 2016[8].

2.10 Neurotransmitter Markers

The procedure given in the instructions of the ELISA kits acquired from CUSABIO Biotech Co, China, was employed for acetylcholinesterase (AChE) and monoamine oxidase (MAO) enzyme activities.

2.11 Pro-inflammatory Markers

Interleukin-1β (IL-1β), interleukin-6 (IL-6), and interferon-γ (IFN-γ) were assayed by the method described in the commercial ELISA kit instructions purchased from CUSABIO Biotech Co, China. Tumour Necrosis Factor-alpha (TNF-α) was assayed according to the method given in the commercial kit procured from Invitrogen, USA.

2.12 Amyloid-beta and Tau Proteins

The levels of amyloid beta-40 (Aβ-40) and amyloid beta-42 (Aβ-42) were quantified in the hippocampus and tau protein in the blood using an ELISA kit (CUSABIO Biotech Co., China) as per the manufacturer's protocol. The absorbance was measured at 450 nm against a blank using an ELISA reader.

2.13 Histopathological Examination

Rat brain samples (cortex and hippocampus) from each group were transversely cut, fixed in 10% formalin solution and processed for section cutting, staining, and histopathological examination. The sections were examined using a QUASMO STAR-7 microscope[8].

2.14 Statistical Analysis

The statistical analysis of the results was done using the Statistical Package for Social Science (SPSS) software program, version 23.0, for windows 11. The parametric data were expressed as Mean ± SD. Statistical analysis between groups was performed using one-way analysis of variance (ANOVA) followed by Tukey's post hoc test for multiple comparisons between groups was performed and a p-value<0.05 was considered statistically significant.

3. Results

3.1 The Effect of *Cassia tora* and $AlCl_3$ on Body and Brain Weight

Our results demonstrated that $AlCl_3$ treatment reduced ($p<0.01$) body weight and relative whole brain weight when compared to the control group. Administration of MECT ($AlCl_3$+CT, Pre-CT, and Post-CT groups) and MEM ($AlCl_3$+MEM and Post-MEM groups) significantly increased the body weight and relative whole brain weight as compared to $AlCl_3$-exposed rats. No significant change was observed in body and brain weight in CT and MEM-groups treated rats (Table 2).

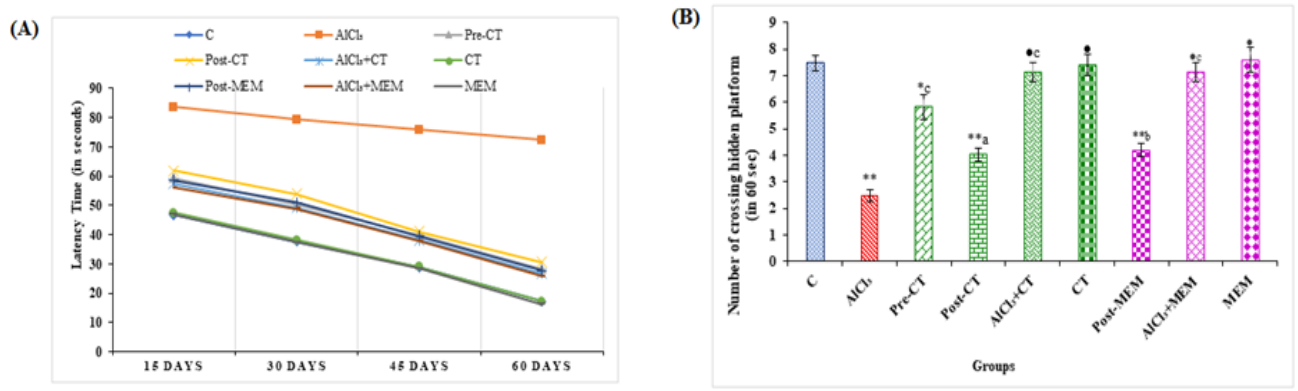
3.2 The Effect of *Cassia tora* and $AlCl_3$ on Cognitive Function

As shown in Figure 1 (A), all rats had the same level of performance at the start of the experiment. The results showed that administration of $AlCl_3$ significantly increased the escape latency time in the second, third, and fourth training days compared to the control group. Treatment with MECT as $AlCl_3$+CT, Pre-CT and Post-CT groups, significantly decreased the escape latency time in the second, third, and fourth training as compared to the $AlCl_3$ group.

Table 2. Effects of $AlCl_3$, *C. tora* and MEM on body weight, absolute brain weight and relative brain weight of Wistar rats

Groups	Initial Body Weight (g)	Final Body Weight (g)	Absolute whole brain weight (g)	Relative whole brain weight (g) (Absolute whole brain weight/ Final Body Weight X 100)
C	198.62 ± 4.11	250.12 ± 4.90	2.263 ± 0.039	0.905 ± 0.004
$AlCl_3$	199.12 ± 3.58	183.62 ± 4.39**	1.589 ± 0.035**	0.866 ± 0.004**
Pre-CT	198.87 ± 4.42	220.50 ± 3.25^{**c}	1.940 ± 0.040^{**c}	0.879 ± 0.005$^{\bullet a}$
Post-CT	198.50 ± 4.32	209.25 ± 2.12^{**c}	1.827 ± 0.037^{**c}	0.872 ± 0.010^{*a}
$AlCl_3$+CT	197.87 ± 3.31	238.12 ± 4.14$^{\bullet}$	2.110 ± 0.029$^{\bullet c}$	0.886 ± 0.007$^{\bullet a}$
CT	199.25 ± 4.46	252.12 ± 5.46$^{\bullet}$	2.244 ± 0.037$^{\bullet}$	0.890 ± 0.007$^{\bullet}$
Post-MEM	197.12 ± 3.81	215.12 ± 2.45^{**c}	1.897 ± 0.031^{**c}	0.881 ± 0.004$^{\bullet a}$
$AlCl_3$+MEM	198.25 ± 4.71	242.12 ± 4.61$^{\bullet c}$	2.143 ± 0.029$^{\bullet c}$	0.886 ± 0.007$^{\bullet a}$
MEM	199.37 ± 4.36	248.12 ± 4.01$^{\bullet}$	2.220 ± 0.030$^{\bullet}$	0.895 ± 0.007$^{\bullet}$

Values are represented as mean ± SE for 8 rats in each group
(·) $p>0.05$, (*) $p<0.05$, (**) $p<0.01$: compared with control
(a) $p>0.05$, (b) $p<0.05$, (c) $p<0.01$: compared with aluminium chloride ($AlCl_3$)



Values are represented as mean ± SE for 8 rats in each group
(·) p>0.05, (*) p<0.05, (**) p<0.01: compared with control
(a) p>0.05, (b) p<0.05, (c) p<0.01: compared with aluminium chloride ($AlCl_3$)

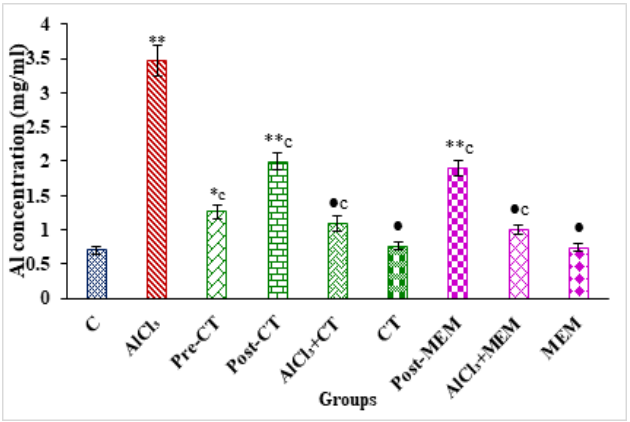
Figure 1. Effects of *C. tora* and MEM on the progress spatial memory performance in Morris water maze test. **(A)** The acquisition trial (time required to reach the platform in seconds) along 60 days and **(B)** the probe trial (number of crossing hidden platform) in Wistar rats

Similarly, MEM treatment ($AlCl_3$+MEM and Post-MEM groups) also reduced the escape latency time. CT and MEM groups did not cause any significant change in the escape latency time as compared to the control group.

In the memory retention test with the MWM probe trial (Figure 1 B), administration of $AlCl_3$ significantly decreased (p<0.01) the time spent in the target quadrant as compared to the control group. MECT ($AlCl_3$+CT, Pre-CT and Post-CT groups) and MEM ($AlCl_3$+MEM and Post-MEM groups) treatments significantly increased the time spent in the target quadrant as compared to the $AlCl_3$ group. There were no significant differences between the control, CT and MEM groups.

3.3 The Effect of *Cassia tora* and $AlCl_3$ on Blood Aluminium Concentration

$AlCl_3$ exposure significantly (p<0.01) increased the blood Al concentration in the group as compared to the control group. MECT treatment significantly attenuated the blood Al concentration in the $AlCl_3$+CT, Pre-CT and Post-CT groups when compared to the $AlCl_3$ group. Similarly, a significant reduction in blood Al concentration was detected in the Post-MEM and $AlCl_3$+MEM groups as compared with the $AlCl_3$ group. Moreover, when the CT and MEM groups were compared to the control group, there was a non-significant elevation in blood Al concentration (Figure 2).



Values are represented as mean ± SE for 8 rats in each group
(·) p>0.05, (*) p<0.05, (**) p<0.01: compared with control
(a) p>0.05, (b) p<0.05, (c) p<0.01: compared with aluminium chloride ($AlCl_3$)

Figure 2. Al concentration in blood of $AlCl_3$, *C. tora* and MEM treated groups in Wistar rats.

3.4 The Effect of *Cassia tora* and $AlCl_3$ on Lipid Peroxidation, Nonenzymatic and Enzymatic Antioxidants

$AlCl_3$ treatment significantly enhanced (p<0.01) the LPO levels and diminished (p<0.01) levels of GSH, CAT, SOD, GPx, GR and GST, respectively, compared to the control group. Treatment of MECT ($AlCl_3$+CT, Pre-CT and Post-CT groups) and MEM ($AlCl_3$+MEM and Post-MEM groups)

Table 3. *C. tora* and MEM attenuate the effects of $AlCl_3$ against lipid peroxidation (LPO), reduced glutathione (GSH), catalase (CAT), superoxide dismutase (SOD), glutathione peroxidase (GPx), glutathione reductase (GR) and glutathione-S-transferase (GST) in the brain of Wistar rats

Groups	LPO (nmolemda/ hr/gram/ tissue)	GSH (µmole/g tissue)	CAT (µmole/min/ mg protein)	SOD (µ mole/ min/mg protein)	GPx (nmole/ min/mg protein)	GR (nmole/min/ mg protein)	GST (µmole/ min/mg protein)
C	4.808 ± 0.442	13.932±0.489	7.610 ±0.380	8.648±0.447	3.592±0.286	4.148±0.297	5.255±0.323
$AlCl_3$	10.707 ± 0.366**	5.819±0.347**	2.528±0.230**	4.081±0.366**	1.129±0.122**	1.081±0.109**	1.655± 0.212**
Pre-CT	7.124 ± 0.419^{*c}	11.798±0.460$^{\bullet c}$	5.820 ±0.468^{*c}	6.813±0.425^{*c}	2.676±0.338$^{\bullet c}$	3.287±0.196$^{\bullet c}$	3.539±0.348^{*b}
Post-CT	8.626 ± 0.437^{**b}	7.589±0.322^{**a}	3.961±0.225^{**a}	5.846±0.366^{**a}	2.153±0.251^{**a}	1.951±0.128^{**a}	3.031±0.315^{**a}
$AlCl_3$+CT	6.549 ± 0.491$^{\bullet c}$	12.402±0.304$^{\bullet c}$	6.883±0.392$^{\bullet c}$	7.971±0.297$^{\bullet c}$	3.015±0.193$^{\bullet c}$	3.574±0.261$^{\bullet c}$	4.874±0.387$^{\bullet c}$
CT	5.021 ± 0.480$^{\bullet}$	13.269±0.607$^{\bullet}$	7.398±0.403$^{\bullet}$	8.515±0.338$^{\bullet}$	3.308±0.272$^{\bullet}$	3.879±0.204$^{\bullet}$	5.168±0.390$^{\bullet}$
Post-MEM	8.445 ± 0.452^{**b}	11.404±0.470^{*c}	4.188±0.239^{**b}	6.074±0.422^{**b}	2.354±0.269^{*b}	2.977±0.268^{*c}	3.406±0.269^{*b}
$AlCl_3$+MEM	6.571 ± 0.349$^{\bullet c}$	12.676±0.708$^{\bullet c}$	7.044±0.327$^{\bullet c}$	8.450 ±0.444$^{\bullet c}$	3.104±0.245$^{\bullet c}$	3.657±0.246$^{\bullet c}$	4.912±0.392$^{\bullet c}$
MEM	4.906 ± 0.496$^{\bullet}$	13.381±0.541$^{\bullet}$	7.498±0.450$^{\bullet}$	8.522±0.420$^{\bullet}$	3.361±0.268$^{\bullet}$	3.996±0.194$^{\bullet}$	5.18±0.510$^{\bullet}$

Values are represented as mean ± SE for 8 rats in each group
(·) p>0.05, (*) p<0.05, (**) p<0.01: compared with control
(a) p>0.05, (b) p<0.05, (c) p<0.01: compared with aluminium chloride ($AlCl_3$)

significantly declined the LPO levels and augmented the levels of GSH, CAT, SOD, GPx, GR and GST as compared to $AlCl_3$-treated animals. Between the control, CT and MEM groups, no significant changes were identified (Table 3).

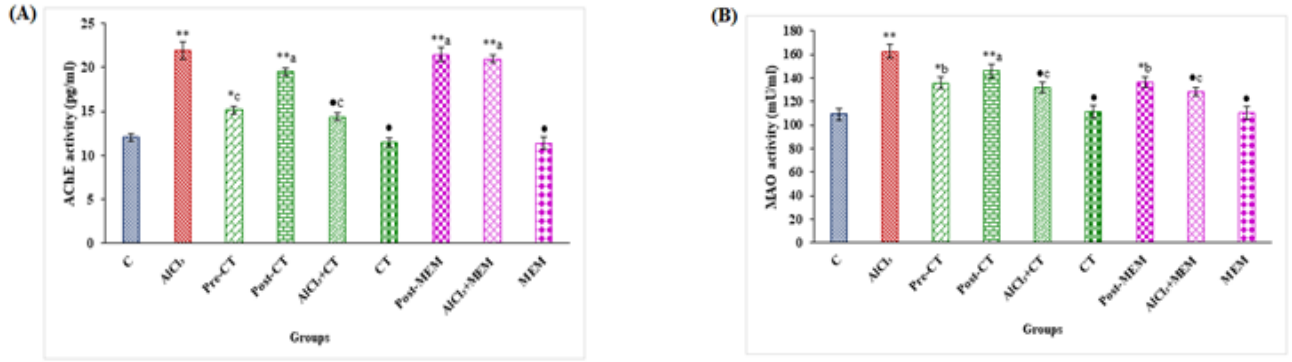
3.5 The Effect of *Cassia tora* and $AlCl_3$ on Neurotransmitter Markers

As shown in Figure 3, $AlCl_3$ intake resulted in an alteration in the activity of neurotransmitter metabolising enzymes. AChE and MAO activities were significantly increased ($p<0.01$) in the brain tissues of $AlCl_3$-treated rats as compared to the control group. On the other hand, their activities were significantly inhibited after the treatment with MECT as $AlCl_3$+CT, Pre-CT and Post-CT groups when compared to $AlCl_3$-treated animals. In contrast, MEM treatment ($AlCl_3$+MEM and Post-MEM groups) reduced the increased levels of MAO but did not alter the elevated AChE levels compared to $AlCl_3$-treated animals. The control, CT and MEM groups showed no significant changes.

3.6 The Effect of *Cassia tora* and $AlCl_3$ on Pro-inflammatory and Amyloidogenic Markers

Exposure to $AlCl_3$ increased ($p<0.01$) the production of pro-inflammatory molecules such as IL-1β, IL-6, TNF-α and IFN-γ compared to the control group (Table 4). However, $AlCl_3$ exposure increased ($p<0.01$) the levels of Aβ-42 protein and suppressed ($p<0.01$) the Aβ-40 levels in brain tissues as compared to the control group (Table 4).

MECT administration normalized the cytokines levels (IL-1β, IL-6, TNF-α and IFN-γ) compared to the $AlCl_3$-treated group. MECT treatment not only significantly decreased Aβ-42 but also induced the anti-amyloidogenic



Values are represented as mean ± SE for 8 rats in each group
(·) p>0.05, (*) p<0.05, (**) p<0.01: compared with control
(a) p>0.05, (b) p<0.05, (c) p<0.01: compared with aluminium chloride ($AlCl_3$)

Figure 3. *C. tora* and MEM prevent the $AlCl_3$-induced overexpression of **(A)** Acetylcholinesterase (AChE) and **(B)** Monoamine oxidase (MAO) activities in Wistar rats.

Table 4. *C. tora* and MEM prevent the overexpression of interleukin-1β (IL-1β), interleukin-6 (IL-6), interferon-γ (IFN-γ), tumor necrosis factor-α (TNF-α), amyloid beta-40 (Aβ-40), amyloid beta-42 (Aβ-42) and tau protein levels induced by $AlCl_3$ in Wistar rats

Groups	IL-1β (pg/ml)	IL-6 (pg/ml)	IFN-γ (pg/ml)	TNF-α (pg/ml)	Aβ-40 (pg/ml)	Aβ-42 (pg/ml)	Tau Protein (pg/ml)
C	118.953±4.748	8.157±0.329	7.516±0.268	48.530 ±3.149	19.863±0.652	5.213±0.351	78.212±3.380
$AlCl_3$	215.518±6.847**	14.756±0.597**	19.476±0.678**	167.348±4.928**	6.306±0.578**	13.306±0.599**	232.782±5.308**
Pre-CT	146.717±6.520*c	10.759±0.561*c	9.715±0.503*c	64.447±3.525*c	16.169±0.871*c	7.782±0.525*c	95.796±3.516*c
Post-CT	169.806±4.702**c	12.560 ±0.652**a	17.529±0.495**a	94.402±3.105**c	8.865±0.599**a	12.227±0.657**a	118.495±3.058**c
$AlCl_3$+CT	142.853±6.092•c	10.150 ±0.669•c	9.106±0.344•c	60.949±2.160•c	18.044±0.886•c	6.757±0.619•c	90.796±2.971•c
CT	125.606±3.236•	8.811±0.364•	8.173±0.380•	56.536±2.715•	19.121±0.584•	5.434±0.312•	85.631±2.828•
Post-MEM	147.076±4.760*c	10.882±0.610*c	9.871±0.522*c	62.862±2.114*c	16.385±0.785*c	7.685±0.532*c	97.237±4.173*c
$AlCl_3$+MEM	142.419±4.480•c	9.980 ±0.540•c	9.255±0.457•c	58.981±1.922•c	18.210 ±0.755•c	6.397±0.488•c	87.218±3.029•c
MEM	122.258±5.736•	8.417±0.418•	7.942±0.423•	53.164±2.685•	18.937±0.706•	5.312±0.473•	82.854±3.686•

Values are represented as mean ± SE for 8 rats in each group
(·) p>0.05, (*) p<0.05, (**) p<0.01: compared with control
(a) p>0.05, (b) p<0.05, (c) p<0.01: compared with aluminium chloride ($AlCl_3$)

protein Aβ-40 in the $AlCl_3$+CT, Pre-CT and Post-CT groups when compared to the $AlCl_3$-treated group. Similar results were also observed in the treatment of MEM in the Post-MEM and $AlCl_3$+MEM groups. No significant differences were observed between the control, CT and MEM groups (Table 4).

3.7 The Effect of *Cassia tora* and $AlCl_3$ on Tau Protein

The hyper-phosphorylation of tau protein was significantly increased (p<0.01) in $AlCl_3$-treated rats as compared to the control group (Table 4). Administration of MECT reduced hyperphosphorylation of tau protein in all *C. tora*-treated

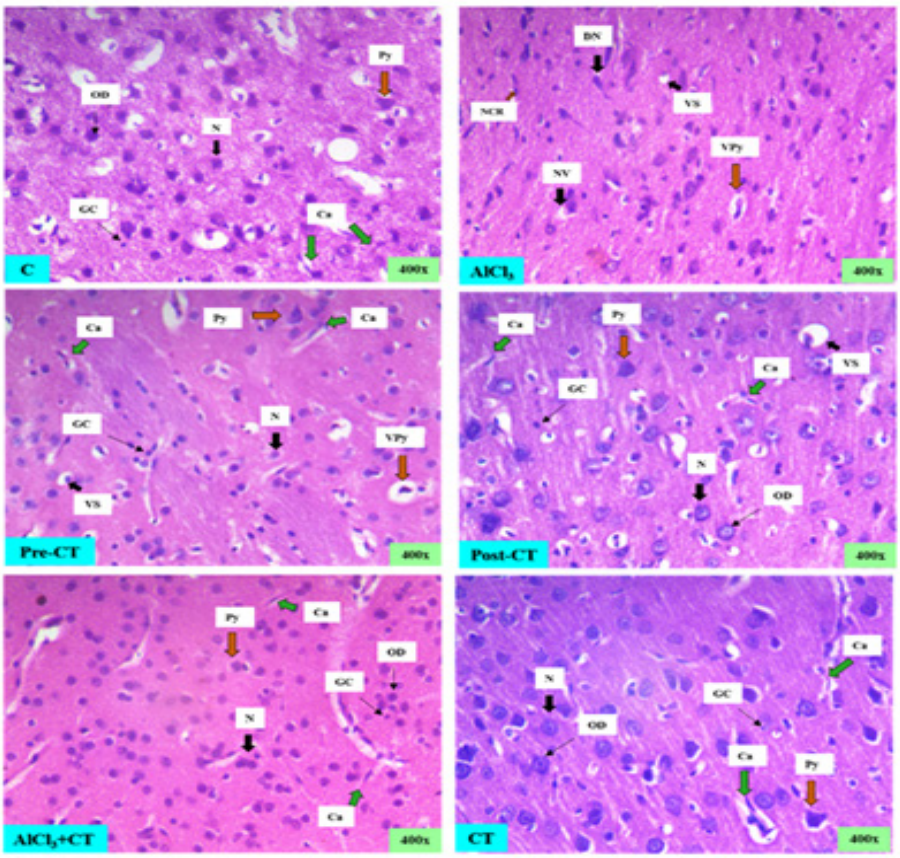
groups ($AlCl_3$+CT, Pre-CT and Post-CT groups) compared to the $AlCl_3$-treated group. Similarly, MEM treatment efficiently ameliorated the $AlCl_3$-induced tau pathology in MEM-treated groups (Post-MEM and $AlCl_3$+MEM groups) when compared to the $AlCl_3$-treated group. There were no significant differences between the control, CT and MEM groups.

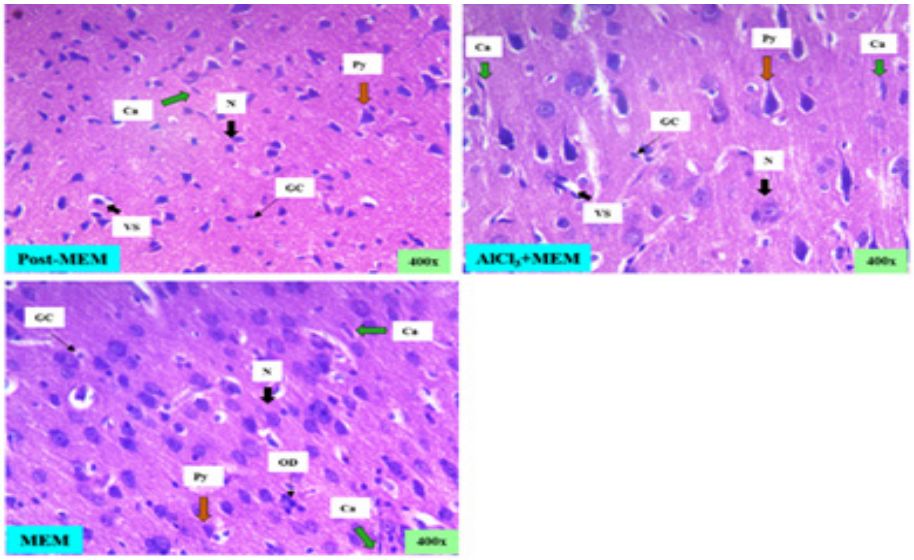
3.8 The Effect of *Cassia tora* and $AlCl_3$ on Brain Histopathology

In the histopathological study, pathological changes in the cortex and hippocampus of $AlCl_3$-intoxicated rats were examined under light microscopy. The micrographs of hematoxylin and eosin (H and E) staining (Figures 4 and 5) showed that the cortex and hippocampal neurons presented a regular arrangement, with a clear nucleus and nucleolus without any pathological alteration in the control, CT and MEM groups. Neuronal loss, spongy degeneration, necrosis and vacuolated cytoplasm were found in the $AlCl_3$ group when compared to the control group. In addition, the number of pyramidal cells and neurons decreased significantly after $AlCl_3$ exposure. Treatment with MECT ($AlCl_3$+CT, Pre-CT and Post-CT groups) showed an improvement effect against $AlCl_3$-induced neurodegeneration. Similar outcomes were seen in the treatment of MEM (Post-MEM and $AlCl_3$+MEM groups).

4. Discussion

Within the scope of the current investigation, the protective effect of MECT was examined against $AlCl_3$-induced neurotoxicity in rats. The results from the present research indicated that $AlCl_3$ exposure decreased the body weight and relative weight of the whole brain as compared to the control group. The reduction in body weight in the $AlCl_3$ exposure group may have been due to a lesser desire for food intake. In addition, the loss of brain weight could be due to spongiosis of the neuropil resulting in retarded brain development in the rats. Similar results were reported by Gol *et al.*, 2019[29]. They stated that $AlCl_3$ exposure resulted in decreased body weight in rats. Tair *et al.*, 2016[8] noticed a reduction in the brain weight of $AlCl_3$-treated rats.

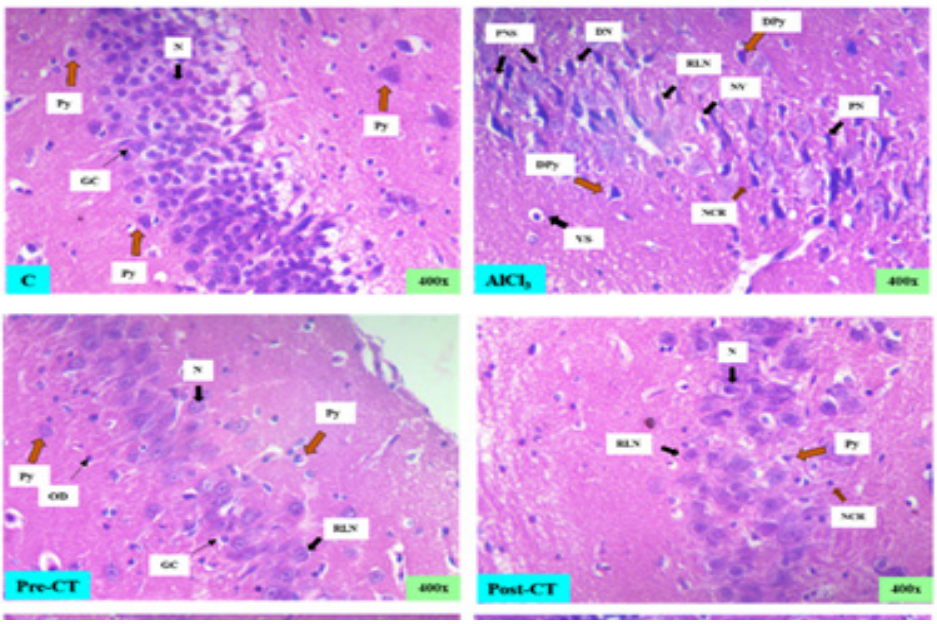


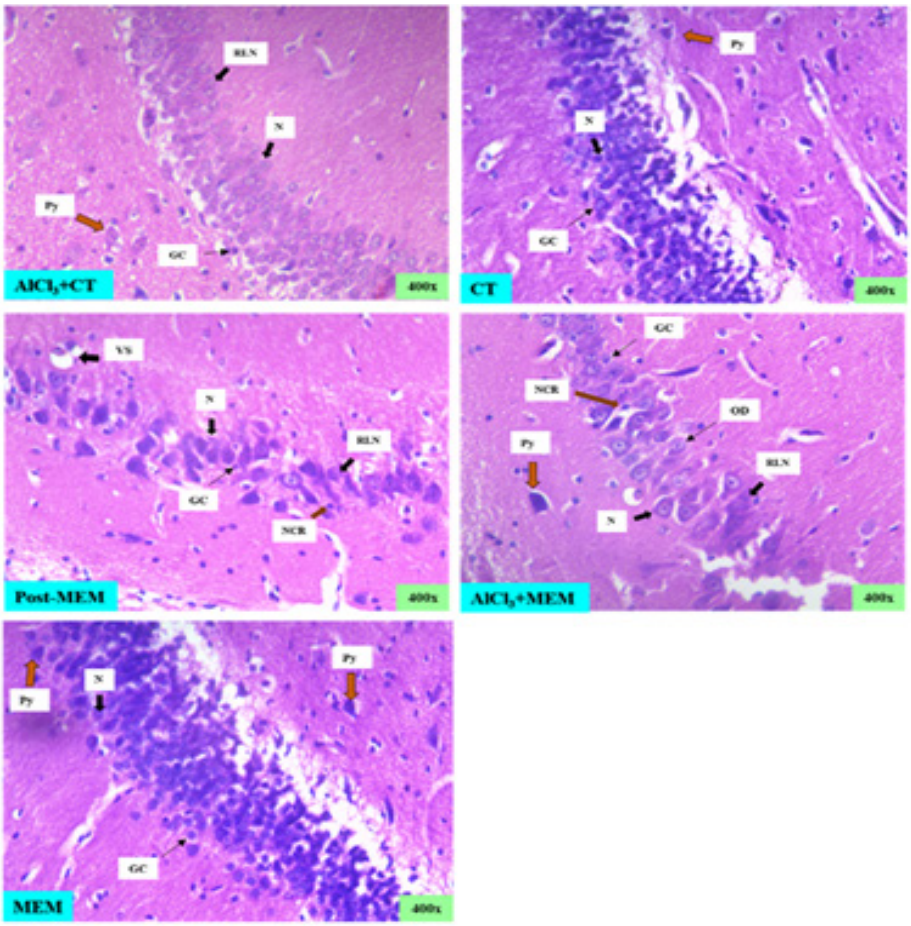


N: Neurons, **Py:** Pyramidal cells, **GC:** Glial cells, **OD:** Oligodendrocytes, **NV:** Neuronal vacuoles, **DN:** Degenerated Neurons, **Ca:** Calcium, **NCR:** Necrosis, **VS:** Vacuolar spaces around cell cytoplasm, **VPy:** Vacuolated Pyramidal cells

Figure 4. Effects of *C. tora* and MEM on $AlCl_3$-induced neurodegeneration in the H and E stained cortex tissue of rats brain.

According to our results, administration of $AlCl_3$ exhibited a marked deterioration of cognitive skills in the MWM test when compared to the control group. Our results are in line with the previous study by Khalaf e*t al.*, 2020[30], where they reported that the administration of $AlCl_3$ to rats led to the decline of spatial learning and recognition memory. Al reaches the brain via blood circulation with the help of transferrin (Tf) and/or citrate and accumulates in various parts of the brain[11]. Accumulation of Al in the brain interferes with long-term potentiating molecules such as cyclic guanosine monophosphate (cGMP), leading to an impairment in the glutamate-NO-cGMP pathway





N: Neurons, **Py:** Pyramidal cells, **GC:** Glial cells, **OD:** Oligodendrocytes, **NV:** Neuronal vacuoles, **DN:** Degenerated Neurons, **PNS:** Pyknotic neurons, **PN:** Pyknotic nuclei, **DPy:** Degenerated Pyramidal cells, **RLN:** Reduced layer of neuronal cell, **NCR:** Necrosis, **VS:** Vacuolar spaces around cell cytoplasm

Figure 5. Effects of *C. tora* and MEM on $AlCl_3$-induced neurodegeneration in the H and E stained hippocampus tissue of rats brain.

that results in cognitive impairments and neurobehavioral deficits in rats[31].

In the current study, ICP-OES analysis results showed that the Al concentration in the blood of $AlCl_3$-treated rats was significantly increased as compared to the control group. It may be due to the fact that transferrin and citrate, which are present in the blood, play a major role in binding to Al, which leads to the circulation of Al through the blood to various parts of the body[11]. In addition, fasting also enhances Al absorption[32].

Due to its prooxidant nature, Al generates ROS and/or inhibits antioxidant enzymes in the brain and plays an essential role in the imbalance between these, further inducing oxidative stress[33,8]. Based on our results, $AlCl_3$ accumulation in the brain resulted in elevated LPO levels and reduced GSH levels, CAT, SOD, GPx, GR and GST activities as compared to the control group. These results agree with Kumar *et al.*, 2011[28] who reported that the administration of $AlCl_3$ alters antioxidant levels in Wistar rats. Skalny *et al.*, 2021[33] mentioned that a reduction in antioxidant enzyme activities is linked to increased brain lipid peroxidation under Al exposure. Natural antioxidant enzymes like SOD, CAT and GPx are present in the first line of defence against free-radical damage under oxidative stress conditions. In oxidative stress situations, SOD presents the first line of defence against superoxide as it converts the superoxide anion to H_2O_2 and O_2. It also has an important role in detoxifying superoxide radicals to H_2O_2, which is then converted to H_2O by CAT and GPx at the expense of GSH. Therefore, $AlCl_3$ exposure increases levels of LPO (Figure

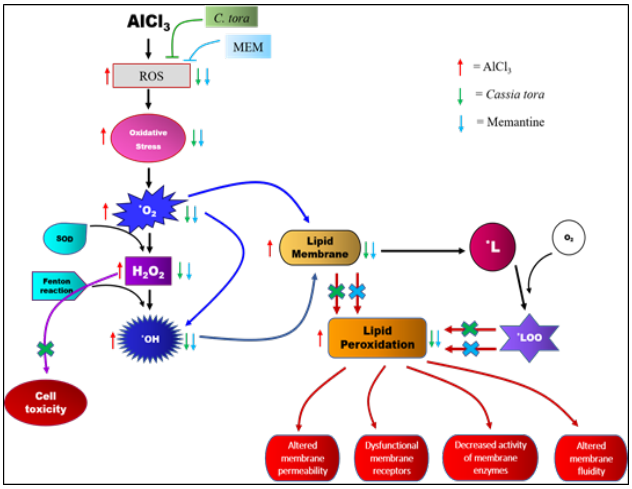


Figure 6. Schematic representation of lipid peroxidation in $AlCl_3$-induced neurotoxicity and possible mechanism of inhibiting effect of *C. tora* and MEM[34].

6)[34] and inhibits the GSH levels, and activities of CAT, SOD, GPx, GR and GST enzymes (Figure 7). In addition, $AlCl_3$ modifies those genes that regulate the activity of the CAT enzyme in rats[12]. Also, Aβ peptides directly bind with the CAT enzyme and deactivate its catalytic activity, creating oxidative stress conditions[35].

AChE is the key enzyme involved in the hydrolysis of acetylcholine in the neuromuscular junction and synapse, which results in the termination of nerve impulse transmission. The impairments in cholinergic transmission are associated with the severity of neurological diseases[31]. Our findings show that $AlCl_3$ exposure increases the AChE activity in the hippocampus. It is probably due to the direct neurotoxic effect of Al that alters the kinetic properties of AChE. The enhanced expression of AChE resulted in a decreased level of acetylcholine, which may lead to the pathogenesis of neurological diseases. A similar finding has also been reported by Khalaf *et al.,* 2020[30], who mentioned that $AlCl_3$ induces aberrant inflammatory reactions and gene expression defects after entering the brain. Increased levels of AChE could be due to the allosteric interaction between the cation (Al^{+3}) and anionic sites of the AChE enzyme, which leads to an alteration in the secondary structure of AChE in the brain and eventually enhances its activity. Increased activity of AChE lead to over-consumption of ACh[31,36]. The possible mechanism for the same is mentioned in Figure 8[38]. In addition, the overproduction of IL-1β promotes the activity and expression of AChE. Khalaf *et al.*, 2020[30] stated that long-term Al^{3+} exposure results in overexpression of the Amyloid Precursor Protein (APP) gene and consequently Aβ production, which increases AChE activity through alpha7-nicotinic acetylcholine receptors. Over-expression of APP is associated with oxidative damage and mitochondrial dysfunction[37].

MAO, a flavin-containing enzyme confined to the superficial membrane of mitochondria, is crucially involved in the metabolism of monoamine neurotransmitters and other amines as well[39]. Our results revealed that the $AlCl_3$ exposure group showed a significant increase in MAO activity compared to the control group. The increased activity of MAO may be due to the direct impact of $AlCl_3$, which activates MAO. Activated MAO is involved in producing H_2O_2, which is a mediator of oxidative stress. The previous study strongly supports our hypothesis that $AlCl_3$ exposure in rats enhanced oxidative stress by increasing the activity of MAO[40].

Neuroinflammation plays a critical role in the pathogenesis of neurodegenerative diseases[37]. Skalny *et al.*, 2021[33] mentioned that Al shows pro-inflammatory activity. Our results confirmed that $AlCl_3$ is responsible for the neuroinflammation and the elevation of inflammatory cytokines such as IL-1β, IL-6, IFN-γ and TNF-α as compared to the control group. These results are in agreement with previous studies that found a positive correlation between

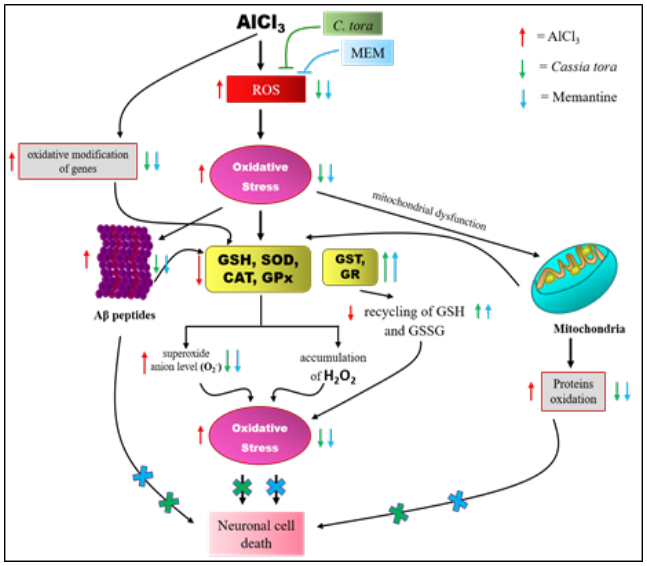


Figure 7. Schematic representation of reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST) and glutathione reductase (GR) enzymes in $AlCl_3$-induced neurotoxicity and possible mechanism of inhibiting effect of *C. tora* and MEM.

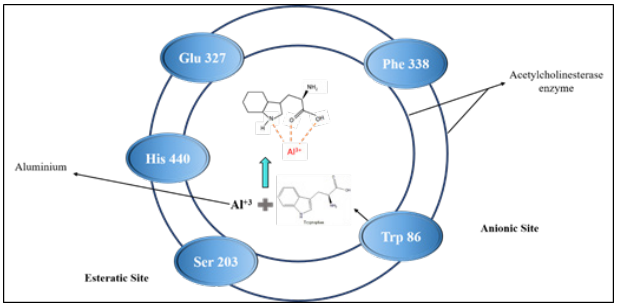


Figure 8. Schematic representation of possible binding sites of acetylcholinesterase enzyme with aluminium[38].

neuroinflammatory cytokine release and the progression of neurological diseases, which suggests these cytokines are involved in the pathophysiology of neurological disorders[19,41]. Administration of $AlCl_3$ activates astrocytes and microglia through ROS generation, which leads to increased levels of IL-1β, IL-6, IFN-γ and TNF-α[19,42]. Combined stimulation with IFN-γ and TNF-α triggers the production of Aβ peptides. Aβ peptides lead to the activation of microglia, which increases the production of neuroinflammatory cytokines[43].

Aβ is an aggregation-prone and toxic polypeptide with 39-43 residues, derived from the proteolysis process of APP in neurological diseases. Among all Aβ isoforms, Aβ-40 and Aβ-42 are believed to be the most important ones[44]. In the current investigation, there was a significant decrease in the Aβ-40 protein levels and an increase in the Aβ-42 protein levels in rats exposed to $AlCl_3$ compared with the control group. The reduced level of Aβ-40 is possibly due to oxidative stress induced by $AlCl_3$, which further inhibits antioxidants and activates inflammatory cytokines. Hussien *et al.*, 2018[37] stated that a mixture of aluminium chloride, cadmium and fluoride decreased Aβ-40 levels in rats. The increased level of Aβ-42 is possibly due to the sequential proteolytic splitting of APP by β- and γ-secretase enzymes. $AlCl_3$ plays an important role in initiating the amyloidogenic pathway with the help of ROS and oxidative stress[45]. Promyo *et al.*, 2020[46] reported that $AlCl_3$ exposure elevated Aβ-42 levels in the brain by upregulating beta-site amyloid precursor protein cleaving enzyme1 (BACE1) expression and suppressing Lipoprotein Receptor-related Protein-1 (LRP1) and neprilysin (NEP) expression and Aβ clearance-related proteins in rats. In addition, Aβ-42 is produced as a monomer but readily aggregates to form amyloid plaques because Aβ-42 is chemically stickier than the other lengths due to the length of 42 amino acids[47].

These amyloid plaques induce hyper-phosphorylation of tau protein[37]. Our results displayed the overexpression of tau protein in the $AlCl_3$-treated group as compared to the control group. Hyper-phosphorylation of tau protein is possibly due to oxidative stress and stimulation of kinase activities (like GSK 3β and AKT) by $AlCl_3$. Our results are in the same line with the study of Rather *et al.*, 2019[1]. Aβ leads to exacerbating synaptic mitochondrial alterations, including increased oxidative stress and decreased respiration that promote the release of cytochrome C (Cyt C). It activates caspase-9 and caspase-3, which cleaves AKT (also known as protein kinase B or PKB), resulting in GSK3β activation. GSK3β, under the control of AKT and PP1, is a critical determinant of the direction of NMDA receptor-dependent plasticity. The active GSK3β isoforms critically contribute to neurodegeneration by hyper-phosphorylation of tau protein[48]. Phosphorylated tau proteins are detached from the microtubules and assembled to form helical filaments. These helical filaments further assemble into insoluble NFTs. As a result, the internal transport within neurons gets lost, and the capability to correspond with other neurons significantly vanishes, which causes neuronal death[1]. Additionally, these NFTs deregulate Fyn enzyme (Proto-oncogene tyrosine-protein kinase Fyn) activity and consequently affect NMDA receptor response[48] (Figure 9).

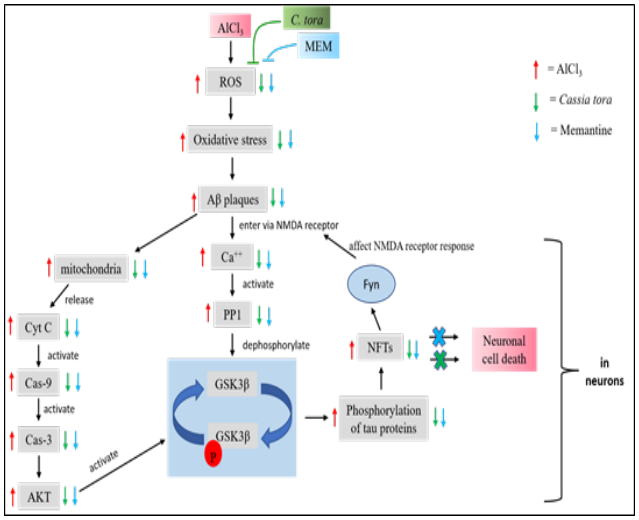


Figure9. Schematic representation of tau phosphorylation in $AlCl_3$-induced neurotoxicity and possible mechanism of inhibiting effect of *C. tora* and MEM[48].

The examination of H and E stained sections revealed that Al produced marked histopathological abnormalities in brain tissues, including neuronal vacuolization, neuronal shrinkage, necrosis, spongiosis, gliosis, cellular rarefaction and disruption in cytoplasmic-Ca^{2+} in the $AlCl_3$ treated group as compared to the control group. As already stated that $AlCl_3$ increases ROS production and mitochondrial dehydrogenase in glial cell generation. On the other hand, $AlCl_3$ accumulation initiates lipid peroxidation and also disturbs the antioxidant mechanism of eliminating free radicals inside the cell. Free radicals are drawn to the double bonds of unsaturated fatty acids because there is a high level of unsaturated fatty acids in the brain. Meanwhile, neurons naturally possess a high aerobic metabolism. These factors come together to make the nervous system prone to oxidative damage and inflammation, which leads to apoptosis and degeneration of neurons (Figures 4 and 5). These results are correlated with those claimed by many authors. $AlCl_3$-induced alterations like neuronal vacuoles, necrosis, reduced neuronal cell layer, etc., are associated with learning-memory impairments. Vacuolated cells are considered as the initial stages of dying cells producing a swollen appearance within distinct boundaries[8,29]. Khafaga, 2017[13] stated that the administration of $AlCl_3$ causes neuronal shrinkage and necrosis in the rat's brains by disturbing the iron-dependent cellular processes.

C. tora leaves contain antioxidant and anti-inflammatory properties[49]. In our previous study, Gas Chromatography-Mass Spectrometry (GC-MS) analysis of MECT was performed. The GC-MS results showed that various compounds such as DL-alpha-tocopherol, linolenic acid, phytol, trehalose, tocopheryl acetate, ethyl palmitate, a 2,3-dihydro benzofuran and stearic acid were present in the *C. tora* leaves. Most of these compounds show antioxidant, anti-inflammatory and neuroprotective properties[18]. The presence of the above phytochemicals in the methanolic extract used in the present study may confer the neuroprotective properties of *C. tora*.

In the present study, MECT helped maintain body and brain weight by reducing the side-effects of Al in the $AlCl_3$+CT, Pre-CT and Post-CT groups as compared to the $AlCl_3$ group. MECT increased food cravings in rats and improved their body weight. It reduces $AlCl_3$-induced alterations in the rat's brain, as confirmed by H and E-stained brain histology micrographs. Also, it significantly improved spatial learning behaviour and recognition memory. Similar findings were reported by Malabade and Ashok, 2015[16], who stated that treatment of *C. tora* significantly reversed the behavioural impairment in scopolamine-induced amnesia in rats. Our results of ICP-OES analysis demonstrated that the blood Al concentration of MECT-treated groups was significantly decreased as compared to the $AlCl_3$-treated group. It could be due to its metal-chelating properties. Arya and Yadav, 2010[50] stated that *C. tora* shows the highest metal chelating activity.

In the present study, MECT enhanced the antioxidant activity, restored mitochondrial function and suppressed oxidative stress via eliminating the harmful ROS induced by the $AlCl_3$ in a neurodegenerative disease model of rats. It increased GSH levels, SOD, CAT, GPx, GR and GST activities while decreasing LPO levels in MECT-treated groups. Treatment of MECT significantly restores hippocampal AChE and MAO activities and reduces the levels of IL-1β, IL-6, IFN-γ and TNF-α. It also significantly elevated the levels of Aβ-40 and decreased Aβ-42 proteins and tau hyper-phosphorylation in MECT-treated groups. These positive alterations by MECT may be due to its antioxidative, anti-inflammatory and metal-chelating properties. It deactivates astrocytes and microglia and restores mitochondrial functions via suppression of $AlCl_3$-induced oxidative stress, which down-regulates the AChE, MAO activities, over-production of pro-inflammatory cytokines and inhibits the aggregation of Aβ oligomers. It further decreases the hyperphosphorylation of tau proteins. These findings seem to be in concordance with the results of some previous studies. Ravi e*t al.*, 2020[9] reported that *C. tora* considerably enhanced the activities of antioxidants and decreased the levels of inflammatory cytokines and Aβ-42 in $AlCl_3$-induced neurotoxicity in rats. Khalifeh e*t al.*, 2021[51] stated that trehalose shows antioxidant and anti-inflammatory properties by scavenging oxygen-free radicals. Trehalose directly impacts neurons and induces autophagy which aids in the removal of misfolded protein aggregates[52]. In another study, Sathya e*t al.*, 2018[53] indicated that phytol prevents the aggregation of Aβ oligomers through its antioxidative and non-cytotoxic properties in Aβ-induced neurotoxicity in Neuro2a cells. Similarly, alpha-tocopherol has antioxidant and anti-inflammatory properties and negatively affects the aggregation of Aβ proteins[54]. Malabade and Ashok, 2015[16] stated that an ethanolic extract of *C. tora* leaves showed AChE inhibitory activity and enhanced the level of ACh in scopolamine-induced amnesia in the rat model. Yamamuro e*t al.,* 2013[55] reported that stearic acid can reduce the activity of MAO. Figures 6, 7 and 9 depict the possible mechanisms of *C. tora*.

In the histopathological examination, our observation is that MECT was able to maintain the integrity of $AlCl_3$-treated neurons, pyramidal neurons, glial cells and cytoplasmic Ca^{2+} in the cortex and hippocampal regions as compared to the $AlCl_3$ group. It significantly mitigated the degenerative shape of the pyramidal cells and increased mature pyramidal cell density, along with the improved alignment of pyramidal cell layers which corroborated with a significant diminution of the number of pyknotic cells and an increase in the proportion of healthy neurons. It might be due to the antioxidant, anti-neuroinflammatory and neuroprotective properties of MECT. Ravi e*t al.*, 2020[9] stated that *C. tora* shows cytoprotective properties against $AlCl_3$-induced neurotoxicity in rats. Figures 4 and 5 show the potential neuroprotective effect of *C. tora* on brain regions.

In the present study, the $AlCl_3$+CT, Pre-CT and Post-CT groups significantly decreased $AlCl_3$-induced neuronal degeneration. The therapeutic impact of early MECT treatment in the Pre-CT group might be able to protect the brain owing to its antioxidative effect that later exposure to $AlCl_3$ is unable to cause extreme damage to neurons. Further the $AlCl_3$+CT group had a greater therapeutic effect against neuronal damage, which might be due to the simultaneous quenching of ROS by MECT that caused a reduction in oxidative stress, superoxide anion and hydroxyl radicals. The lower protective effect of MECT treatment in the Post-CT group might be because early $AlCl_3$ exposure resulted in more damage to the neuronal cells via oxidative stress, superoxide anion, hydroxyl radicals and misfolded proteins. Further treatment of MECT was unable to reverse it. The $AlCl_3$+CT group exhibited a more protective impact than other MECT-treated groups (Pre-CT and Post-CT groups).

MEM is used to treat variety of neurological disorders[56]. In the present study, treatment of MEM ($AlCl_3$+MEM and Post-MEM groups) restored body and brain weight and reversed spatial learning and memory impairment in rats exposed to $AlCl_3$. According to the results of our ICP-OES analysis, the blood Al concentration in the MEM-treated groups was significantly lower than that of the $AlCl_3$-exposure group. In addition, MEM administration considerably re-equilibrated the levels of LPO, GSH and activities of SOD, CAT, GPx, GR, and GST enzymes in $AlCl_3$+MEM and Post-MEM groups. These positive impacts of MEM are probably due to its antioxidant property, which plays an essential role in reducing oxidative stress. Our results are in line with the previous study by Abdel-Aal e*t al.*, 2011[20]. They stated that MEM treatment significantly improved body weight and memory performance in $AlCl_3$-induced neurotoxicity in rats. Several studies reported that MEM increased the GSH levels, SOD, CAT, GPx, GR and GST activities and decreased LPO levels in neurotoxicity rat models[57-59].

Our results revealed that treatment with the MEM drug did not show a noticeable decrease in AChE levels in the Post-MEM and $AlCl_3$+MEM groups as compared to the $AlCl_3$ treated group. MEM does not affect the AChE level because the target of MEM is NMDA receptors. Gothwal e*t al.*, 2019[60] reported that the MEM treatment does not cause significant changes in AChE activity in $AlCl_3$ administered group. On the contrary, the MEM drug led to a considerable decrease in MAO activity in MEM-treated groups when compared to the $AlCl_3$-treated group. The reduction in the MAO activity caused by MEM administration is probably due to the inhibition of ROS generation. Onogi e*t al.*, 2009[61] reported similar results in their study. MEM administration with $AlCl_3$ in rats significantly reduced IFN-γ, IL-1β, IL-6, and TNF-α levels. The levels of these cytokines reduced by MEM administration might be due to the inhibition of the nuclear factor-kappa B (NF-κB) pathway and blockade of the NMDA receptor, and it also prevents T-cell activation. These findings were in line with the study of Shata e*t al.*, 2020[62]. Our results showed that the treatment of MEM restored the levels of Aβ-40 and Aβ-42 proteins the $AlCl_3$+MEM and Post-MEM groups as compared to the $AlCl_3$-treated group. It is possibly due to stabilizing APP on the cell surface and decreasing secretase enzymes (β and γ secretase) activity, which further reduces Aβ peptides. MEM also significantly decreases the hyper-phosphorylation of tau protein. It is possibly due to reversing the activation of multiple kinase activities, including GSK-3β and associated signalling mechanisms. Similar to our results, Khalaf e*t al.*, 2019[56] reported that MEM decreases APP levels by reducing beta-site amyloid precursor protein cleaving enzyme1 (BACE1) activity. It also inhibits the prolonged influx of Ca^{2+} ions via modulating NMDA receptors, which downregulates the expression of tau protein.

In the current study, our histopathological results indicated that MEM drug treatment ($AlCl_3$+MEM and Post-MEM groups) ameliorated $AlCl_3$-pathological alterations in the hippocampus and cortex regions. The reduced neuronal alteration in the brain region caused by MEM treatment is probably due to its antioxidative and anti-inflammatory properties. The present morphometrical results were in line with Shata e*t al.*, 2020[62], reported that MEM administration reduces neuronal degeneration in $AlCl_3$-treated rats.

The $AlCl_3$+MEM group showed a greater therapeutic effect against neuronal degeneration than the Post-MEM group. It may be due to the antioxidant property of MEM, which can prevent further damage by $AlCl_3$. In comparison, the Post-MEM group showed lighter degeneration with slighter cell distortion. It could be because chemical changes happen before morphological changes.

5. Conclusion

In the present study, MECT ameliorated $AlCl_3$-induced neurodegenerative alterations in rats. The neuroprotective activity of MECT was comparable with the standard drug MEM. Treatment with MECT successfully restored all parameters as well as AChE activity, while MEM treatment was unable to restore the activity of AChE. The present study justified the ethnomedicinal use of *Cassia tora* by the local ethnic people as a vegetable.

6. Acknowledgements

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7. Conflict of Interest

The authors declare that there is no conflict of interest.

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