

Research Article



Neuroprotective Effect of Ethanolic Extract of *Galinsoga parviflora* Plant against Aluminium Chloride (AlCl₃) induced Neurotoxicity in Rats

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ABSTRACT

Aluminium chloride (AlCl₃) has been proven neurofilament changes in the cerebral cortex, hippocampus, brain stem, and spinal cord, in addition biochemical changes were observed in many neurodegenerative diseases. Oxidative stress plays a significant role in AlCl₃ induced neurotoxicity. The amyloid hypothesis, which states that oxidative stress causes beta amyloid deposition by causing free radical production, lipid peroxidation (LPO), Protein and DNA damage and neurotoxicity. The cholinergic hypothesis was based on reduced synthesis of the neurotransmitter acetylcholine (Ach). In the present research investigation, the administration of AlCl₃ (50 mg/kg/day) orally for 7 days has induced oxidative stress and its implications possesses significant neurotoxicity was evaluated by behavioral deficits, Motor incoordination and memory deficits which were also supported biochemically marked as increased in lipid peroxidation (LPO) with decreased Glutathione (GSH), Superoxide dismutase (SOD) and Total protein (TP) levels and thereby confirms the oxidative model of cognitive deficits. Histopathological studies of cerebral cortex area recognized as worsening and visualized as increased glial cells. The ethanolic whole plant extract of *Galinsoga parviflora* treatment (Low dose 200 mg/kg and High dose 400 mg/kg) was significantly prevented the altered behavioral, biochemical and histopathological changes associated with AlCl₃ induced neurotoxicity. The ethanol extract of *Galinsoga parviflora* plant and its strong antioxidant potential due to various bioactive chemical constituents might have neuroprotective potential in AlCl₃ induced neurotoxicity. High dose of ethanolic extract of *Galinsoga parviflora* plant (400 mg/kg) has shown maximum neuroprotection.

Keywords: Neurodegenerative diseases, Aluminium chloride (AlCl₃), *Galinsoga parviflora*, Neuroprotective activity, Neuroprotective drugs, Neurotoxicity, Antioxidants.

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INTRODUCTION

Heavy metals like Aluminium has proven to have an effect with learning, understanding, memory, concentration and decision making, which affect everyday life of people in urban areas.¹ Aluminium is a well-known neurotoxicant involved in the development of neurodegenerative diseases such as Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS) and Huntington's disease (HD).² Aluminium supplementation causes neurodegeneration and apoptotic neuronal loss, along with cognitive dysfunction, as it is a potent cholinotoxin.³ Generally, cholinergic activity is essential for the acquisition and retrieval of learning and memory skills. Numerous animal studies have similarly shown that prolonged exposure to aluminum can cause neurochemical, neurobehavioral and neuropathological changes in the brain that reduce the rats' ability for learning.⁴ The preference of using

aluminum chloride (AlCl₃) was compounded on the fact that it is found in many food products, toothpastes, medicines and purified drinking water.⁵ Experimentally, long-term exposure to Aluminium had been shown to cause not only neurological symptoms that mimic advanced neurodegeneration but also neurofilament alterations in the cerebral cortex, hippocampus, brain stem, and spinal cord, in addition to biochemical changes observed in many Neurodegenerative diseases.⁶ Oxidative stress plays a significant role in induction of AlCl₃ induced neurotoxicity.⁷ Two major competing hypotheses exist to explain the AlCl₃ cause of the neurodegenerative diseases: the cholinergic hypothesis is due to reduced synthesis of the neurotransmitter acetylcholine (Ach) and the amyloid hypothesis where oxidative stress induces β amyloid (Aβ) deposition that is manifested by lipid peroxidation, protein and DNA oxidation, free radical formation and neurotoxicity.^{8,9} Furthermore, for a suitable neuroprotectant, a very important property can its ability to cross the blood-brain barrier (BBB), so that it reaches the target sites of the brain. Polyphenols have been shown to have multiple targets in the brain, so they could potentially be used to treat neurological disorders. These include polyphenols, flavonoids, quinones and coumarins, catechins, terpenoids, ascorbic acid, alpha-tocopherol, beta-



carotene, vitamin C and vitamin E. Herbal medicines and their constituents have been proven to be a potent neuroprotectant against various brain pathologies including Alzheimer's disease, Parkinson's disease, Huntington's disease, multiple sclerosis, stroke, traumatic brain injury and epilepsy. Herbal medicines containing anti-inflammatory/anti-oxidative compounds and their constituents are proving to be a powerful neuroprotectant against various brain pathologies.¹⁰ In view of all the points, *Galinsoga parviflora* plant (Family: Asteraceae) has been selected to investigate the neuroprotective activity based on its antioxidant activity and use in traditional systems of medicine which contains all the potent constituents as Alkaloids, Flavonoids, Phenols, Terpenoids, Carbohydrates, Proteins, Tannins etc. which may have capability to treat the common neurological disorders.¹¹

In the current study, oxidative stress indicators such as reduced glutathione (GSH), lipid peroxidation (LPO), superoxide dismutase (SOD), and total protein (TP) were used to evaluate the neuroprotective effect of an ethanolic extract of the *Galinsoga parviflora* plant in AlCl₃ induced neurotoxicity.

MATERIALS AND METHODS

Chemicals used during experiments

Acetate solution, Acetic anhydride (Merk), Aluminium chloride (AlCl₃) (Inducing agent), Ammonia (SD Fine Chem Ltd.), Chloroform (Merk), Conc. H₂SO₄, Conc. HCl, Ethanol (SD Fine Chem Ltd), Ferric Chloride (SRL), Ferric chloride solution, Formalin (Merk), Ethanolic extract of *Galinsoga parviflora* plant (Test drug), Glacial acetic acid, Mayer's reagent (Merk), Molish reagent, Ninhydrin reagent, Normal saline (Vehicle for control group), Piracetam (Standard drug), Wagner's reagent, all other chemicals were of the highest purity commercially available.

Apparatus/Equipments/Glasswares

Actophotometer, Beakers, Centrifuge (REMI Cooling Centrifuge. C-24 BL), Conical flask, cylindrical vessel, Digital balance (ACCULAB- Sartorius group), Elevated plus maze, Homogenizer (REMI Homogeniser Mumbai. Type RQ 127 A), Motor and pestle, Oral feeding needle (18 gauge), Polypropylene cage, Refrigerator, Rota rod, round bottom flask, Soxhlet apparatus, Steel wire (2 mm diameter and 80 cm in length), Surgical cotton, Test tubes, UV Spectrophotometer (SHIMATZU, UV-16100), variable micropipette.

Collection and authentication of plant material

For the study, the *Galinsoga parviflora* plant was collected from the surrounding garden area at Mooganayakanakote. The sample was identified and authenticated by Dr. P.E. Rajasekharan, Principal scientist and Nodal officer GAC, Division of Plant genetic Resources, Indian institute of horticultural research, Hesaraghatta lake post, Bangalore, Karnataka.

Ethanolic extraction method of *Galinsoga parviflora* whole plant

The *Galinsoga parviflora* plant was freshly collected, washed and dried at room temperature after which they (whole dried plant except root) made into coarse powder.

Soxhlet extraction

Air dried and powdered plant (45 gm) were extracted with 95% ethanol (1 liter) in a Soxhlet apparatus (30°C - 40°C) for 72 hours and filter, filtrate was kept for evaporation at room temperature.¹²

Phytochemical studies in various fractions of *Galinsoga parviflora* plant extract

Preliminary phytochemical screening of an ethanolic extract of *Galinsoga parviflora* plant was done by using various types of chemical tests including Dragendorff's, Mayer's, Hager's and Wagner's tests for alkaloids, Molisch's, Fehling's, Benedict's, Barfoed's, and Biuret's tests for carbohydrates, Biuret and Millon's tests for proteins, Ninhydrin's test for amino acids, Salkowski and Libermann-Burchard's reactions for steroids, Borntrager's test for anthraquinones glycosides, Foam test for saponins; Shinoda and alkaline tests for Flavonoids glycosides, and Ferric chloride, Lead acetate tests for tannins, and Phenols.

Experimental animals

Adult Healthy male albino rats weighing between 180-220g used for this study. Air condition rooms with optimal air changes per hour, humidity, temperature and elimination cycle set to 12 hours light and 12 hours in dark. The suitable standard conditions were maintained for animals in an animal house approved by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA). The study protocol of the project was approved by the Institutional Animal Ethics Committee (IAEC) of Mallige College of Pharmacy, Bangalore. Reg No.1432/PO/RE/S/11/ relative CPCSEA & 27/05/2017. **Approval no:** MCP 083/2019-20.

Acute oral toxicity studies

Acute toxicity was carried out to determine lethal dose (LD₅₀) according to Organization of Economic Co-Operation and Development (OECD) no 425 guidelines. The oral administration of extracts of *Galinsoga parviflora* plant up to a dose of 3200 mg/kg neither showed any mortality nor any adverse signs with regard to 72 hours of food and water uptake in the animals after treatment of plant extracts. The LD₅₀ of extracts was recorded as 3617.20 mg/kg (*p.o.*) for *Galinsoga parviflora* plant.³³ Based on the results obtained from this study, the dose of further pharmacological studies was fixed to be 200 mg/kg for low dose and 400 mg/kg for high dose.



Experimental Design**Aluminium chloride (AlCl₃) induced neurotoxicity in rats**

Total 30 rats were randomly divided into five groups of 6 rats each and treated for 7 days as follows:

Table 1: Method of AlCl₃ induced neurotoxicity in rats^{13,14}.

Sl. No.	Group	Treatment	Duration of treatment
1.	Negative control	Normal Saline	Daily for 7days
2.	Positive control	AlCl ₃ 50mg/kg/day (p.o) dissolved in water (oral)	Daily for 7days
3.	<i>Galinsoga parviflora</i> plant extract (low dose) -200mg/kg/day (p.o).	AlCl ₃ 50mg/kg/day (p.o) + Low dose of <i>Galinsoga parviflora</i> plant extract (p.o)	Daily for 7days
4.	<i>Galinsoga parviflora</i> plant extract (high dose) - 400mg/kg/day (p.o).	AlCl ₃ 50mg/kg/day (p.o) + High dose of <i>Galinsoga parviflora</i> plant extract (p.o)	Daily for 7days
5.	Standard (Piracetam)	AlCl ₃ 50mg/kg/day (p.o) + Piracetam 200 mg/kg/day (p.o)	Daily for 7days

The ethanolic extract of *Galinsoga parviflora* plant was administered before 1 hour administration of AlCl₃ 50 mg/kg (p.o). During the drug treatment, rats were observed for the behavioral changes for 50 min daily. On 8th day after 1 hour of AlCl₃ administration, rats were evaluated for General behavioral studies such as string test for grip strength, Locomotor activity, Rota-rod performance assessment for motor coordination and elevated plus maze test. On 9th day, rats were sacrificed humanly under mild anesthesia and brain were isolated for estimation of different biochemicals such as Reduced Glutathione (GSH), Lipid peroxidation (LPO), Superoxide dismutase (SOD), Total protein, and histopathological study.¹⁵

Monitored Parameters**Changes in body weight measurement**

Animals body weight was measured and noted on the first day and last day of the experimentation. The body weight was calculated in comparison to the initial body weight on the first day of the experimentation.¹⁶

Movement analysis (Neurological changes)

Neurotoxins cause various types of motor disturbances which prevent normal walking or movement of the animals. The severity of the motor abnormalities in neurotoxin-treated animals was therefore assessed by using a quantitative neurological scale for locomotion. A neurological score was determined for each animal on 8th day of AlCl₃ after 4 h of last dose in comparison to control animals. (Score = 0, normal behavior; score = 1, general slowness of displacement resulting from moderate impairment to the hind limbs; score = 2, in coordination and marked gait abnormalities; score = 3, hind limb paralysis; score = 4, incapacity to move resulting from fore limb and hind limb impairment; score =5).¹⁷

Locomotor activity

Using a photo actometer with infrared sensitive photocells, the spontaneous locomotor activity was recorded. The apparatus was set up in a dark, light, and

well-ventilated testing room. Animals were first placed individually into the activity meter for 2 minutes for habituation. Thereafter, their locomotor activity was recorded during a 5-minute test session. Locomotor activity was expressed as the number of total photo beams/5 min.¹⁸

Elevated plus maze (EPM) test for special memory

The spatial long-term memory of rats was assessed by using the EPM test. A typical EPM apparatus consists of two open arms (length = 500 mm × width = 100 mm), two close arms (length = 500mm × width = 100 mm × height = 400 mm), and central square connecting the four arms.

The maze was elevated to a height of 500 mm above the floor. In the acquisition trial, each rat was placed individually in the end of one of the open arms and the time it took to move from the end of the open arm to either of closed arms was recorded as ITL using a stopwatch. If the rat did not enter into one of the closed arms within 300 seconds, it was pushed to the back into one of the enclosed arms and the transfer latency was recorded as 300 seconds. Later, the rat was allowed to freely explore the maze for 30 seconds to become familiar with the maze and return to its home cage. The retention trial followed 24 hours later in which time it took to move from the open arm and re-entered into either of the closed arms were recorded as RTL using a stopwatch. The spatial longterm memory" refers to the capability of a rat to remember where it has been in a maze.¹⁹

Rota-rod performance assessment for motor coordination

The Rota-rod (rotating rod) testing was commonly used to assess rodent motor function, including coordination and balance. The Rota rod apparatus consisted of a rotating rod 75 mm in diameter, which was divided in to 4 equal sections, so that 4 rats could be tested simultaneously. Rats were placed on the rod at a speed of 25 rpm and their ability to stay on the rats were subjected 3 times to training trials at 3 to 4 hours intervals on two different days for acclimatation purposes. During the test



session, the rat was placed on the rotarod and its performance time was recorded.²⁰

String test for grip strength

A rat was allowed to grasp a steel wire (2 mm diameter and 80 cm long) at a height of 50 cm above a cushion support. The length of time that the rat could hold the wire was measured. The Latency to the grip loss was used as an indirect measure of the grip strength cutoff time. It was taken as 90 seconds.²¹

Estimation of antioxidant enzyme levels in brain tissue

Preparation of tissue homogenate

The entire brain dissected out, stained dry and immediately weighed. The brain sections cerebral cortex (Ct), cerebellum (Cb), hippocampus (Hc) and striatum (St) were subsequently dissected from the intact brain carefully on ice plate ($4 \pm 2^\circ\text{C}$). A 10% brain homogenate was prepared with ice-cold phosphate buffered saline (0.1 M, pH 7.4) using Teflon-glass homogenizer. The homogenate was centrifuged at 10,000 rpm at -4°C for 15 min and the pellet discarded. The supernatant obtained was used for the quantification of antioxidant levels like GSH, CAT, LPO, SOD, total protein levels.²²

Lipid peroxidation (LPO)

Briefly, the reaction mixture contained 0.1 ml of brain regions homogenate (1 mg protein), 1.5 ml of 20% acetic acid (pH 3.5), 1.5 ml of 0.8% Thiobarbituric acid (0.8 % w/v) and 0.2 ml Sodium dodecyl sulphate. Following these additions, tubes were mixed and heated at 95°C for 1 hour on a water bath and cooled under tap water before mixing 1 ml of distilled water and 5 ml mixture of n-butanol and pyridine (15:1). The mixture was centrifuged at 2200 g for 10 minutes. The amount of malondialdehyde (MDA)/Thiobarbituric acid (TBA) pink chromogen Thiobarbituric acid reactive substances (TBARS) formed was measured by the absorbance of the organic layer above a wavelength of 532 nm. The results are expressed in nmol MDA/mg of protein. The absorbance of the clear pink color supernatant was measured at 532 nm against appropriate blank. The amount of lipid peroxidation was determined by using molar extinction coefficient $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ and the results were expressed as n moles MDA/g of protein.²³

Reduced Glutathione (GSH)

The assay was based on the principle of Ellman's reaction. The sulfhydryl group of glutathione reacts with DTNB (5, 5'- dithiobis-2-nitrobenzoic acid) and produces a yellow colored 5-thio- 2-nitrobenzoic acid (TNB). Measuring the absorbance of TNB at 412 nm provides an accurate

estimate of glutathione in a sample. In short, 0.5 ml of homogenate was mixed with 0.1 ml of 25% trichloroacetic acid (TCA) to precipitate proteins and centrifuged at 4000 rpm for 5 min. Then 0.3 ml of the supernatant was mixed with 0.5 ml of 0.1 M phosphate buffer (pH 7.4) and 0.2 ml of 10 mM DTNB. This mixture was incubated for 10 minutes, and the absorbance was measured at 412 nm against appropriate blanks. The glutathione content was calculated using the expansion coefficient $13.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$. Values are expressed in n moles/mg protein.²⁴

Superoxide dismutase (SOD)

Mixture of 0.1 ml of sample +1.2 ml de tampon pyrophosphate de sodium (pH 8,3, 0,052 M) + 0.2 ml of Nicotinamide Adenine Dinucleotide Hydrogen (NADH) (750 μm) + 0.3 ml of blue nitro tetrazolium (300 μm) + 0.1 ml of phenazine methosulphate (186 μm). By the addition of NADH the reaction was started. Incubated at 300°C for 90 sec, by the addition of 1 ml of glacial acetic acid the reaction was stopped. Permits to stand for 10 min. Chromogen color intensity was measured at 560 nm relative to blank and SOD concentration was expressed as units/min/mg protein.^{25, 26.}

Total protein (TP)

The total protein of brain tissue was determined by biuret method in Event-Related Brain Activation (brain function test method) (ERBA) diagnostic kit.

Total protein (g/dL) = Absorbance of test / Absorbance of standard \times concentration of standard (g/dL).^{27,28.}

Histopathological study of rat brain

A section of the brain was fixed with 10% formalin and embedded in paraffin wax and cut into sections 5 μm thick. Sections were stained with hematoxylin and eosin stain for histopathological observations. Depending on the model, cerebral cortex region was observed for morphological changes.²⁹

Statistical significance

The values observed and expressed as mean \pm SEM. Statistical difference in mean was analyzed by using one way ANOVA followed by Dunnet's multiple comparison tests. * $P < 0.5$, ** $P < 0.01$, *** $P < 0.001$ was considered statistically significant.

RESULTS AND DISCUSSION

Phytochemical Analysis

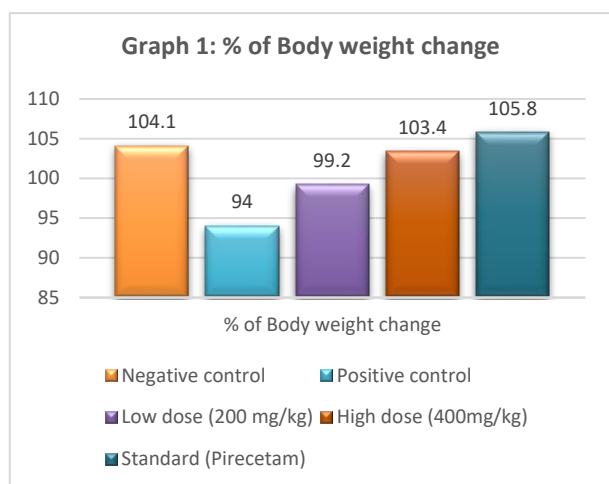
The ethanolic extract of *Galinsoga parviflora* plant subjected for phytochemical study showed the presence of Flavonoids, Alkaloids, proteins or amino acids, cardiac glycosides, phenols, Terpenoids, carbohydrates.



Table 2: % of body weight changes in AlCl₃ induced neurotoxicity in rats

Treatment	Negative Control	Positive control	Low dose (200 mg/kg)	High dose (400 mg/kg)	Standard (200 mg/kg)
Before	204.3±14.79	196.83±5.173	191.8±4.24	195±4.89	197.5±4.59
After	212.8±3.721	185.16±14.31	193.33±3.75	201.66±4.37	209±3.67
% Body weight change	104.1	94***	99.2##	103.4#	105.8

Values are expressed as mean ± SEM (n = 6) and analysed by one-way ANOVA followed by Dunnett's multiple comparison test. Level of significance *** (P < 0.001) when compared to negative control rats and ## (P < 0.01), # (P < 0.05) when compared to standard group of rats.



Administration of AlCl₃ (50 mg/kg p.o for 7 days) resulted in change in body weight when compared to normal rats. In case of AlCl₃ induced rats, the body weight was significant (P<0.001) decrease in initial body weight. Pretreatment with ethanolic extract of *Galinsoga parviflora* plant (200 and 400 mg/kg p.o) markedly prevented the AlCl₃ induced decrease in body weight. The effect of ethanolic extract of *Galinsoga parviflora* plant at 400 mg/kg was found to be much better (p<0.05) than its lower dose when compared to standard group of rats (Table no. 2 and Graph no. 1).

Table 3: Effect of ethanolic extract of *Galinsoga parviflora* plant on Behavioral characters in AlCl₃ induced neurotoxicity of rats.

Treatment	Neurological Score	Locomotor activity (counts/5min)	Plus maze (transfer latency time sec)		Rotarod performance (S)	Grip strength test (S)
			ITL	RTL		
Negative control	0.0	527.16±2.33	42.5±1.335	39.8±1.24	34.5±1.18	30.16±0.980
Positive control	3±0.365***	295.5±3.019**	78.66±1.40	81.33±1.40***	15±0.764***	15.5±0.764***
Low dose (200mg/kg)	2±0.258###	336.3±2.231	59.8±1.249	56.16±1.10##	22.67±0.66###	21.16±1.046##
High dose (400mg/kg)	1.5±0.422###	487.8±2.076#	39.1±2.02	36.9±1.83#	29.5±0.80##	29.5±0.764#
Standard (pirecetam)	1.33±0.333	500±3.45	38.3±1.78	36±1.732	30.33±0.760	29.33±0.760

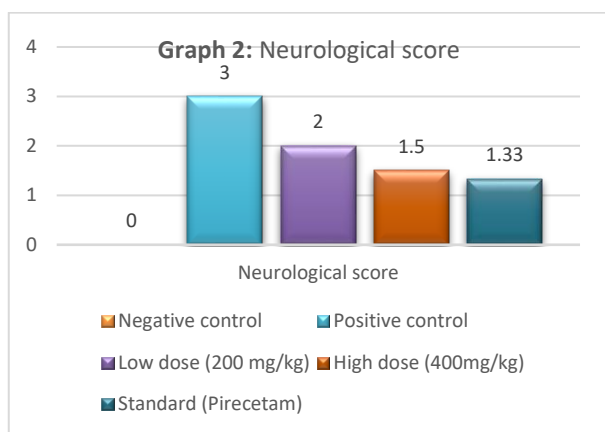
Values are expressed as mean ± SEM (n = 6) and analysed by one-way ANOVA followed by Dunnett's multiple comparison test. Level of significance *** (P < 0.001), ** (P < 0.01) when compared to negative control rats and ### (P < 0.001), ## (P < 0.01), # (P < 0.05) when compared to standard group of rats.

Effect of *Galinsoga parviflora* plant extract on Behavioral characters in AlCl₃ induced neurotoxicity in rats

Neurological scoring

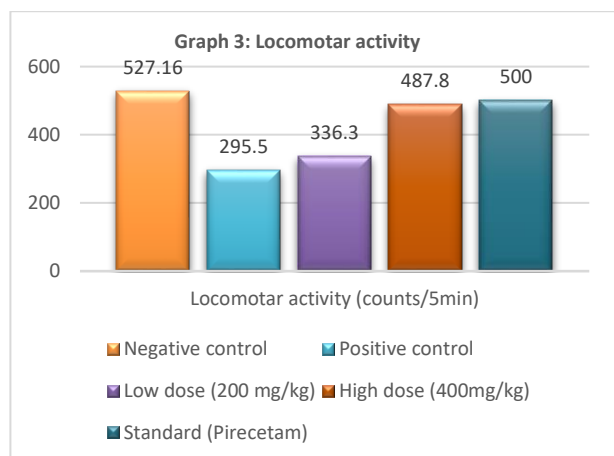
In AlCl₃ administrated group, resulted significant (P<0.001) motor abnormalities, out of six rats two rats showed in-coordination and hind limb paralysis, two rats showed hind limb and two rats showed marked gait

abnormalities. They showed an increase in neurological score compared to normal control rats. Pretreatment with *Galinsoga parviflora* extract (200 and 400 mg/kg) in AlCl₃ induced rats showed a significant (P<0.001 and P<0.01) improvement in behavioral changes when compared to standard group of rats (Table no. 3 and graph no. 2).



Locomotor activity

Administration of AlCl₃ from 1-7 days resulted in significant (P<0.01) decreased locomotor activity compared to normal control animals. Animals Pretreated with *Galinsoga parviflora* extract (200 and 400 mg/kg) in AlCl₃ induced rats prevented significantly (High dose P<0.05) decreased locomotor activity compared to standard group of rats (Table no. 3 and Graph no. 3).

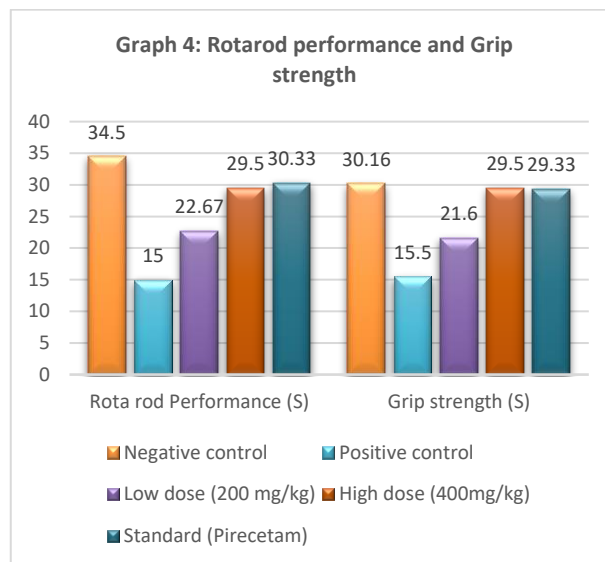


Hanging wire test (Grip strength test)

Animals treated with AlCl₃ from 1-7 days resulted in significantly (P<0.001) decrease in grip strength when compared to normal control animals. Pretreatment with *Galinsoga parviflora* plant extract (200 and 400 mg/kg) in AlCl₃ induced rats improved significantly (P<0.001 and P<0.05) the grip strength compared to standard group of rats (Table no. 3 and Graph no. 4).

Rota rod test

Administration of AlCl₃ from 1-7 days significantly (P<0.001) decreased motor coordination and body balance compared to normal control rats. Pretreatment with *Galinsoga parviflora* plant extract (200 and 400 mg/kg) in AlCl₃ induced rats significantly (P<0.001 and P<0.01) improved the motor coordination and body balance and showed increase in latency to balance on the beam (Table no. 3 and Graph no. 4).



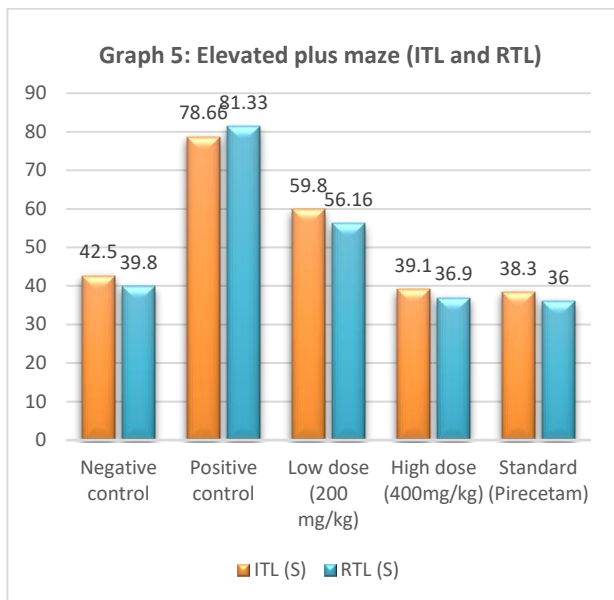
Elevated plus maze test

In the present experiment, mean initial transfer latency (ITL) on 7th day was relatively stable in all the animals within the group. AlCl₃ alone administered rats for 7 days showed significant (P<0.001) increased mean retention transfer latency (RTL) compared to normal control animals. Normal control animals entered rapidly with the arm closed and the average RTL was shorter than their own ITL (initial transfer latency). In contrast, AlCl₃ treated rats performed poorly and showed an increased mean RTL compared to its own ITL. This indicates there is cognitive dysfunction in AlCl₃ treatment. The pretreatment with *Galinsoga parviflora* Plant extract (200 and 400 mg/kg p.o.) in AlCl₃ treated rats showed significant (P<0.01 and P<0.05) improvement in memory performance when compared to standard group of rats (Table no. 3 and Graph no. 5).

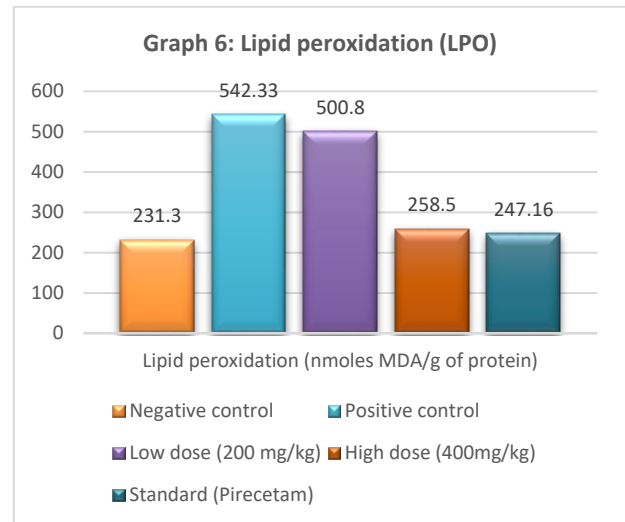
Table 4: Effect of *Galinsoga parviflora* extraction antioxidant levels in AlCl₃ induced neurotoxicity in rats

Treatment	Lipid peroxidation (n moles MDA/g of protein)	Reduced glutathione (nmoles/min/mg of protein)	Super oxide dismutase (units/min/mg of protein)	Protein estimation (g/dl of total protein)
Negative control	231.3±1.14	1.83 ±0.076	20.5±0.98	8.28±0.39
Positive control	542.33±1.745***	0.758±0.042***	10.15±0.79***	3.5±0.24***
Low dose (200mg/kg)	500.8±1.74###	1.142±0.027###	13.18±0.62##	6.84±0.13##
High dose (400mg/kg)	258.5±2.23##	1.31±0.031###	15.98±1.056	7.61 ±0.37#
Standard	247.16±2.75	1.52±0.023	17.85±1.08	7.78±0.19





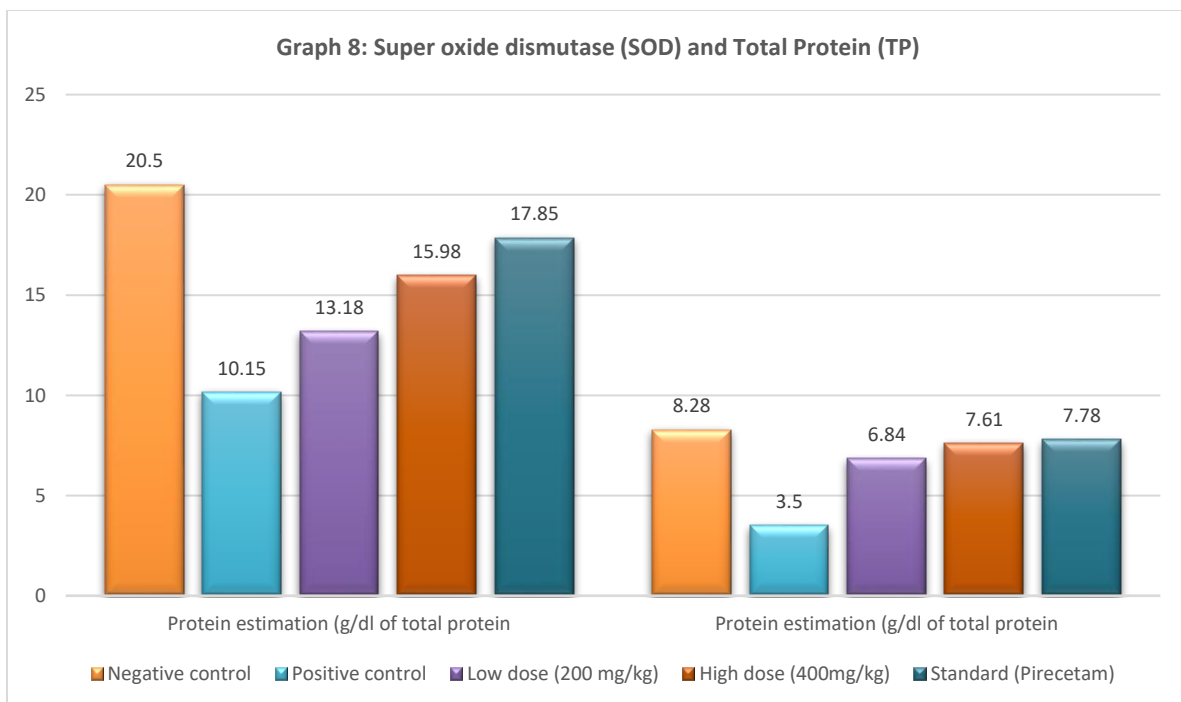
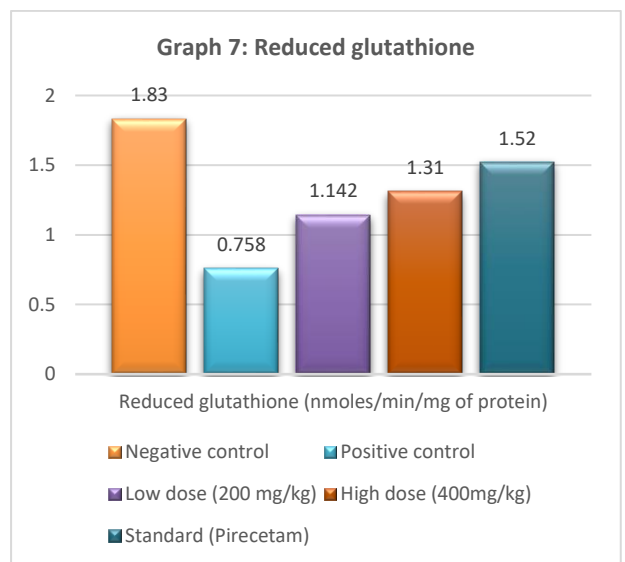
0.001), ## (P < 0.01), # (P < 0.05) when compared to standard group of rats.



Effect of ethanolic extract of *Galinsoga parviflora* plant on levels of LPO, GSH, SOD, and Total protein

The administration of AlCl₃ has significantly elevated lipid peroxide with decreased GSH, SOD and total protein as compared to negative control animals (Table no. 4 and Graph no. 6). However, co-administration of *Galinsoga parviflora* plant ethanolic extract (200 and 400 mg/kg) with AlCl₃ significantly reduced oxidative damage, indicated by reduction in lipid peroxidation, with elevated Total protein, SOD and GSH levels (Table no. 4, Graph no. 7 and 8). High dose of ethanolic extract of *Galinsoga parviflora* plant (400 mg/kg) has shown maximum protection.

Values are expressed as mean ± SEM (n = 6) and analysed by one-way ANOVA followed by Dunnett’s multiple comparison test. Level of significance *** (P < 0.001) when compared to negative control rats and ### (P <



HISTOPATHOLOGY**The effect of *Galinsoga parviflora* extract on $AlCl_3$ induce neurotoxicity in rats brain**

The brain section was stained by Hematoxylin-Eosin and observed (Magnification 400x) histological structure of cerebral cortex region.

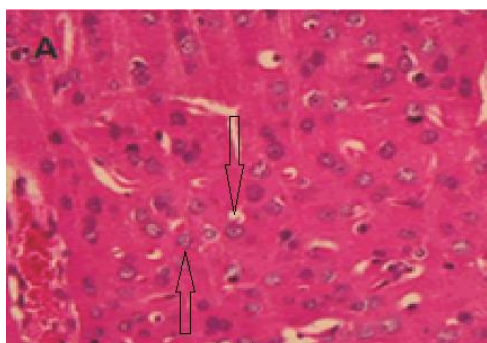


Figure A [Negative control (normal)]

In negative control group, normal histological features with well-formed neurons mature cells in cerebral cortex region of the brain.

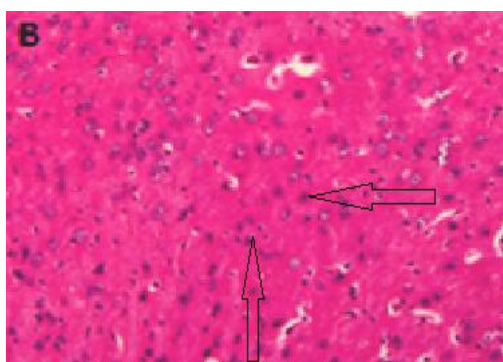


Figure B [Positive control ($AlCl_3$ 50mg/kg induced group)] In Positive control group of rat's brain, abnormal cellular morphology accompanied by cellular infiltration, recruitment of macrophages, with damaged cerebral cortex regions with increased number of glial cells was observed in $AlCl_3$ -induced neurotoxicity.

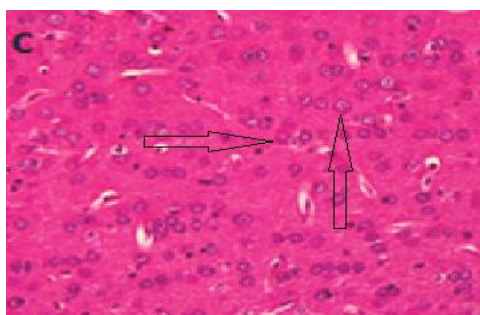


Figure C [Low dose of ethanolic extract of *Galinsoga parviflora* plant 200 mg/kg + $AlCl_3$ 50mg/kg]

Galinsoga parviflora plant extract Low dose (200mg/kg) shows normal morphology of brain regions with increased number of mature cells and a few glial cells like in control group.

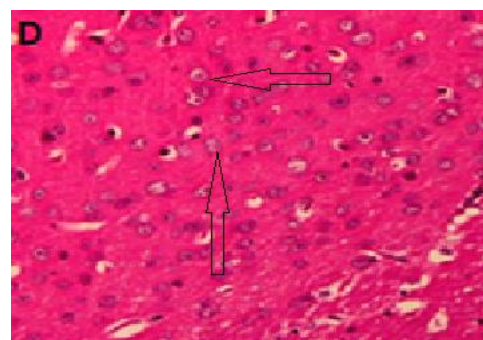


Figure D [High dose [High dose of ethanolic extract of *Galinsoga parviflora* plant 400 mg/kg + $AlCl_3$ 50mg/kg]] *Galinsoga parviflora* plant extract high dose (400mg/kg) shows protected cellular morphology and also exhibited significantly reduced morphologic abnormalities in brain with increased number of mature cells.

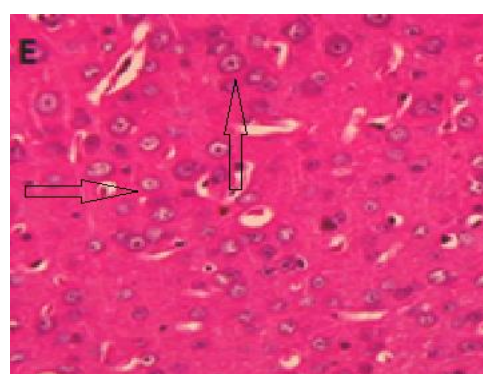


Figure E [Standard (Piracetam 200mg/kg + $AlCl_3$ 50mg/kg)]

Piracetam 200 mg/kg shows significantly reduced morphologic abnormalities in regions of brain with recovery in tissues and well-formed mature cells without irregular features.

DISCUSSION

Aluminium interacts with the cholinergic system, acting as a cholinotoxin (neurotoxin) that provokes functional alterations in the cholinergic, dopaminergic and noradrenergic neurotransmission; consequently, it has the affinity to cause impaired cholinergic transmission by influencing the synthesis and release of neurotransmitters.³⁰ Literature reports confirmed that its persistent use of aluminium leads to oxidative worsening of cellular lipids, proteins and DNA. Lipid peroxidation is one, it can damage tissues under chronic use. Therefore, aluminium can be considered as a contributing factor in neurodegenerative diseases. Later $AlCl_3$ administration, it accumulates in all brain regions with more quantity in cortex and damaged it. These regions play an important role in learning and memory.³¹

The role of free radical in the neurodegenerations and cognitive decline has been studied previously and findings emphasize the ROS role in the brain and found to improve the neuronal function. From the other studies, it is apparent that the hypothesis of memory deficits occurred after aluminum chloride administration was due to the

mitochondrial dysfunction which is appealed to be the key factor for ROS production and finally inducing oxidative injury to the neurons. Hence, oxidative stress was considered as one of the main causes for cognitive impairment. Apart from chronic stress, Additionally, it promotes oxidative stress and disturbs antioxidant defense mechanism of brain.³²

In the current research investigation, the administration of AlCl₃ has induced oxidative stress and damages as indicated in increased lipid peroxidation (MDA) and reduction of antioxidant enzymes such as SOD and GSH and thus confirms the oxidative theory of cognitive deficits and its implications.

The present study was investigated the neuroprotective potential of ethanol extract of *Galinsoga parviflora* plant in AlCl₃ induced neurotoxicity. AlCl₃ (50 mg/kg/day) administered orally for 7 days possesses significant neurotoxicity as assessed by behavioral deficits. Additionally, Motor incoordination and memory deficits, which were also supported biochemically as there was marked increase in lipid peroxidation with reduced GSH, SOD and total protein levels. Histopathological studies of cerebral cortex area endorsed marked deterioration visualized as increased glial cells. The ethanolic extract of *Galinsoga parviflora* plant treatment significantly prevented the altered behavioural, biochemical and histopathological outcomes associated with AlCl₃ induced neurotoxicity.

CONCLUSION

The detailed result of the study with justification was strong antioxidant potential of ethanol extract of *Galinsoga parviflora* plant was responsible for improvement in behavioral, biochemical and histopathological results in AlCl₃ induced neurotoxicity. Another significant finding of the study was after administration of ethanolic extract of *Galinsoga parviflora* plant was decreased the abnormal cerebral cortex area of rat's brain and another possible neuroprotective effect of *Galinsoga parviflora* plant extract may be decreased the AlCl₃ transport into the brain across Blood brain barrier (BBB). Overall result was concluded that the ethanolic extract of *Galinsoga parviflora* plant and its strong antioxidant potential due to various bioactive chemical constituents might have neuroprotective potential in AlCl₃ induced neurotoxicity. High dose of *Galinsoga parviflora* plant ethanol extract (400 mg/kg) has shown maximum neuroprotection.

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