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Neuroprotective Activity of *Galinsoga parviflora* Plant Ethanolic Extract against 3-Nitropropionic Acid (3-NP) Induced Neurotoxicity in Rats



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**Madhu S U^{1*}, Harshitha S², Hariprasad M G³,
Honnesh⁴**

1* M Pharmacy, Dept of Pharmacology, Mallige College of Pharmacy, Bangalore-560090, India. 2 B Pharmacy, Mallige College of Pharmacy, Bangalore-560090, India. 3 Professor and HOD, Dept of Pharmacology, KLE College of Pharmacy, Bangalore-560010, India. 4 B Pharmacy, RR College of Pharmacy, Bangalore-560090, India.

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ABSTRACT

This research investigated whether ethanolic whole plant extracts of *Galinsoga parviflora* could protect against neurodegeneration in 3-Nitropropionic acid (3-NP) induced neurotoxicity rats. The ethanol extract of *Galinsoga parviflora* (G p) was administered orally at two different doses (200 and 400 mg/kg) to neurotoxic rats. The time gap between 3-NP and *Galinsoga parviflora* extract administration was 2 hours. Rats were observed for behavior changes for 50 minutes every day during the treatment period. On the 14th day after 4 hours of 3-NP 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 the 15th day, rats' brain was isolated for estimation of Reduced Glutathione (GSH), Lipid peroxidation (LPO), Superoxide dismutase (SOD), Total protein, and histopathological study. 3-NP treatment showed a significant decrease in brain antioxidant enzymes such as SOD, GSH, and Total protein and increased LPO. It was clear from the results that 3-NP administration caused mitochondrial dysfunction, which led to ROS production and eventually neuronal damage. *Galinsoga parviflora* plant extract significantly prevented this raise in levels of LPO and significantly increased GSH, SOD, and Total protein in extract-treated groups. *Galinsoga parviflora* plant extract at a high dose (400 mg/kg) protects cells from damage and helps recover damaged cells. These results suggested that ethanolic extract of *Galinsoga parviflora* may have potential therapeutic value in the treatment of some neurological disorders, probably by its anti-inflammatory and antioxidant properties.



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1. INTRODUCTION:

Neurodegenerative diseases include Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis (ALS), and Huntington's disease (HD) which are characterized by different etiologies with distinctive morphological and pathophysiologic features.¹ There is a large amount of evidence suggesting that these diseases arise from multiple causes including abnormal protein folding with defective protein aggregation, oxidative stress and free radical production, impaired energy metabolism and mitochondrial dysfunction, exposure to toxic metals and pesticides and environmental factors associated with aging have also been implicated in causing neurodegeneration.² 3-Nitropropionic acid (3-NP) is a neurotoxin that is produced by various fungal species and is naturally present in leguminous plants which are commonly used to feed animals and can poison grazing livestock. 3-NP causes biochemical and morphological alterations in human and animal brains.³ 3-NP is an irreversible inhibitor of succinate dehydrogenase and interferes with mitochondrial adenosine triphosphate (ATP) synthesis.⁴ 3-NP can cause excitotoxic compound (cell death by excessive stimulation of glutamate receptors) and it can be characterized as an impairment of mitochondrial energy components which produced progressive degeneration of neurons in the lateral striatum, hippocampus (CA1 and CA3) lesions in the brain of rat characterized by cell body shrinkage and Deoxyribonucleic acid (DNA) fragmentation. Adding glutamate antagonists during 3-NP exposure did not prevent neuronal death.⁵ The 3-NP can permeable through the blood-brain barrier, it shows its toxic effect by irreversible inhibition of mitochondrial complex II enzyme, succinate dehydrogenase (SDH) which leads to interruption of oxidative phosphorylation (Mitochondrial cellular respiration) and ultimately inhibit the production of ATP in the brain cells.^{6,7} Administration of 3-NP induces selective striatal neuronal loss combined with behavioral deficits and involved in inhibition of the transfer of electrons and generates reactive oxygen species.^{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 powerful neuroprotectants against various brain pathologies.¹⁰ because of all the points, *Galinsoga parviflora* has been selected to investigate the neuroprotective activity based on its antioxidant activity and use in traditional systems of medicine which contain all the potent constituents as Alkaloids, Flavonoids, Phenols, Terpenoids, Carbohydrates, Proteins, Tannins, etc. which may have the capability to treat the common neurological disorders.^{11,12.}

2. MATERIALS AND METHODS:

2.1 Chemicals used during experiments:

Acetate solution, Acetic anhydride (Merk), 3-Nitropropionic acid (3-NP) (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), *Galinsoga parviflora* plant Extract (Test drug), Glacial acetic acid, Mayer's reagent (Merk), Molish reagent, Ninhydrin reagent, Normal saline (Vehicle for the control group), Piracetam (Standard drug), Wagner's reagent, all other chemicals were of the highest purity commercially available.

2.2 Apparatus/Equipment/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 (SHIMADZU, UV-16100), variable micropipette.

2.3 Collection and authentication of plant material:

For the study, The *Galinsoga parviflora* 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.

2.4 Whole plant ethanolic extraction of *Galinsoga parviflora*:

The plant of *Galinsoga parviflora* plant will be freshly collected, washed, and dried at room temperature after which they (whole dried plant except root) are made into a 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, the filtrate was kept for evaporation at room temperature.¹³

2.5 Phytochemical studies in various fractions of *Galinsoga parviflora* extract:

Preliminary phytochemical screening of the extract 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.

2.6 Experimental animals:

Adult Healthy male albino rats weighing between 180-220g were 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 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.

2.7 Acute oral toxicity studies:

Acute toxicity was carried out to determine the lethal dose (LD₅₀) according to Organization of Economic Co-Operation and Development (OECD) no 425 guidelines. The oral administration of extracts of *Galinsoga parviflora* plants up to a dose of 3200 mg/kg neither showed any mortality nor any adverse signs about 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*.³⁸ Based on the results obtained from this study, the dose of further pharmacological studies was fixed to be 200 mg/kg for low doses and 400 mg/kg for high doses.

2.8 Experimental Design: 3-Nitropropionic acid (3-NP) induced neurotoxicity:

3-NP was diluted with saline (adjust pH 7.4) and administered *intraperitoneal (i.p)* to rats for 7 days to induce toxicity. A total of 30 rats were randomly divided into five groups of 6 rats each and treated as follows: ¹⁴

Negative control group (vehicle + Normal saline): Control group vehicle alone for 7 days (oral) and from 8th-day onward vehicle followed by normal Saline *per oral (p.o)* after 2 hours daily for 7 days.

Positive control [Normal saline + 3-NP 10 mg/kg (i.p)]: Normal saline alone for 7 days (oral) and from 8th day onward saline followed by 3-NP 10 mg/kg (*i.p*) after 2 hours daily for 7 days.

***Galinsoga parviflora* extract low dose 200 mg/kg (p.o) + 3-NP 10 mg/kg (i.p):** Pretreatment for 7 days with *Galinsoga parviflora* extract low dose (oral) and from 8th day onward *Galinsoga parviflora* extract followed by 3-NP 10 mg/kg (*i.p*) after 2 hours daily for 7 days in the test group.

***Galinsoga parviflora* extract high dose 400 mg/kg (p.o) (p.o) + 3-NP 10 mg/kg (i.p):** Pretreatment for 7 days with *Galinsoga parviflora* extract high dose (oral) and from 8th day onward *Galinsoga parviflora* extract followed by 3-NP 10 mg/kg (*i.p*) after 2 hours daily for 7 days in the test group.

3-NP 10mg/kg (i.p)+ Piracetam 200mg/kg (p.o): Pretreatment for 7 days with Piracetam 200mg/kg (*p.o*) and from 8th day on ward Piracetam followed by 3-NP 10 mg/kg (*i.p*) after 2 hours daily for 7 day in standard group.

The gap between 3-NP and *Galinsoga parviflora* extract administration is 2 h. During the drug, treatment rats were observed for behavioral changes for 50 min daily. On the 14th day after 4 hours of 3-NP 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 the 15th day, rats were sacrificed humanly under mild anesthesia, and brains were isolated for estimation of Reduced Glutathione (GSH), Lipid peroxidation (LPO), Superoxide dismutase (SOD), Total protein, and histopathological study.¹⁵

2.9 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 that 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 the 14th day of 3-NP after 4 h of the last dose in comparison to control animals. (Score = 0, normal behavior; score = 1, general slowness of displacement resulting from mild hind limb impairment; 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: The spontaneous locomotor activity was monitored using a photo actometer equipped with infrared-sensitive photocells, and the apparatus was placed in darkened, light and sound attenuated, and 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 5minute test session. The locomotor activity was expressed in terms of total photo beam counts/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 a 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 at 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 the closed arms was recorded as ITL using a stopwatch. If the rat did not enter 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 which time it took to move from the open arm and re-entered into either of the closed arms was recorded as RTL using a stopwatch. The spatial longterm memory” refers to the ability 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 into 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 was subjected 3 times to training trials at 3 to 4 hours intervals on two different days for acclimatization purposes. During the test session, the rat was placed on the rota-rod 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 50cm above 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

2.10 Estimation of antioxidant enzyme levels in brain tissue:

Preparation of tissue homogenate:

The whole brain was dissected out, blotted dry, and immediately weighed. The brain regions cerebral cortex (Ct), cerebellum (Cb), hippocampus (Hc), and striatum (St) were subsequently dissected from the intact brain carefully on an 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 a Teflon-glass homogenizer. The homogenate was centrifuged at 10,000 rpm at -4°C for 15 min and the pellet was discarded. The supernatant obtained was used for the quantification of antioxidant levels like GSH, CAT, LPO, SOD, and 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 min. The amount of malondialdehyde (MDA)/Thiobarbituric acid (TBA) pink chromogen, Thiobarbituric acid reactive substances (TBARS) formed was measured by the absorbance of the upper organic layer at a wavelength of 532 nm. The results are expressed as nmol MDA/mg protein. The absorbance of the clear pink color supernatant was measured at 532 nm against an appropriate blank. The amount of lipid peroxidation was determined by using the molar extinction coefficient $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$ and the results were expressed as 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). Measurement of the absorbance of TNB at 412 nm provides an accurate estimation of glutathione in a sample. Briefly, 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 0.1M phosphate buffer (pH 7.4) and 0.2 ml of 10 mM DTNB. This mixture was incubated for 10 min and the absorbance was measured at 412 nm against appropriate blanks. The glutathione content was calculated by using an extension coefficient of $13.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$. The values are expressed as nmol/mg protein.²⁴

Superoxide dismutase (SOD):

Mixture of 0.1 ml of sample +1.2 ml of sodium pyrophosphate buffer (pH 8.3, 0.052M)+ 0.2 ml of Nicotinamide Adenine Dinucleotide Hydrogen(NADH) (750 μm)+ 0.3 ml of nitro blue tetrazolium (300 μm) + 0.1 ml of phenazine methosulphate (186 μm). With 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. The color intensity of the chromogen was measured at 560 nm against blank and the concentration of SOD was expressed as units/min/mg of protein.^{25, 26}

Total protein (TP):

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

Total protein (g/dl) = Absorbance of test/Absorbance of standard × concentration of standard (g/dl).^{27,28}

2.11 Histopathological study of rat brain:

A section of the brain was fixed with 10% formalin and embedded in paraffin wax and cut into sections of 5µm thickness. The sections were stained with hematoxylin and eosin dye for histopathological observations. Depending on the model, either hippocampal (CA1 and CA3 region) or striatal neurons region were observed for morphological changes.²⁹

2.12 Statistical significance:

The values observed will be expressed as mean ± SEM. Statistical differences in mean will be analyzed using one-way ANOVA followed by Dunnett's multiple comparison tests. **P*<0.5, ***P*<0.01, ****P*<0.001 will be considered statistically significant.

3. RESULT:

3.1 Phytochemical Analysis:

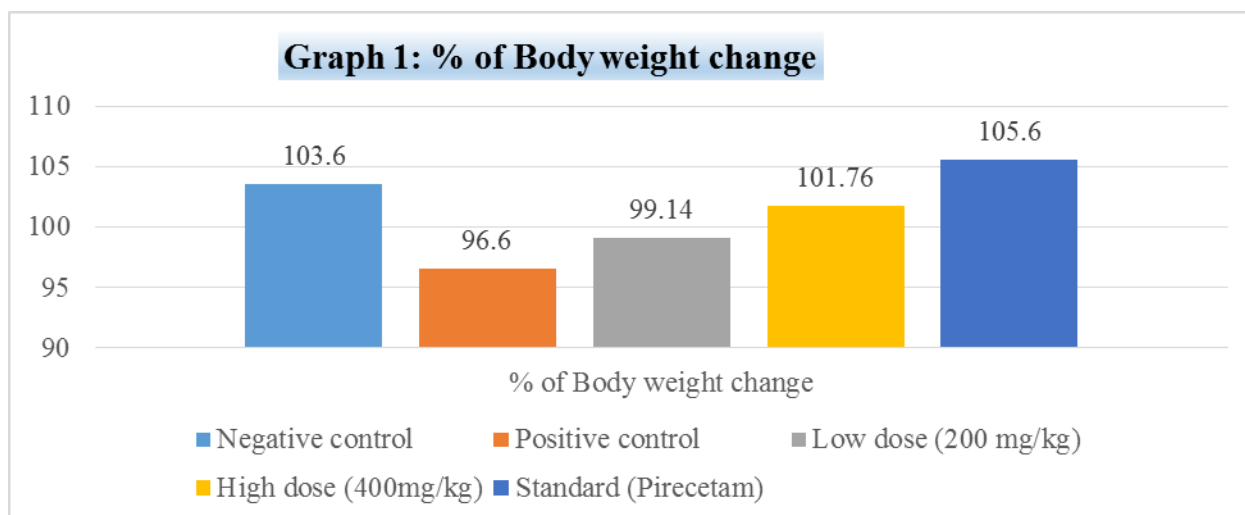
The plant extracts subjected to the phytochemical study showed the presence of Flavonoids, Alkaloids, proteins or amino acids, cardiac glycosides, phenols, Terpenoids, and carbohydrates.

3.2 Body weight changes in 3NP-induced neurotoxicity in rats :

Table No. 1: % of body weight changes in 3NP induced neurotoxicity in rats

Treatment	Negative Control	Positive control	Low dose (200 mg/kg)	High dose (400 mg/kg)	Standard (200 mg/kg)
Before	195.6±4.26	199.16±4.59	195.16±4.60	198±4.23	200±4.12
After	203.16±4.2	192.16±3.58	193.5±4.59	201.5±4.07	211.2±4.27
%Body weight change	103.6	96.6***	99.14##	101.76#	105.6

Values are expressed as mean ± SEM (n = 6) and analyzed 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.



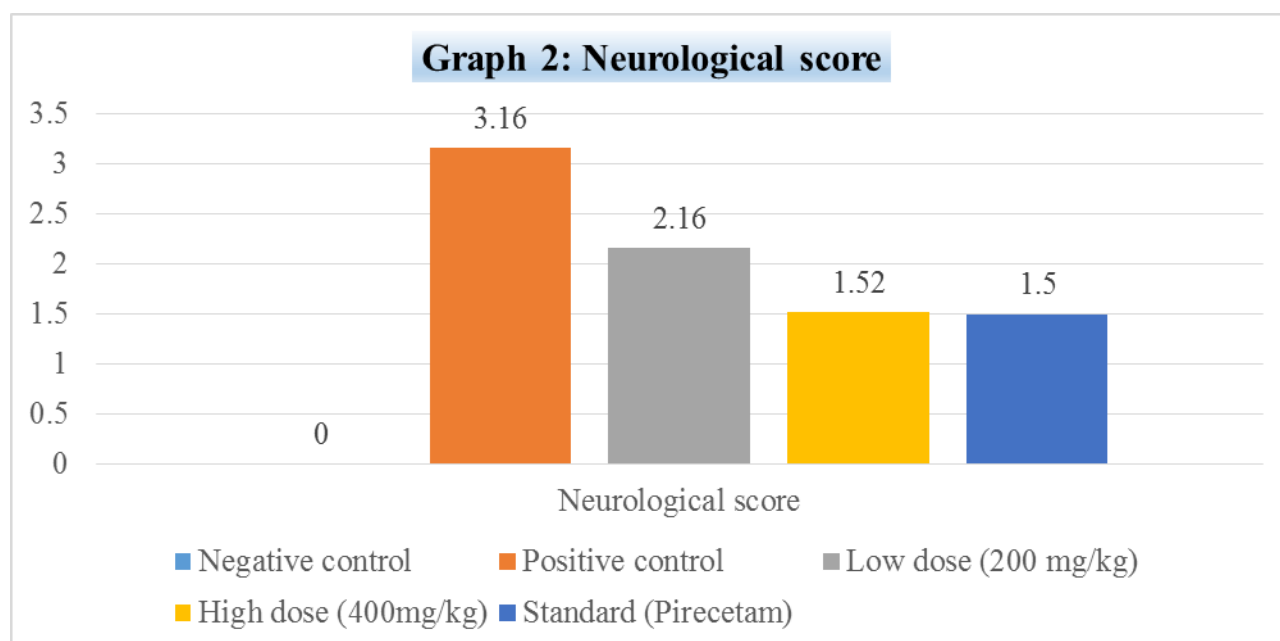
Effect of *Galinsoga parviflora* extracts on body weight change in 3NP induced neurotoxicity Administration of 3-NP (10 mg/kg *i.p* for 7 days) resulted in a change in body weight when compared to normal rats. The body weight of 3-NP-induced rats was significantly ($P<0.001$) reduced compared to the control group. Treatment with *Galinsoga parviflora* Extract (200 and 400 mg/kg *p.o*) markedly prevented the 3-NP induced decrease in body weight. The effect of *Galinsoga parviflora* extract at 400 mg/kg was found to be much better ($p<0.05$) than its lower dose when compared to 3-NP (Table 1 and Graph 1).

Table No. 2: Effect of *Galinsoga parviflora* on Behavioral characters in 3NP induced neurotoxicity in rats

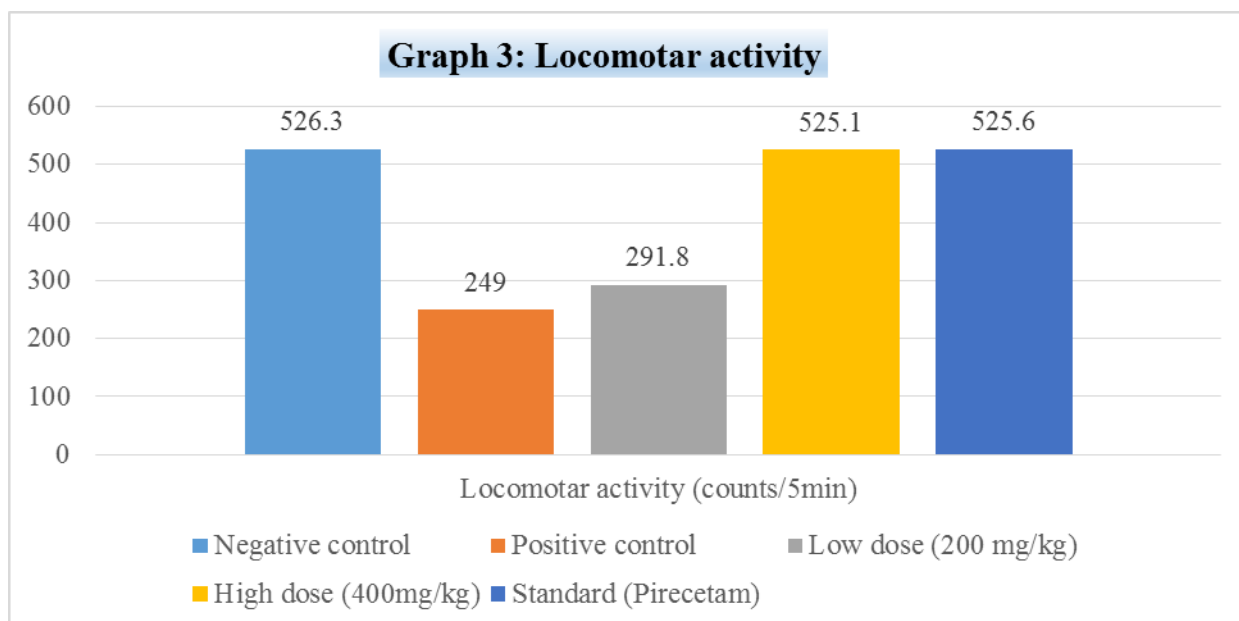
Treatment	Neurological Score	Locomotor activity (counts/ 5 min)	Plus maze (transfer latency time sec)		Rotarod performance (S)	Grip strength test (S)
			ITL	RTL		
Negative control	0.0	526.3±1.90	44.33±1.35	42±1.39	32.16±1.64*	32.33±1.72
Positive control	3.16±0.307***	249±3.0***	80.33±1.38	82.16±1.27***	17.6±1.47***	17.16±0.60***
Low dose (200mg/kg)	2.16±0.308###	291.8±2.54###	61.35±1.13	63.66±0.88###	24.33±0.89##	22±1.065###
High dose (400mg/kg)	1.52±0.304#	525.1±2.24#	43±2.06	45±1.983##	28.83±0.80#	30.33±1.17#
Standard (Piracetam)	1.50±0.224	525.6±3.67	40±1.65	42.16±1.74	29.33±0.91	31.50±1.25

Values are expressed as mean \pm SEM (n = 6) and analyzed 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 < 0.001), ## (P < 0.01), # (P < 0.05) when compared to standard group of rats.

Neurological scoring: Intra-peritoneal administration of 3-NP resulted in 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 increased neurological scores when compared to normal control rats. Pretreatment with *Galinsoga parviflora* extract (200 and 400 mg/kg) in 3-NP induced rats showed a significant (P<0.001 and P<0.05) improvement in behavioral changes when compared to the standard group of rats (Table 2 and Graph 2).

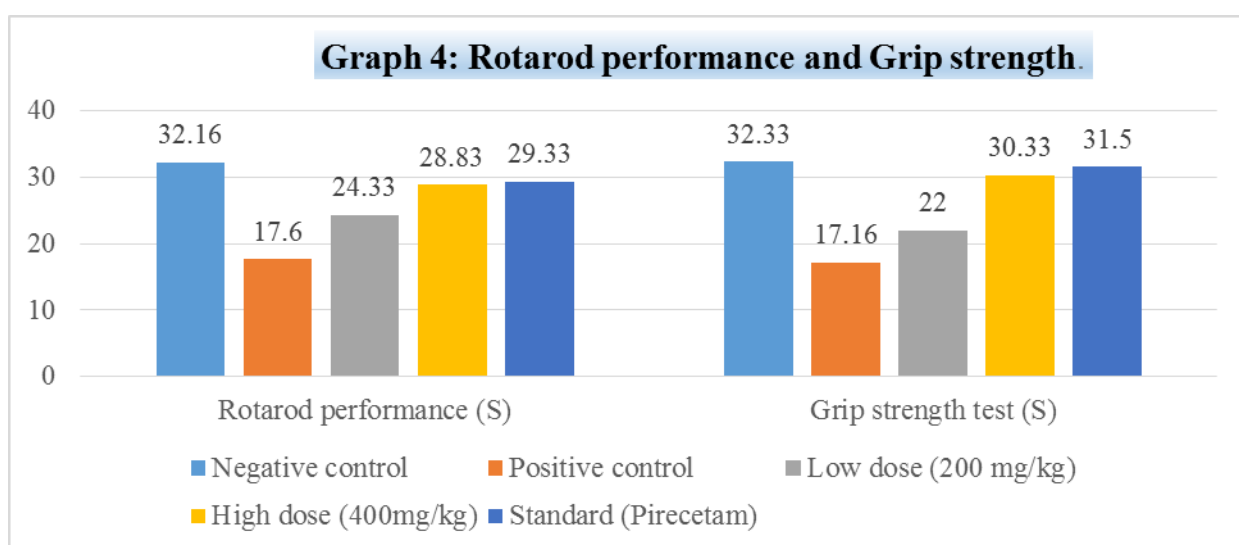


Locomotor activity: Administration of 3-NP from 8-14 days resulted in a significant (P<0.001) decrease in locomotor activity when compared to normal control animals. Animals pretreated with *Galinsoga parviflora* extract (200 and 400 mg/kg) in 3-NP induced rats prevented significantly (P<0.001 and P<0.05) increases locomotor activity compared to the standard group of rats (Table 2 and Graph 3).



Rota rod test: Administration of 3-NP from 8-14 days significantly ($P<0.001$) decreased motor coordination and body balance when compared to normal control rats. Pretreatment with *Galinsoga parviflora* extract (200 and 400 mg/kg) in 3-NP induced rats significantly ($P<0.01$ and $P<0.05$) improved the motor coordination and body balance and showed an increase in latency to balance on the beam (Table 2 and Graph 4).

Hanging wire test (Grip strength): Animals treated with 3-NP from 8-14 days resulted in a significant ($P<0.001$) decrease in grip strength when compared to normal control animals. Pretreatment with *Galinsoga parviflora* extracts (200 and 400 mg/kg) in 3-NP induced rats improved significantly ($P<0.001$ and $P<0.05$) the grip strength compared to the standard group of rats (Table 2 and Graph 4).



Elevated plus maze test: In the present experiment, mean initial transfer latency (ITL) on day 14th day was relatively stable in all the animals within the group. 3-NP alone administered to rats for 7 days showed a significant ($P<0.001$) increase in mean retention transfer latency (RTL) compared to normal control animals. Normal control animals entered closed arms quickly and mean RTL was shorter when compared to its ITL (initial transfer latency). In contrast, 3-NP treated rats performed poorly and showed an increased mean RTL compared to their ITL. This indicates there was cognitive dysfunction in 3- NP treatment. Chronic pretreatment with *Galinsoga parviflora* extract (200 and 400 mg/kg *p.o.*) to 3-NP treated rats showed significant ($P<0.001$ and $P<0.01$) improvement in memory performance when compared to the standard group of rats (Table 2 and Graph 5).

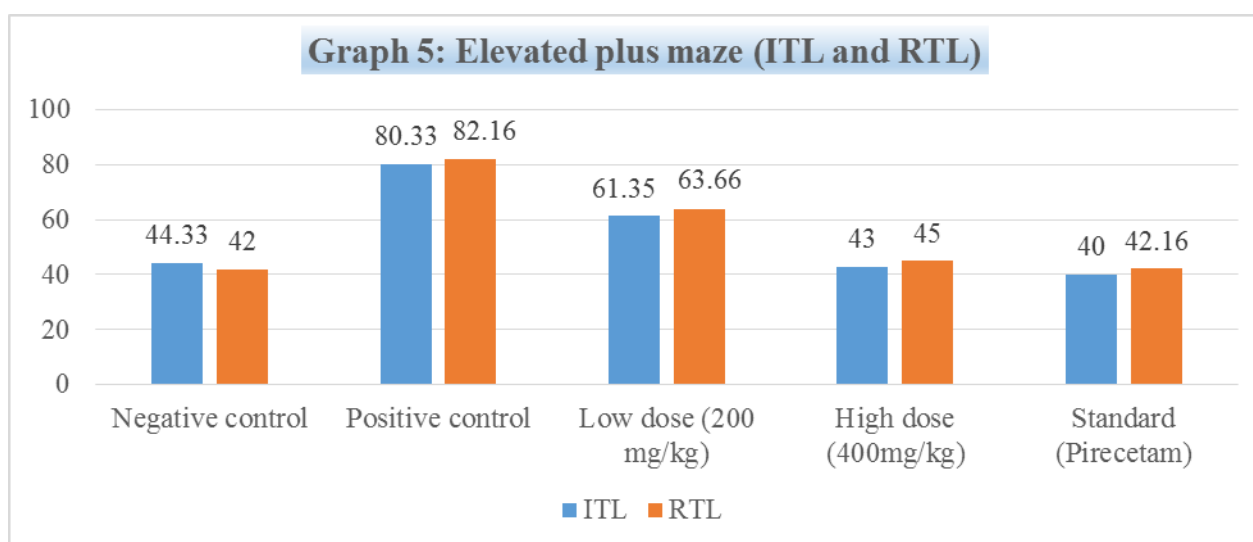


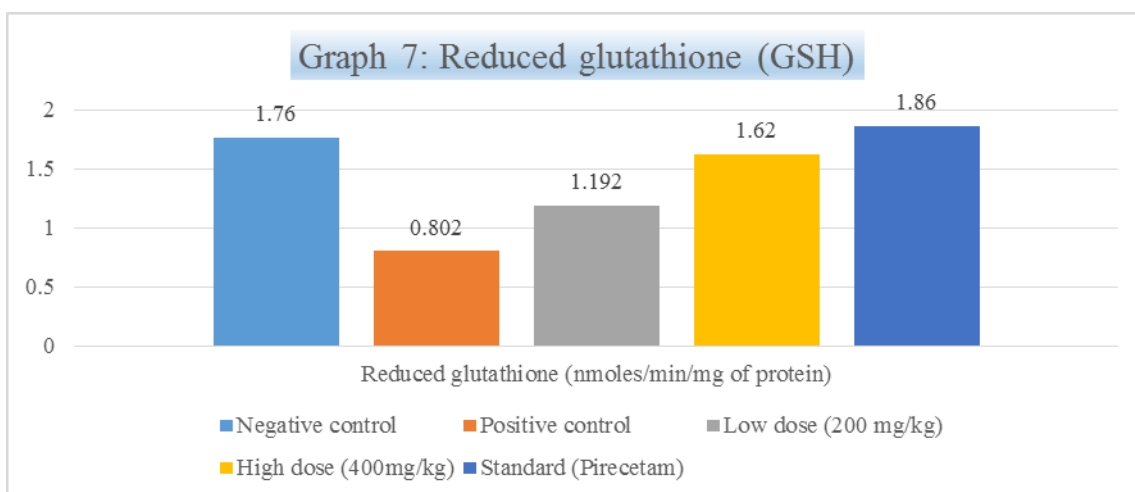
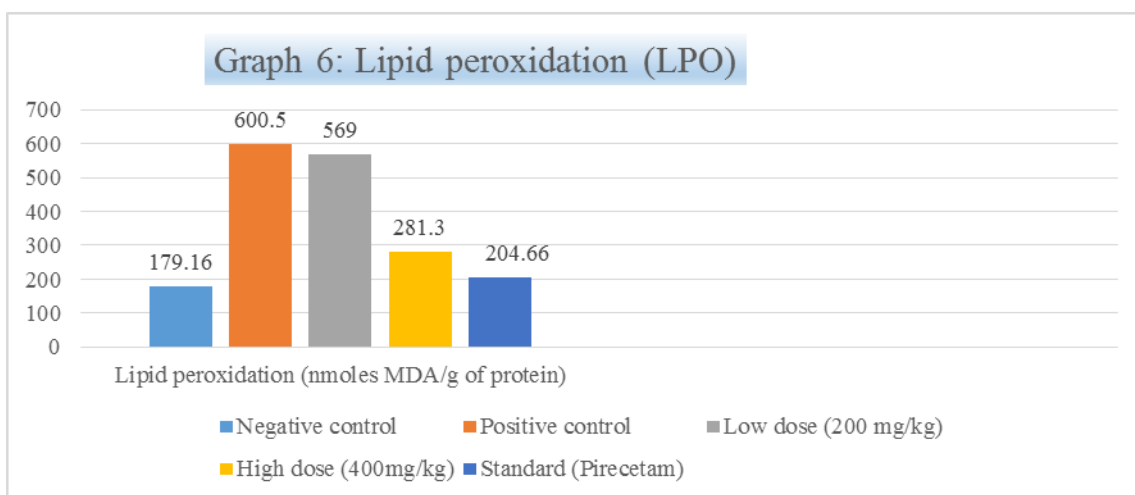
Table No. 3: Effect of *Galinsoga parviflora* extraction antioxidant levels in 3-NP induced neurotoxicities in rats

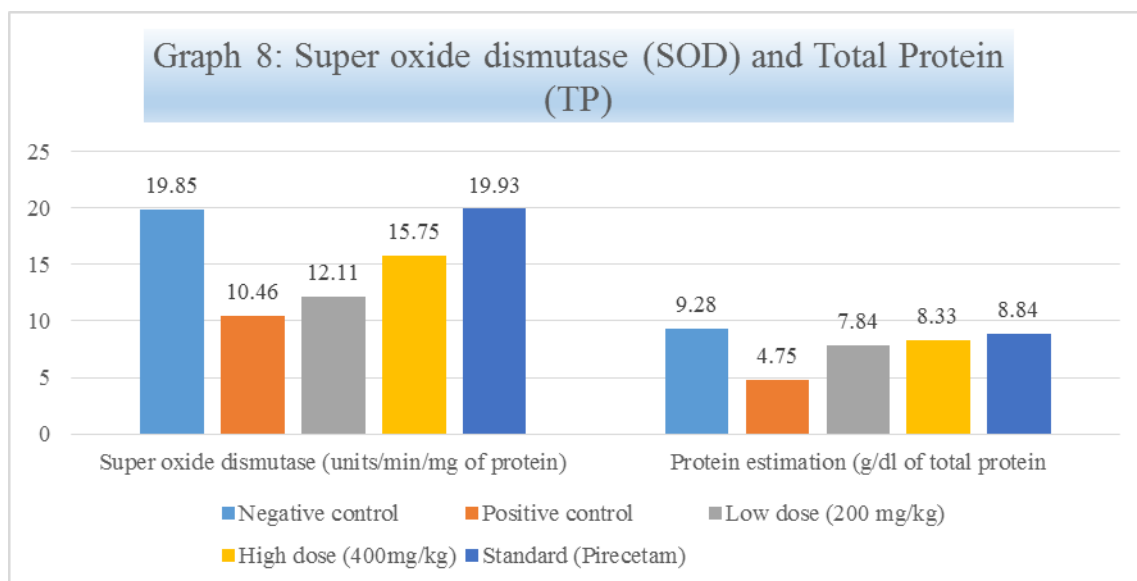
Treatment	Lipid peroxidation (nmol MDA/g of protein)	Reduced glutathione (nmoles/min/mg of protein)	Superoxide dismutase (units/min/mg of protein)	Protein estimation (g/dl of total protein)
Negative control	179.16±5.26	1.76 ±0.075	19.85±0.65	9.28±0.38
Positive control	600.5±6.75***	0.802±0.040***	10.46±0.51***	4.75±0.26***
Low dose (200mg/kg)	569±6.08###	1.192±0.041###	12.11±0.86###	7.84±0.23
High dose (400mg/kg)	281.3±2.23###	1.62±0.057##	15.75±0.69##	8.33 ±0.31#
Standard	204.66±2.72	1.86±0.052	19.93±0.77	8.84±0.32

Values are expressed as mean \pm SEM (n = 6) and analyzed 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 < 0.001), ## (P < 0.01), # (P < 0.05) when compared to standard group of rats.

3.3 Effect of *Galinsoga parviflora* Extract on levels of LPO, GSH, SOD, and TP:

The results revealed that LPO increase with the levels of Malondialdehyde (MDA) in the 3-NP induced toxic group compared to the control group (Table 3 and Graph 6). Treatment with *Galinsoga parviflora* extract (200mg and 400mg) significantly prevented this raise in levels. GSH, SOD, and TP content have significantly increased in extract-treated groups whereas the toxic group has shown a significant decrease in levels (Table 3, Graph 7, and Graph 8).





3.4 Histopathology:

The brain section was stained with Hematoxylin-Eosin and observed (Magnification 400x) histological structure of the Hippocampus region. **1A & 1B:** Negative control (normal control), **2A & 2B:** Positive control (3-NP 10mg/kg *i.p*), **3A & 3B:** Low dose (200mg/kg + 3-NP 10mg/kg *i.p*), **4A & 4B:** High dose (400mg/kg + 3-NP 10mg/kg), **5A & 5B:** Standard (Piracetam 200mg/kg + 3-NP 10mg/kg).

Figure 1A & Figure 1B [Control group]: The CA3 region shows Normal pyramidal cells in tight clusters [Figure 1A]. The interconnected neuropil fibers in the CA3 region appear normal or not damaged. The CA1 region shows Normal pyramidal cells along with normal neuropil fibers [Figure 1B].

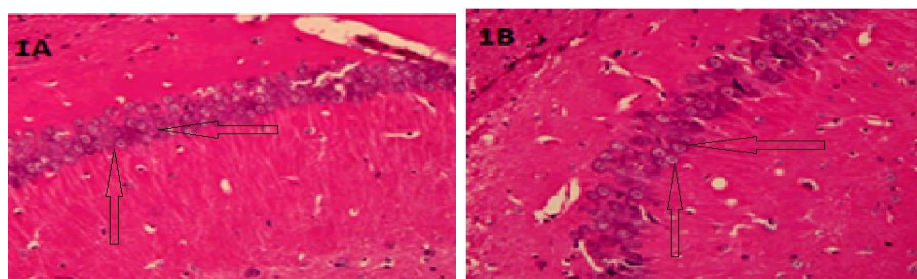


Figure 2A & Figure 2B [3-NP induced group]: The CA3 region shows normal pyramidal cells in clusters [Figure 2A]. The interconnected neuropil fibers in the CA3 region appear normal or not damaged and damaged pyramidal cells. The CA1 region shows a decreased number of normal pyramidal cells and most of the pyramidal cells show degenerative changes along with a decrease in neuropil fibers [Figure 2B].

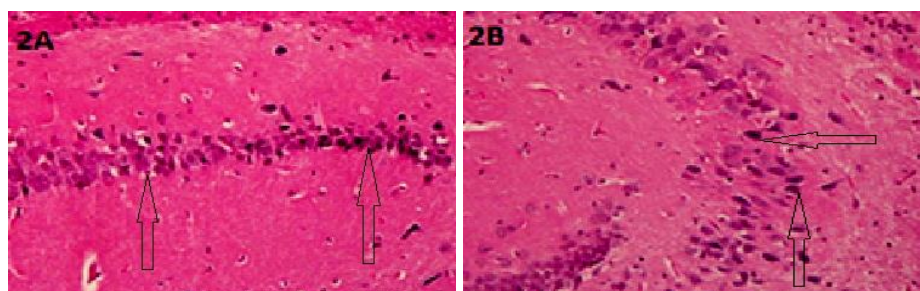


Figure 3A & Figure 3B [Low dose *Galinsoga parviflora* extract 200 mg/kg +3-NP 10 mg/kg *i.p.*]: The CA3 region shows some normal pyramidal cells in clusters and some damaged pyramidal cells [Figure 3A]. The interconnected neuropil fibers in the CA3 region appear normal or not damaged. The CA1 region shows a moderate number of normal pyramidal cells and some of the pyramidal cells show degenerative changes along with normal neuropil fibers [Figure 3B].

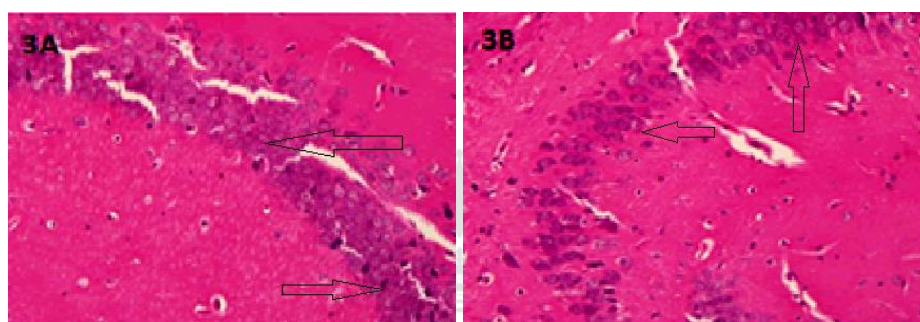


Figure 4A & Figure 4B [High dose *Galinsoga parviflora* extract 400 mg/kg +3-NP 10 mg/kg *i.p.*]: The CA3 region shows normal pyramidal cells and some damaged pyramidal cells [Figure 4A]. The interconnected neuropil fibers in the CA3 region appear smaller than normal. The CA1 region shows pyramidal cells and a few of the pyramidal cells show degenerative changes along with decreased neuropil fibers [Figure 4B].

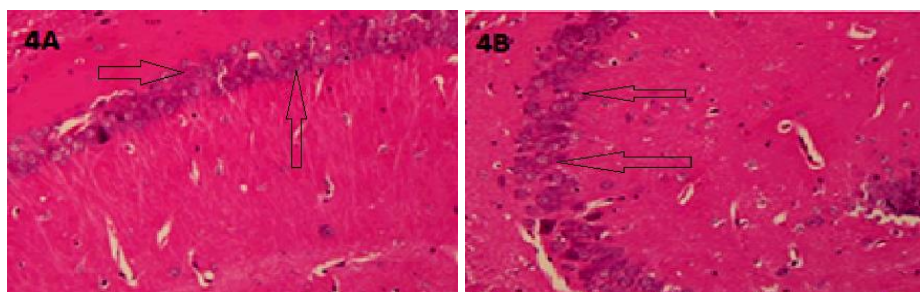
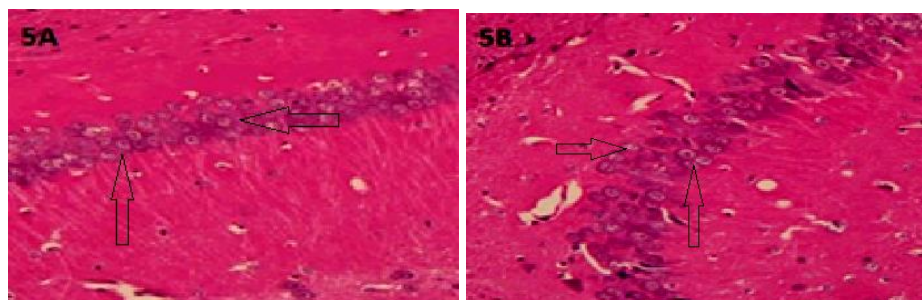


Figure 5A & Figure 5B [Standard (Pirecetam 200 mg/kg) + 3-NP 10 mg/kg *i.p.*]: The CA3 region shows Normal pyramidal cells and some damaged pyramidal cells [Figure 5A]. The interconnected neuropil fibers in the CA3 region appear smaller than normal. The CA1 region

shows pyramidal cells and only some of the pyramidal cells show degenerative changes along with fewer neuropil fibers [Figure 5B].



4. DISCUSSION:

Lipid peroxidation plays an important role in the development of neurodegenerative disorders, especially Alzheimer's disease (AD). Excitatory neurotransmitters, such as acetylcholine (ACh), are more imperious for neuronal communications. AChE enzyme is responsible for the degradation of ACh and the level of this degradative enzyme is higher in Alzheimer's patients.³⁰

Induction of apoptosis may be due to the accumulation of 3 NP in neurons following cell depolarization, which leads to inhibition of $\text{Na}^+/\text{Ca}^{2+}$ exchange and thereby induces an excessive accumulation of mitochondrial Ca^{2+} . Further, an increase in intramitochondrial Ca^{2+} levels can lead to an opening of mitochondrial transmembrane potential, with the release of cytochrome c and subsequent apoptosis resulting from activation of the caspase family of proteases.³¹

Cells in the nervous system can defend themselves against oxidative stress by using several different resources, including vitamins, antioxidants, enzymes (such as superoxide dismutase, glutathione peroxidase, heme oxygenase 1, and NAD (P)H quinone oxidoreductase 1), and lipoic acid and redox-sensitive protein transcription factors (such as AP-1, Nrf2, and HSF) Hsp70, also referred to as Hsp72, which has a cytoprotective function induced in many neurodegenerative disorders.³²

3 NP is well known that increased oxidative stress contributes significantly. Thus, pharmacological Systemic administration of 3-NP induces an important increase in free radicals formation, leading to a degenerative process in the hippocampal or striatal region.³³ The *i.p* administrations of 3-NP in rats, significantly attenuated 3-NP-induced motor impairments and striatal or hippocampal oxidative stress. Notably, these effects occurred

independently of mitochondrial complex II activity, suggesting that 3-NP may induce oxidative stress through mechanisms independent of mitochondrial dysfunction.³⁴

Mitochondrial dysfunction linked to increased oxidative stress can be considered one of the deleterious events that occurred during the experimental induction of Huntington's disease. Neurochemical analysis of cortical, striatal and hippocampal levels/activities of lipid peroxidation revealed that 3-NP administration consecutive days significantly increased lipid peroxidation (MDA level) and reduced antioxidant enzyme activity in the mentioned brain areas.³⁵ The role of free radicals in neurodegenerations and cognitive decline has been studied previously and findings emphasize the ROS role in the brain and found to improve neuronal function. Hence, oxidative stress was considered one of the main causes of cognitive impairment.³⁶ Oxidative damage of the hippocampus and cerebral cortex in rats during aging and oxidative stress are thought to contribute to impairment of cognitive functions, for example, learning and memory deficits.³⁷

5. CONCLUSION:

In the present study, rats after 3-NP treatment showed a significant decrease in brain antioxidant enzymes such as SOD, GSH, and Total protein and increased lipid peroxidation. It was clear from the results that 3-NP administration caused mitochondrial dysfunction, which led to ROS production and eventually neuronal damage.

However, treatment with a high dose of *Galinsoga parviflora* extract has produced a more significant reversal of GSH, and SOD than a low dose. This activity was comparable with the anti-inflammatory activity of extract of *Galinsoga parviflora* due to antioxidant activity. Taken together; these results suggest that its antioxidant properties might be one of the contributing factors to its neuroprotectant action. 3-NP-induced neurotoxicity there was a direct relationship between inflammation and excitotoxicity, due to various inflammatory mediators, and glutamate-induced excitotoxic activation results in neuronal death.

Galinsoga parviflora plant extract significantly prevented this raise in levels of LPO. GSH, SOD, and Total protein content have significantly increased in extract-treated groups whereas the toxic group has shown a significant decrease in levels compared to the control group.

Histopathological observation of the Brain shows that the *Galinsoga parviflora* extract at the dose of 200 and 400 mg/kg *p.o* prevented the damage caused to the brain and helped in neuroprotective to neurotoxic induce albino rats. The observed results leading to

neurodegeneration were also confirmed by the histopathological differences between the treatment and the 3-NP control group. There was a reversal of the brain damage observed in *Galinsoga parviflora* extract treated animals and it prevented neuron loss. *Galinsoga parviflora* plant extract at a high dose (400 mg/kg) shows protected cellular morphology exhibiting significantly reduced morphologic abnormalities in all regions with significant recovery in tissues and well-formed nuclei without irregular features.

These results suggested that ethanolic extract of *GALINSOGA PARVIFLORA* may have potential therapeutic value in the treatment of some neurological disorders, probably by its anti-inflammatory and antioxidant properties.

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<p><i>Image</i> <i>Author -1</i></p>	<p><i>Corresponding Author: Madhu S U</i> <i>Author Affiliation: M Pharmacy, Dept of Pharmacology.</i> <i>Author Address/Institute Address: Mallige College of Pharmacy, Siluvepura, Chikkabanavara Post, Bangalore-560090, India.</i></p>
<p><i>Image</i> <i>Author -2</i></p>	<p><i>Author Name: Harshitha S</i> <i>Author Affiliation: B Pharmacy</i> <i>Author Address/Institute Address: Mallige College of Pharmacy, Siluvepura, Chikkabanavara Post, Bangalore-560090, India.</i></p>
<p><i>Image</i> <i>Author -3</i></p>	<p><i>Author Name: Dr. Hariprasad M G</i> <i>Author Affiliation: Professor and HOD, Dept of Pharmacology</i> <i>Author Address/Institute Address: KLE College of Pharmacy, 2nd Block, Rajajinagar, Bangalore, Karnataka 560010, India.</i></p>
<p><i>Image</i> <i>Author -4</i></p>	<p><i>Author Name: Honnesh</i> <i>Author Affiliation: B Pharmacy.</i> <i>Author Address/Institute Address: RR College of Pharmacy, Chikkabanavara, Bangalore-560090, India.</i></p>