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EVALUATION OF NISOLDIPINE FOR ITS NEUROPROTECTIVE ACTIVITY IN RATS – A DRUG RE-PURPOSING STUDY

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Abstract: **Neurodegenerative disorders pose significant challenges due to their progressive neuronal loss and resultant functional impairments. This study investigated the neuroprotective potential of Nisoldipine, a calcium channel blocker, against AlCl3 and MSG-induced neurotoxicity in Wistar rats. Evaluation through behavioral tests, biochemical assays, and histopathological examination revealed that Nisoldipine co-treatment ameliorated cognitive deficits, improved motor coordination and locomotor activity, and reduced acetylcholinesterase activity and lipid peroxidation. Furthermore, it enhanced antioxidant defenses and mitigated neuronal damage. These findings suggest that Nisoldipine may offer therapeutic benefits by modulating intracellular calcium levels, highlighting its promise as a neuroprotective agent in managing neurodegenerative disorders.**

I. INTRODUCTION

Neurons are the structural and functional building blocks of the nervous system. They are highly specialized cells that transport signals from one area of the body to another via nerve impulses. The three main components of a neuron are the cell body, dendrites, and axon. Because neurons don't often multiply or replace themselves, the body is unable to repair or replace them when they are injured or die. [1][2] Neurodegenerative disorders are distinguished by the progressive and irreversible loss of neurons from specific areas of the brain. Parkinson's disease (PD) and Huntington's disease (HD), where the loss of neurons from basal ganglia structures results in abnormalities in movement control; Alzheimer's disease (AD), where the loss of hippocampal and cortical neurons leads to impairment of memory and cognitive ability; and amyotrophic lateral sclerosis (ALS), where muscular weakness results from degeneration of spinal, bulbar, and cortical motor neurons. [3] These illnesses are relatively frequent and pose a significant medical and societal issue.
Neurodegenerative diseases are chronic central nervous system disorders marked by cognitive, motor, or behavioral impairment [5]. Neurodegenerative diseases are genetic and sporadic conditions that cause progressive nervous system dysfunction. These illnesses are frequently linked with atrophy of the afflicted central or peripheral nervous system structures. Alzheimer's and other dementias, brain cancer, degenerative nerve disease, encephalitis, epilepsy, genetic brain illness, head and brain malformation, hydrocephalus, stroke, Parkinson's Diseases, Multiple Sclerosis, Amyotrophic lateral sclerosis, Huntington's Disease, and others are among them.[6] Excitotoxicity, oxidative stress, and inflammation are the mechanisms that cause neuronal degeneration. Controlling glutamate release, NMDA receptor antagonists, calcium channel blockers, free radical scavengers, anti- inflammatory drugs, NO synthase inhibitors, and other medicines have therefore been proposed for their treatment.[7,8] One of the neurotoxins found in foods is monosodium glutamate. Glutamate causes neuronal death via two mechanisms: excitotoxic and oxidative stress. The excitotoxic pathway involves the overactivation of glutamate receptors, namely the N-Methyl-D-Aspartate (NMDA) type of receptor, which results in calcium excess and excitotoxicity. The oxidative stress route is characterized by the breakdown of the glutamate-cystine antiporter and a decrease in glutathione levels, which results in the generation of neurotoxic reactive oxygen species (ROS) and neuronal damage. [9] Ischemia is another cause of neuronal death. Cerebral ischemia is caused by a decrease in the availability of glucose and oxygen to brain cells, resulting in a cellular energy crisis. Pathological alterations that occur as a result of cerebral ischemia include free radical production, excitotoxicity, and inflammatory activation.[10] Drug repurposing, also known as drug repositioning or drug reprofiling, is becoming more common in the drug development process. Serendipity, observations of adverse effects, target hunting, or novel ideas can lead to the repurposing of old medications, which can function via the same mechanism of action as their original use or via new mechanisms. [11] [12] Calcium homeostasis can be altered during an acute injury or as we age, leading to neuronal dysfunction. Pharmacologic therapies

that modulate calcium may have neuroprotective effects in theory. Increased intraneuronal Ca2+ concentrations caused by cerebral ischemia and traumatic brain injury set off a chain of metabolic reactions that can lead to cell lysis and death. Calcium channel blockers reduce the rise in intracellular Ca2+ in some experimental models of localized and global ischemia. (a) the brain has the largest density of [3H] nitrendipine binding sites (i.e., voltage-sensitive calcium channels) in the body. (b) Calcium channel blockers, particularly those in the dihydropyridine class, are lipophilic chemicals that pass the blood-brain barrier. Previously, it was discovered that dihydropyridine compounds were more effective on the biogenic amine metabolism of the rat brain than verapamil or diltiazem. Nisoldipine had no effect on the hypothalamic biogenic amines and metabolites. The voltage- sensitive calcium channel (VSCC) in the brain is blocked by nisoldipine. Nitrendipine crosses the blood-brain barrier more easily. However, it is known that Nisoldipine has a high affinity for the dihydropyridine binding site on the VSCC of the rat brain.

II. RESEARCH METHODOLOGY

ANIMALS: Adult albino Wistar rats of either sex, weighing 200-250 g will be collected from the Drug Testing Lab, Drugs Control Department, Bengaluru. The animals will be housed under standard laboratory conditions, maintained on a 12 h light:12 h dark cycle, and free access to food and water.

EXPERIMENTAL MODELS:

The following two models were used to screen the neuroprotective activity

1. Monosodium Glutamate (MSG) induced neurotoxicity model
2. Aluminum chloride (AlCl3) induced neurotoxicity model

1. Monosodium Glutamate (MSG) induced neurotoxicity model: A total of 36 rats will be randomly divided into six groups of 6 rats each and treated for 7 days. Monosodium Glutamate solution was prepared in distilled water. Test drug Nisoldipine for oral injections was prepared as a stock solution of 5%CMC. The test drugs Nisoldipine and Standard Vit E are administered before 1 hour to the administration of Monosodium glutamate for 7 Consecutive days as mentioned above. On the 8th day, rats will be evaluated for Locomotor activity, Cognitive ability, and Motor performance (Grip strength) using Actophotometer, Elevated plus maze, and Rotor rod apparatus respectively. On the 9th day, rats will be sacrificed and the brain will be isolated for estimation of AchE, CAT, GSH, LPO, Nitrite, and Total protein. Two brains were fixed in 10% formalin for histopathological studies to determine the degree of brain damage.
2. Aluminium Chloride (AlCl3) induced neurotoxicity model: A total of 36 rats will be randomly divided into six groups of 6 rats each and treated for 14 days. Aluminum chloride solution was prepared in distilled water. Test drug Nisoldipine for p.o injections was prepared as a stock solution of 5%CMC. The test drugs Nisoldipine and Standard Vit E are administered before 1 hour to the administration of Aluminium chloride (AlCl3) for 14 Consecutive days as mentioned above. On the 15th day, rats will be evaluated for Locomotor activity, Cognitive ability, and Motor performance (Grip strength) using Actophotometer, Elevated plus maze, and Rotor rod apparatus respectively. On the 16th day rats will be sacrificed and the brain will be isolated for estimation of Ache, CAT, GSH LPO, Nitrite, and Total protein. Two brains were fixed in 10% formalin for histopathological studies to determine the degree of brain damage.

IN-VIVO PARAMETERS:

1. Locomotor activity: (11) Using an actophotometer, which runs on photoelectric cells connected in a circuit with a counter, it is simple to detect locomotor activity. When the animal interrupts the light beam hitting the photocell, a count is made. The animal moves within a circular or square arena on an actophotometer. For experimental purposes using this apparatus, both rats and mice may be employed. The anatomical and operational cornerstones of the nervous system are neurons. They are highly specialized cells that use nerve impulses to move signals from one part of the body to another. The cell body, dendrites, and axon are the three primary parts of a neuron. The body cannot repair neurons because they rarely grow or replace themselves.
2. Cognitive ability: (12) The raised plus maze device was made up of two closed walls (each measuring 50 by 10 cm), two open arms (each measuring the same dimensions), and walls that were 40 cm high. The EPM's two arms were joined by a central square (10 x 10 cm), which was kept 50 cm off the ground. The animal was positioned at the end of the arm during the acquisition phase, facing away from the centre square. Initial transfer latency (ITL), which measures how long it takes an animal to transition from an open arm to a closed arm, was measured. Retention transfer latency was evaluated on the last day. The percent retention of memory was evaluated by the formula ITL-RTL/RTL *100
3. Motor performance (Grip strength):(13) Rats and mice's motor skills can be usually evaluated using the rotarod test (Zausinger et al. 2000; Luesse et al. 2001; Jeong et al. 2003; Karl et al. 2003). The test gauges a rat's ability to stay balanced on a rod that rotates quickly. Animals are placed on the testing rod in the trials at an initial speed of 4 revolutions per minute (rpm). Then, over ninety seconds, the rod speed progressively increased to 44 rpm. Each animal's time on the rod is immediately recorded. BIOCHEMICAL ESTIMATION:

1. Preparation of mitochondrial supernatant: [14] Following decapitation, the brain was taken and homogenized as 10% w/v in cold phosphate buffer after being washed in cooled 0.9 percent saline and maintained on ice. The cerebrum, hippocampus region, and cortex were then isolated (0.05 M, pH 7.4). The homogenates were spun at 10,000 g for 10 min at 4°C, and the post- mitochondrial supernatant (PMS) was kept in a freezer at 80°C until it was time to be analyzed.
2. Estimation of acetylcholinesterase levels in rat brain: [15] Using the Ellman et al. approach, AChE levels in the whole brain were measured to evaluate cholinergic dysfunction (1961). In brief, 100 l of Ellman's reagent (0.5 mM, 19.8 mg DTNB, and 0.1M

sodium phosphate, pH 7.2) was added to a cuvette containing 0.4 ml of supernatant, 2.6 ml of sodium phosphate buffer (0.1 M, pH R = 5.74 (10 7.2), and absorbance was measured in a spectrophotometer at 412 nm until the increasing absorbance became stable. Then, 20 l of acetylthiocholine iodide (the substrate) was introduced, and changes in absorbance per minute were noted for 10 minutes. This steady absorbance was then reset to zero. It was computed how much the absorbance changed each minute. The rate was determined using the following method. R = 5.74(10-4) × A / Co Where, R is the rate, in moles substrate hydrolyzed per minute per g of tissue; A is a change in absorbance per minute; Co is the original concentration of tissue i.e., 20 mg/ml.

3. Estimation of reduced glutathione in rat brain:[16] Reduced glutathione was assayed by the method of Jollow et al. (1974). Briefly, 1.0 ml of post mitochondrial supernatant (10%) was precipitated with 1.0 ml of sulfosalicylic acid (4%). The samples were kept at 4°C for at least 1 h and then subjected to centrifugation at 1,200 g for 15 min at 4°C. The assay mixture contained 0.1 ml supernatant, 2.7 ml phosphate buffer (0.1 M, pH 7.4), and 0.2 ml 5,5, dithiobis-(2-nitro benzoic acid) (Ellman's reagent, 0.1 mM, pH 8.0) in a total volume of 3.0 ml. The yellow color developed was read immediately at 412 nm, and GSH levels were calculated using a molar extinction coefficient of 1.36×104 M −1 cm −1) and expressed as micromole per milligram protein.

4. Estimation of Lipid peroxidation (LPO) in rat brain:[17] The rate of lipid peroxidation was calculated using reactive species of thiobarbituric acid (TBARS). Briefly, 2.0 ml of the TCA- TBA-HCl reagent (15 percent w/v TCA, 0.375 percent w/v TBA, and 0.25 N HCl) was added to 0.1 ml of the tissue homogenate. The mixture was then heated for 15 minutes, cooled, then centrifuged for 10 minutes at 1000 rpm. The malondialdehyde concentration of the sample was determined using an extinction value of 1.56 105 /M/cm and the absorbance of clear supernatant at 535 nm.

5. Estimation of Superoxide Dismutase (SOD) in rat brain:[18] Briefly, equal volumes of tissue homogenate and Griess reagent (5% phosphoric acid containing 0.1% NEDD, 1% sulfanilamide) were mixed and incubated in dark for 10 min at room temperature. Then the absorbance of the reaction mixture is measured at 540 nm. Nitrite concentration was calculated using a standard curve for sodium nitrite. Nitrite levels were expressed as micrograms per milliliter.

6. Estimation of Catalase (CAT) in rat brain:[19] Catalase activity was assayed by the method of Claiborne (1985). Briefly, the assay mixture consisted of 1.95 ml phosphate buffer (0.05 M, pH 7.0), 1.0 ml hydrogen peroxide (0.019 M), and 0.05 ml post mitochondrial supernatant (10%) in a final volume of 3.0 ml. Changes in absorbance were recorded at 240 nm. Catalase activity was quantified using the millimolar extinction coefficient of H2O2 (0.07 mM) and expressed as micromoles of H2O2 decomposed per minute per milligram protein.[21]

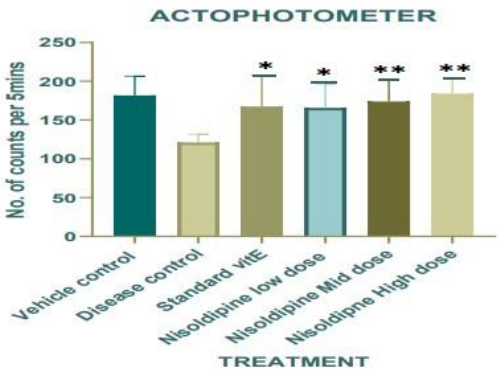
7. Histopathological Studies: At the end of the study, all the rats were sacrificed by approved anesthesia and the brains were dissected out, and washed in ice-cold saline. A section of the brain was immediately fixed in a 10% buffered neutral formalin solution. After fixation, tissues were embedded in paraffin serial sections were cut and each section was stained with hematoxylin and eosin for histopathological studies.

III. RESULTS AND DISCUSSION

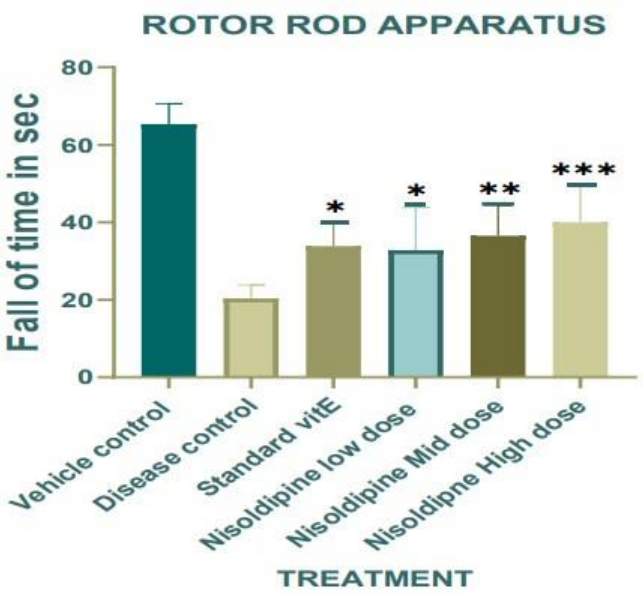
1. MONOSODIUM GLUTAMATE-INDUCED NEUROTOXICITY MODEL:

Effect of Nisoldipine on Neurobehavioral in – vivo parameters in MSG-treated rats:

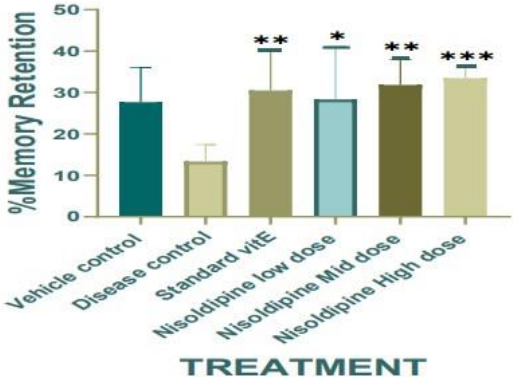
a. Locomotor Activity – Actophotometer



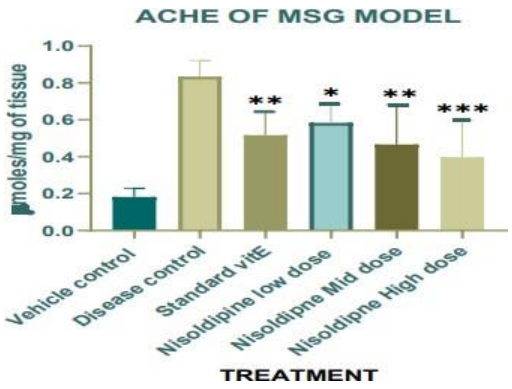
b. Grip Strength – Rotor Rod Apparatus:

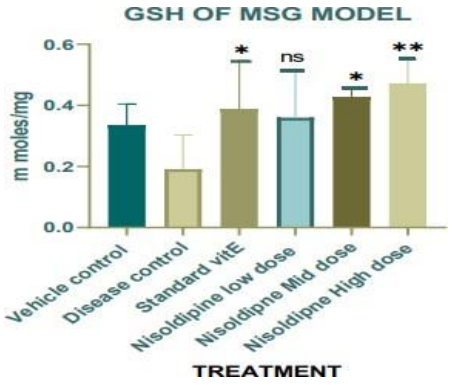


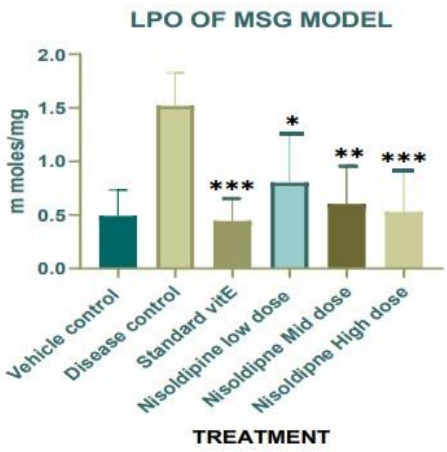
c. Cognitive Ability – Elevated Plus Maze

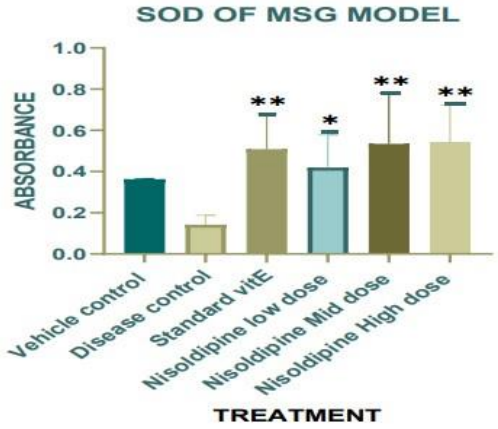


Effect of nisoldipine on biochemical parameters inMSG treated rats









➢ Representation of Histopathological changes in cortical regions in MSG rat brain:

➢ Disease control: The section studied shows brain parenchyma consisting of neuroglial cells having round to oval vesicular nuclei with abundant cytoplasm and inconspicuous nucleoli. The glial cells (Moderate to severe) show degenerative changes (necrotic and pyknotic) (Fig,1, Arrow). The blood vessels appear congested (Fig.2, Arrow) with plump endothelial cells. The background neuropil appears intact.

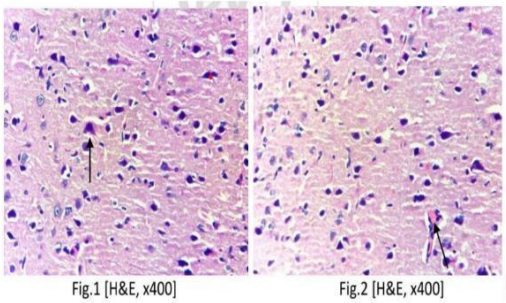


Fig.1 [H&E, x400]

Fig.2 [H&E, x400]

➢ Standard vit E +MSG : Section studied shows brain parenchyma consisting of neuroglial cells having round to oval vesicular nucleus with abundant cytoplasm and inconspicuous nucleoli. The glial cells(Mild) show degenerative changes (necrotic and pyknotic) (Fig.,1, Arrow). Mild mixed inflammation noted. The blood vessels appear intact (Fig.2, Arrow). The background neuropil appears intact.

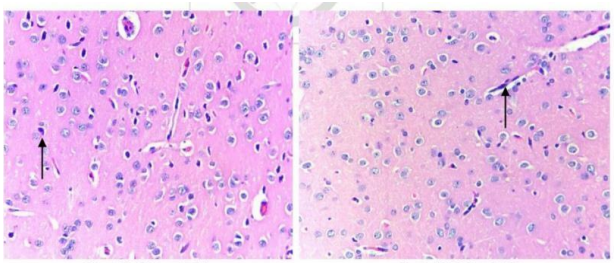


Fig.1 [H&E, x400] Fig.2 [H&E, x400]

➢ Nisoldipine Low Dose + MSG Section studied shows brain parenchyma consisting of neuroglial cells having round to oval vesicular nucleus with abundant cytoplasm and inconspicuous nucleoli. The glial cells (Moderate) show degenerative changes (necrotic and pyknotic) (Fig.,1, Arrow). The blood vessels appear congested (Fig.2, Arrow) with plump endothelial cells. The background neuropil appears intact.

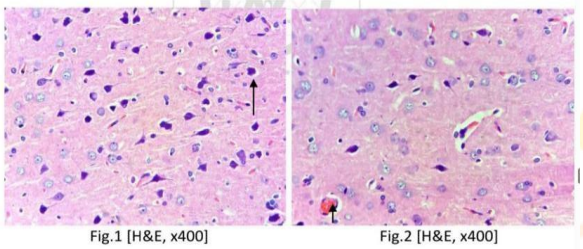


Fig.1 [H&E, x400] Fig.2 [H&E, x400]

➢ • Nisoldipine Mid Dose + MSG: Section studied shows brain parenchyma consisting of neuroglial cells having round to oval vesicular nucleus with abundant cytoplasm and inconspicuous nucleoli. The glial cells(Mild to Moderate) show degenerative changes (necrotic and pyknotic) (Fig.,1, Arrow). The blood vessels appear congested (Fig.2, Arrow) with plump endothelial cells. The background neuropil appears intact.

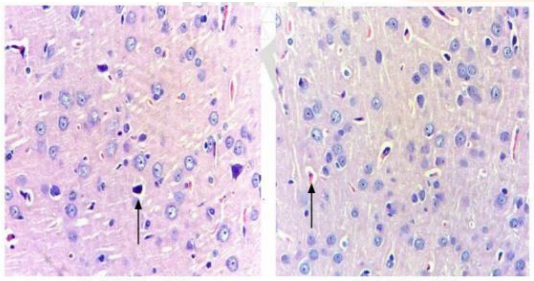


Fig.1 [H&E, x400] Fig.2 [H&E, x400]

➢ • Nisoldipine High Dose + MSG Section studied shows brain parenchyma consisting of neuroglial cells having round to oval vesicular nucleus with abundant cytoplasm and inconspicuous nucleoli. The glial cells (Mild) show degenerative changes (necrotic and pyknotic) (Fig.,1, Arrow). The blood vessels appear congested (Fig.2, Arrow) with plump endothelial cells. The background neuropil appears intact.

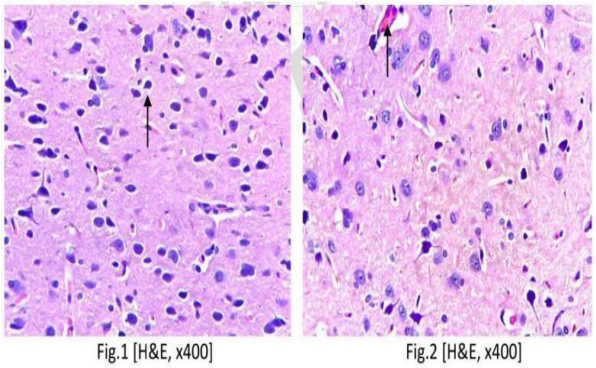


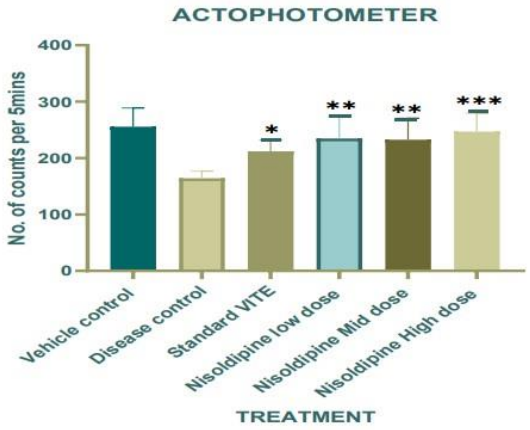
Fig.1 [H&E, x400] Fig.2 [H&E, x400]

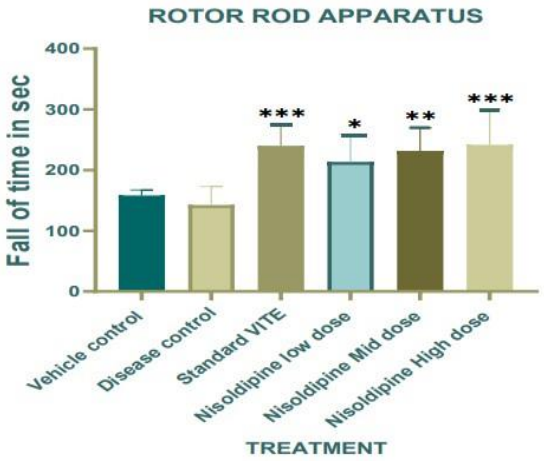
2. ALCL3 INDUCED NEUROTOXICITY MODEL:

Effect of Nisoldipine on neurobehavioral in – vivo parameters in AlCl3 treated rats.

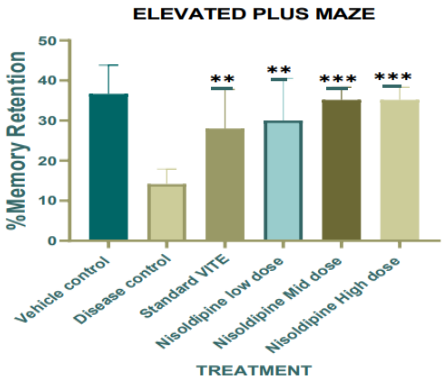
a. Locomotor Activity – Actophotometer

b. Grip Strength – Rotor Rod Apparatus:

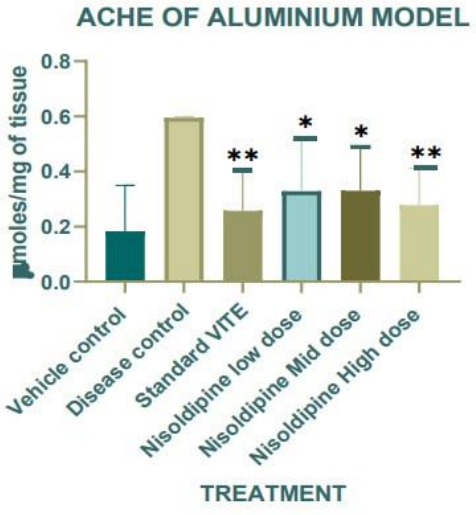


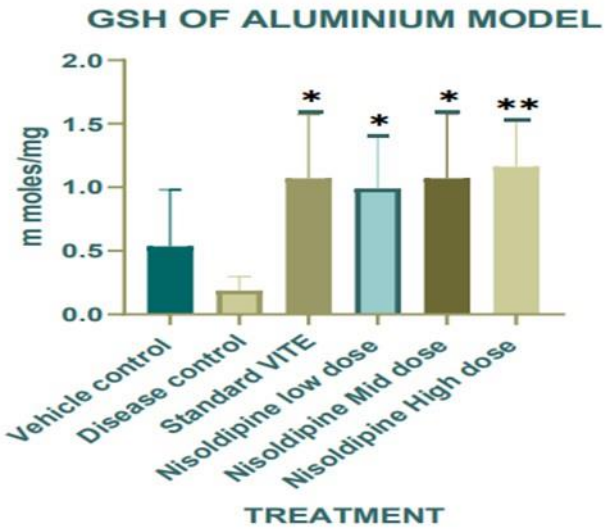


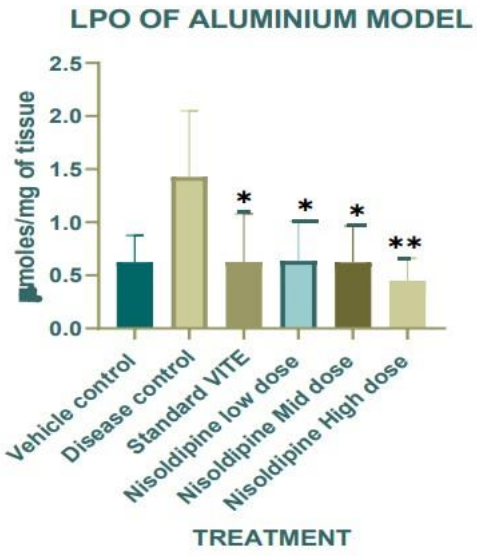
c. Cognitive Ability – Elevated Plus Maze

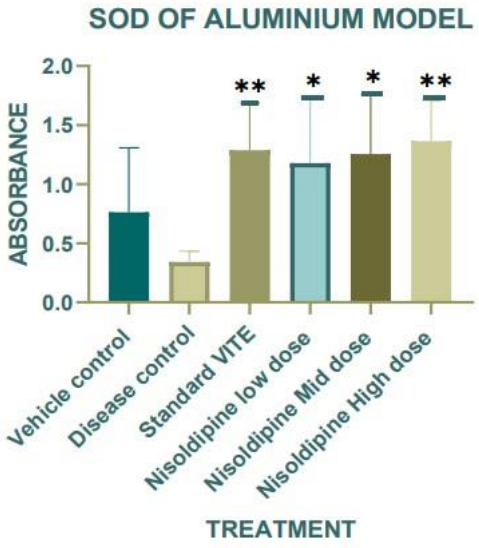


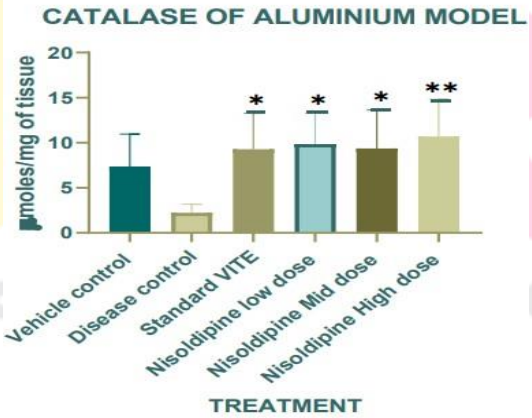
➢ Effect of nisoldipine on acetylcholine esterase, glutathione, lipid peroxidase, superoxide dismutase, total protein and catalase levels in AlCl3 treated rat:











Representation of Histopathological changes in cortical regions in $AlCl_3$ rat brain:

- Vehicle control Section studied shows brain parenchyma (Fig.1, Arrow) consisting of neuroglial cells having round to oval vesicular nucleus with abundant cytoplasm and inconspicuous nucleoli, granular cells and interact blood vessels (Fig.,2Arrow).

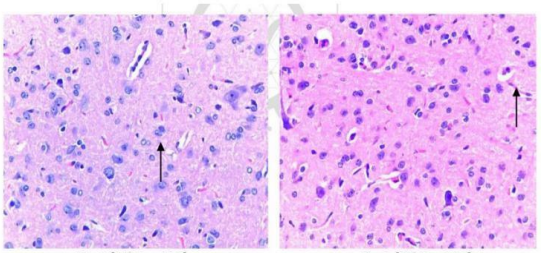


Fig.1 [H&E, x400] Fig.2 [H&E, x400]

• Disease control Section studied shows brain parenchyma consisting of neuroglial cells having round to oval vesicular nucleus with abundant cytoplasm and inconspicuous nucleoli. The glial cells(Moderate to severe) show degenerative changes (necrotic and pyknotic) (Fig.,1, Arrow). The blood vessels appear congested (Fig.2, Arrow) with plump endothelial cells. The background neuropil appears intact.

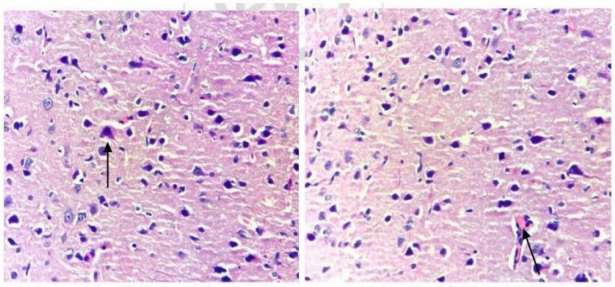


Fig.1 [H&E, x400] Fig.2 [H&E, x400]

• Standard vitE + Nisoldipine : Section studied shows brain parenchyma consisting of neuroglial cells having round to oval vesicular nucleus with abundant cytoplasm and inconspicuous nucleoli. The glial cells(Mild) show degenerative changes (necrotic and pyknotic) (Fig.,1, Arrow). Mild mixed inflammation noted. The blood vessels appear intact (Fig.2, Arrow). The background neuropil appears intact.

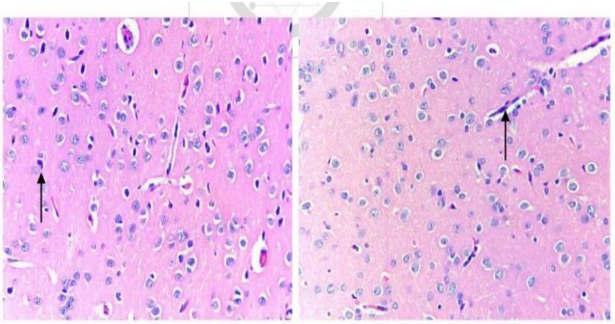


Fig.1 [H&E, x400] Fig.2 [H&E, x400]

• Nisoldipine low dose: Section studied shows brain parenchyma consisting of neuroglial cells having round to oval vesicular nucleus with abundant cytoplasm and inconspicuous nucleoli. The glial cells (moderate) show degenerative changes (necrotic and pyknotic) (Fig.,1, Arrow). The blood vessels appear congested (Fig.2, Arrow) with plump endothelial cells. The background neuropil appears intact.

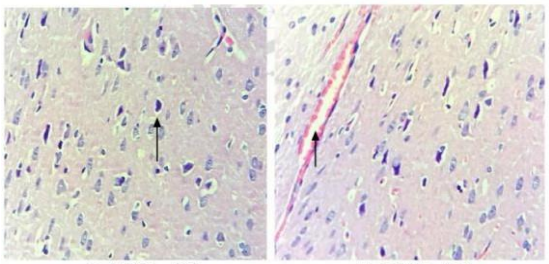


Fig.1 [H&E, x400] Fig.2 [H&E, x400]

• Nisoldipine mid dose Section studied shows brzyain parenchyma consisting of neuroglial cells having round to oval vesicular nucleus with abundant cytoplasm and inconspicuous nucleoli. The glial cells(Mild to moderate) show degenerative changes (necrotic and pyknotic) (Fig.,1, Arrow). The blood vessels appear congested (Fig.2, Arrow) with plump endothelial cells. The background neuropil appears intact.

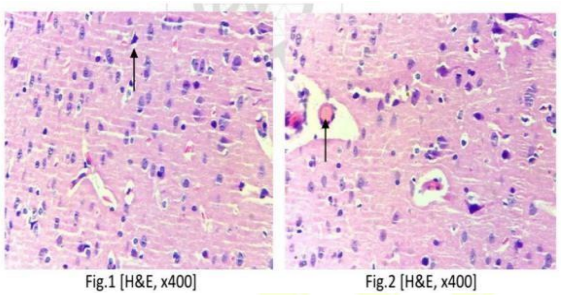


Fig.1 [H&E, x400] Fig.2 [H&E, x400]

• Nisoldipine high dose: Section studied shows brain parenchyma consisting of neuroglial cells having round to oval vesicular nucleus with abundant cytoplasm and inconspicuous nucleoli. The glial cells(Mild) show degenerative changes (necrotic and pyknotic) (Fig.,1, Arrow). The blood vessels appear congested (Fig.2, Arrow) with plump endothelial cells. The background neuropil appears intact.

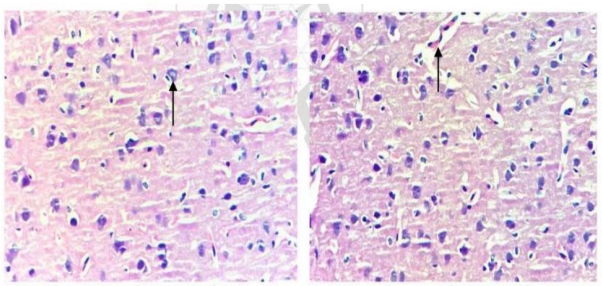


Fig.1 [H&E, x400] Fig.2 [H&E, x400]

This study was undertaken to evaluate potential neuroprotective effects of Nisoldipine using a model of neurotoxicity induced by Monosodium glutamate and Aluminium chloride. The administration of monosodium glutamate (MSG) in rats for a period of 7 days resulted in aggressive and fighting behavior. Furthermore, the study found that MSG significantly reduced locomotor activity, as indicated by a decrease in counts per 5 minutes in the Actophotometer for the disease control group. However, groups treated with Nisoldipine and vitamin E showed an increase in locomotor activity. The increase in locomotor activity was dose- dependent in Nisoldipine-treated animals. MSG administration led to increased oxidative stress in the brain, as evidenced by elevated lipid peroxidation (LPO) levels in treated rats compared to the treatment group. Conversely, Nisoldipine treatment resulted in a dose-dependent decrease in LPO levels, with the high dose showing the most significant reduction. In rats treated with MSG alone, levels of antioxidant enzymes such as GSH, catalase, and SOD were found to decrease (GSH: 0.19±0.04, catalase: 3.63±0.23, SOD: 0.42±0.04). However, in rats treated with Nisoldipine, the levels of these antioxidant enzymes, including GSH and catalase, were found to increase (GSH: 0.29±0.04, $p<0.05$; catalase: 7.88±0.601, $p<0.01$; SOD: 0.4±0.05, $p<0.001$). The histopathological examination revealed degenerative changes in the MSG and Nisoldipine-treated groups, with varying degrees of severity depending on the dose of Nisoldipine administered.

The study demonstrated that administration of aluminum chloride (AlCl3) to rats for 15 days resulted in a decline in cognitive function, muscle strength, and locomotor activity. AlCl3 binding to brain phospholipids and its ability to increase acetylcholinesterase (AChE) activity contributed to these effects. However, treatment with Nisoldipine and vitamin E showed improvement in locomotor activity, cognitive function, and muscle strength. Nisoldipine treatment led to dose-dependent increases in these parameters. The study also found that AlCl3 increased oxidative stress, as indicated by elevated lipid peroxidation (LPO) levels, but Nisoldipine treatment reduced LPO levels in a dose-dependent manner. Furthermore, AlCl3 treatment decreased levels of glutathione (GSH), catalase, and superoxide dismutase (SOD), while Nisoldipine treatment increased their levels. Histological examination revealed degenerative changes in brain tissue due to AlCl3, which were mitigated by Nisoldipine and vitamin E treatment. These findings suggest that AlCl3 administration to rats can induce neurotoxic effects, but Nisoldipine and vitamin E show potential neuroprotective effects in this model.

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