Journal of Neonatal Surgery
ISSN(Online): 2226-0439
Vol. 14, Issue 32s (2025)
https://www.jneonatalsurg.com

OPEN ACCESS

Reprofiling Of Ormeloxifene For Its Neuroprotective Activity Against Monosodium Glutamate And Aluminium Chloride Induced Neurotoxicity In Rats

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Cite this paper as: Suma G, Mallappa Shalavadi, Shubham Teli, Mounashree U, Rajashekhar N, Shivaraj Hiremath, Basavaraj H, (2025) Reprofiling Of Ormeloxifene For Its Neuroprotective Activity Against Monosodium Glutamate And Aluminium Chloride Induced Neurotoxicity In Rats. *Journal of Neonatal Surgery*, 14 (32s), 5031-5047.

ABSTRACT

Background: Monosodium glutamate (MSG) and Aluminium chloride ($AlCl_3$) are known to induce neurotoxicity, which can lead to cognitive deficits and neuronal damage. Due to the neuroprotective role of Ormeloxifene, it is evaluated against chemically (MSG) and ($AlCl_3$) induced neurotoxicity in rats.

Materials & Methods: Male Wistar albino rats were divided into two neurotoxicity models with 60 rats in each model. Rats were administered ormeloxifene at different doses (2.5, 5.1 and 10.2 mg/kg p.o) for 14 days $AlCl_3$ model and 21 days MSG model. Neurobehavioral parameters such as locomotor activity, muscle coordination and spatial memory were carried out. Antioxidant enzyme estimated such as lipid peroxidation (LPO) and glutathione (GSH) along with acetylcholinesterase (AChE). Histopathology studies were also carried out.

Results: Ormeloxifene co-treatment significantly reduced cognitive deficits, improved locomotor activity, muscle coordination, reference memory and spatial memory. It also reduced acetylcholinesterase (AchE) and lipid peroxidation (LPO) activity, while increasing glutathione (GSH) concentration. Histopathology reports showed reduced neuronal damage.

Conclusion: Ormeloxifene demonstrated neuroprotective effects against MSG and $AlCl_3$-induced neurotoxicity in rats, potentially due to its ability to activate kinases and inhibit nuclear factor (NF)-kB induced transcription. These findings suggest Ormeloxifene as a potential therapeutic agent for neuroprotection.

Keywords: Neuroprotective, Ormeloxifene, Monosodium glutamate, Aluminium chloride, Memory..

1. INTRODUCTION:

Neurodegenerative diseases are marked by a progressive dysfunction and loss of neurons. Functional systems are involved to differing extents in various diseases, and their involvement correlates with a diverse array of clinical manifestations. A major element is the accumulation of proteins whose physicochemical properties have altered, commonly known as misfolded proteins. These misfolded proteins may result in modifications to its function or in the buildup of potentially harmful compounds either inside or outside of cells and they are characterized by worsening dysfunction and loss of neurons [1]. The involvement of functional systems varies across diseases and is associated with a wide array of clinical manifestations. This behavior following viral infections leads to neurodegenerative diseases such as Alzheimer's disease (AD), multiple sclerosis (MS), Parkinson's disease (PD), and amyotrophic lateral sclerosis (ALS), which primarily impact the elderly population. Viruses can lead to neurodegeneration by either directly destroying neurons or triggering apoptosis [2]. The seven main types of neuropathological deposition or changes include tau-neurofibrillary tangles, TDP-43 inclusions, amyloid plaques, neuronal loss, angiopathy, gliosis, and alpha-synuclein inclusions [3]. Neurological disorders were the second greatest cause of mortality in 2015, accounting for 9.1 to 9.7 million deaths worldwide and the main cause of disability adjusted life years (DALYs) 250.7 [95% uncertainty range (UI) 229.1 to 274.7] million, or 10.2% of all DALYs [4].

In the CNS, vesicular glutamate transporter (vGlut) family is responsible for the transfer of glutamate, an excitatory

neurotransmitter into synaptic vesicles. Neurodegenerative illnesses are linked to its excitotoxic actions. Likewise, monosodium glutamate (MSG), commonly referred to as Aji-no-moto, has been implicated in neurotoxicity, which results in the destruction of neurons. The excitotoxicity of MSG can cause excessive calcium ion influx by overstimulating glutamate receptors (NMDA and AMPA). This leads to the production of reactive oxygen species (ROS) and the death of neurons by inducing mitochondrial stress and damaging enzyme activity (endonucleases, phospholipases, and proteases like calpain). Excitotoxic injury and neuronal degeneration are caused by elevated glutamate levels that are higher than the ideal level of about 1 mM [5,6].

Well-known neurotoxins like aluminum is also being connected to neurodegenerative illnesses. Apart from oxidative stress, apoptosis, cholinergic dysfunction, glutamate excitotoxicity, mitochondrial damage, microglial activation, protein aggregation, and the formation of neurofibrillary tangles, it also disrupts metal homeostasis, increases reactive oxygen species, and causes inflammation and neurodegeneration [7].

Since neurons cannot regenerate, current treatments focus on disease management rather than cure. As the prevalence of neurodegenerative diseases rises, particularly with aging populations, there is an urgent need for innovative therapeutic strategies [8]. Current pharmacotherapies often provide minimal benefits, highlighting the need for novel interventions. Drug repurposing, finding new uses for existing drugs, offers a lower risk of failure due to established safety profiles and Several drugs, including isradipine, ambroxol, and metformin, have shown potential in clinical studies for various neurodegenerative disorders. This makes the author to choose ormeloxifene, a selective estrogen receptor modulator (SERM), which has shown potential beyond its traditional uses in contraception and osteoporosis. It has shown neuroprotective potential by regulating synaptic transmission, oxidative stress, excitotoxicity, apoptosis, and inflammation, suggesting its use in neurodegenerative disease therapy [9].

2. Materials and Methods

2.1 Chemicals and reagents: All chemicals used were of analytical grade and commercially available. Specifically, the following compounds were utilized: acetylthiocholine iodide, aluminum chloride, concentrated hydrochloric acid (HCl), concentrated sulfuric acid (H2SO4), di-sodium hydrogen phosphate, Ellman's reagent, ormeloxifene, hydrogen peroxide, monosodium glutamate, N-(1-Naphthyl) ethylenediamine dihydrochloride, sodium chloride, sodium dihydrogen orthophosphate, sulfanilamide, sulfosalicylic acid, thiobarbituric acid, trichloroacetic acid, a total protein kit, vitamin E, and Folin reagent.

2.2 Animals: Wistar albino male adult rats weighing 200–250 g were obtained from the Drug Testing Lab of the Drug Control Department in Bengaluru. The animals were housed at room temperature (22–28 oC) with 55±5% relative humidity for 12-hour cycles of light and dark, and they were given normal laboratory feed and unlimited water. Under the number GCP/IAEC/DOP/2022-2023/80, the experiment protocol was authorized by the Institutional Animal Ethical Committee.

2.3 Dose selection: The dose of the test drug Ormeloxifene and standard drug vitamin E were selected on the basis of acute oral toxicity studies in the previous literatures [10].

2.4 Experimental protocol for Aluminium Chloride ($AlCl_3$) induced neurotoxicity model.

Total 60 Adult male Wistar albino rats were divided into six groups of 10 rats each and treated as follows:

GROUPS	TREATMENT
Normal control	Distilled water (p.o) (n=10)
Disease control	$AlCl_3$ (200 mg/kg i.p) + Vehicle (p.o) (n=10)
Vitamin E 100mg/kg	Vitamin E mg/kg (100 mg/kg p.o) + $AlCl_3$ (200mg/kgp.o) (n=10)
Ormeloxifene 2.5mg/kg	Ormeloxifene (2.5 mg/kgp.o)+$AlCl_3$ (200mg/kgp.o) (n=10)
Ormeloxifene 5.1mg/kg	Ormeloxifene (5.1 mg/kgp.o)+$AlCl_3$ (200mg/kgp.o) (n=10)
Ormeloxifene 10.2 mg/kg	Ormeloxifene (10.2 mg/kgp.o)+ $AlCl_3$ (200mg/kgp.o) (n=10)

An hour before to administering $AlCl_3$, the normal vitamin E solution in virgin coconut oil was given. For 14 days in a row, the test medication Ormeloxifene was given twice a week prior to the administration of $AlCl_3$.

2.5 Experimental protocol for MSG induced neurotoxicity model.

Total 60 Adult male Wistar albino rats were divided into six groups of 10 rats each and treated as follows:

GROUPS	TREATMENT
Normal control	Distilled water (p.o) (n=10)
Disease control	MSG (2g/kg i.p) +Vehicle (p.o) (n=10)
Vitamin E 100mg/kg	Vitamin E mg/kg (100mg/kg p.o) +MSG (2g/kg i.p) (n=10)
Ormeloxifene 2.5mg/kg	Ormeloxifene (2.5mg/kgp.o)+MSG (2g/kg i.p) (n=10)
Ormeloxifene 5.1mg/kg	Ormeloxifene (5.1mg/kgp.o)+MSG (2g/kg i.p) (n=10)
Ormeloxifene 10.2 mg/kg	Ormeloxifene (10.2 mg/kgp.o)+ MSG(2g/kg i.p) (n=10)

For 21 days in a row, the usual vitamin E dissolved in virgin coconut oil and Ormeloxifene (twice a week) were given. For ten days, MSG was given alternately.

2.6 Neurobehavioral assessment in $AlCl_3$ and MSG induced neurotoxicity model.

2.6.1 Spontaneous locomotor activity

According to Idris et al., the spontaneous locomotor activity was measured using an actophotometer. In a square, enclosed field arena (30 cm × 30 cm × 30 cm) with six photocells in the exterior wall, each animal was observed for five minutes. Photocell beam interruptions (locomotor activity) were recorded using a six-digit counter. To observe the locomotor activity, the actophotometer was turned on, and each rat was housed in the activity cage independently for five minutes. In both the AlCl3 and MSG models, the baseline reading is taken before to treatment, and the count is noted when the model is complete [11].

2.6.2 Grip strength

The rotarod test is frequently used to assess the motor skills of rats and mice. The test evaluates a rat's ability to remain upright on a rapidly rotating pole. For the testing trials, the animals are placed on the testing rod at a beginning speed of four revolutions per minute (rpm). The rod speed then gradually increased to 44 rpm over the next 90 seconds. The amount of time each animal spends on the rod is automatically recorded [12].

2.6.3 Reference memory

Rats' openness to exploring new regions is measured using a behavioral test known as the Y-Maze. Rats typically prefer to explore a fresh arm of the labyrinth rather than returning to an arm they have already visited. The hippocampus, septum, prefrontal cortex, and basal forebrain are among the brain regions involved in this task. The Y-maze device had three arms, each measuring 8 × 30 × 15 cm, and they were positioned 120 degrees apart. The Y-maze test's two trials were separated by an hour. The first attempt was given ten minutes. The rats could only explore the start arm and the familiar arm of the labyrinth; the third arm, the new arm, was barred. In the second trial, rats were placed back in the maze and given free access to all three arms for five minutes. The total amount of time spent in the new arm was monitored and analyzed using a camera mounted on the ceiling [13].

2.6.4 Spatial memory

The Morris water navigation task, also known as the Morris water maze (MWM), is a behavioral procedure mostly used with rodents. In behavioral neuroscience, it is frequently used to investigate memory and spatial learning. It makes it possible to study learning, memory, and spatial working with high precision. It may also be used to measure damage to specific brain cortical regions [14]. The Morris Water Maze (MWM) uses distal visual cues to evaluate memory and spatial learning. Animals find a hidden platform by navigating an opaque circular pool. Memory retention is measured in a probing trial by evaluating preference for the platform region following its removal, whereas spatial learning is examined over a number of trials. The test allows comparisons between normal, illness, standard, and treatment groups since it is closely related to NMDA receptor activity and hippocampus synaptic plasticity.

Apparatus: A circular pool (150 cm diameter, 60 cm height) filled with water (25°C) is divided into four quadrants (N, E, W, S). A hidden platform (28 cm height, 10 cm width) is placed in the target quadrant, slightly submerged, and remains fixed during training.

Procedure: Animals undergo four trials daily for 4 days, starting from each quadrant. They navigate the pool for up to 1 minute to find the platform. Escape latency (time to reach the platform) is recorded. If unsuccessful, animals are guided to

the platform. After training, the platform is removed, and animals explore the pool for 1 minute. Memory retention is measured by the time spent in the target quadrant [15].

2.7 Biochemical estimation in Aluminium chloride and MSG induced neurotoxicity model

2.7.1 Preparation of post mitochondrial supernatant

The animals were scarified and the brain was taken out and cleaned in chilled 0.9% saline, placed on ice, and then blotted on filter paper. It was then weighed and homogenized as 10%w/v in cold phosphate buffer (0.05 M, pH 7.4). Lipid peroxidation was estimated using the post-mitochondrial supernatant (PMS), which was kept in a freezer at -20°C after the homogenates were centrifuged for 10 minutes at 4°C at 10,000 rpm. The supernatant was centrifuged once more for one hour at 4 oC and 15,000 rpm. Glutathione and acetylcholinesterase levels were further estimated using the supernatant [16].

2.7.2 Acetylcholinesterase

Acetylcholinesterase (AchE) is an enzyme splitting the neurotransmitter acetylcholine in cholinergic synapsis into choline and acetic acid. AchE's sensitivity to neurotoxic substances, such as nerve agents, insecticides, and anti-Alzheimer's medications, can be used to measure them. Thiocholine, which is produced from acetylthiocholine during enzymatic hydrolysis, reacts with Ellman's reagent to produce yellow 5-thio-2-nitrobenzoate, which may be measured by spectrophotometry at 412 nm. This is the basis of a commonly used approach. The acetylcholinesterase activity of tissue extracts, homogenates, cell suspensions, etc., can be measured photometrically. By monitoring the rise in yellow color that results from thiocholine's reaction with the dithiobis-(nitrobenzoate) ion, the enzyme activity is determined. It is predicated on these reactions partnering. The assay is sensitive, and the latter reaction happens quickly. Using the Ellman et al. (1961) approach, AChE levels throughout the entire brain were measured to evaluate cholinergic dysfunction. In short, 100 µl of Ellman's reagent (0.5 mM, 19.8 mg DTNB, and 0.1M sodium phosphate, pH 7.2 to make 100 ml) was added to a cuvette containing 2.6 ml of sodium phosphate buffer (0.1 M, pH R = 5.74 (10 7.2) and 0.4 ml of supernatant. The absorbance was then measured in a spectrophotometer at 412 nm until the increasing absorbance stabilized. After setting this steady absorbance to zero, 20 µl of the substrate, acetylthiocholine iodide, was introduced, and for ten minutes, the absorbance variations were recorded. The absorbance change per minute was computed. The rate was calculated by using following formula and AChE activity was measured as µM/l/min/g tissue.

$R = 5.74(10\text{-}4) \times A / Co$

Where, R is rate, in moles substrate hydrolyzed per minutes per g of tissue; A is change in absorbance per minutes; Co is the original concentration of tissue i.e., 20 mg/ml [17].

2.7.3 Glutathione (GSH)

The assay is based on the interaction of GSH with DTNB (also called Ellman's reagent), which results in the oxidized glutathione–TNB adduct (GS–TNB) and TNB chromospheres, which have a peak absorbance at 412 nm. The content of GSH in the sample is directly correlated with the rate at which TNB is formed, as measured at 412 nm. The Sedlak et al. (1974) method was used to measure reduced glutathione. In short, 1.0 ml of sulfosalicylic acid (4%), mixed with 1.0 ml of post-mitochondrial supernatant (10%), precipitated the mixture. After being stored at 4°C for at least an hour, the samples were centrifuged at 1,200 g for 15 minutes at 4°C. The assay combination measured 3.0 milliliters and included 0.1 milliliter of supernatant, 2.7 milliliters of phosphate buffer (0.1 M, pH 7.4), and 0.2 milliliters of 5,5 dithiobis-(2-nitrobenzoic acid) (Ellman's reagent, 0.1 mM, pH 8.0). GSH levels were determined using a molar extinction coefficient of 1.36×104 M−1 cm−1, and the result was reported as micromole per milligram of protein. The yellow color formed was read at 412 nm right away [18].

2.7.4 Lipid peroxidation (LPO)

The measurement of lipid peroxides is a useful and direct indicator of the oxidative state of tissues (membranes) or biosystems that contain polyunsaturated fatty acids. In the study of atherosclerosis, oxidatively changed human serum low density lipoprotein (LDL) has recently attracted more attention. The testing of so-called thiobarbituric acid reactive substances (TBARS) has been widely used to assess lipoproteins for products of lipid peroxidation. This is based on the reaction of malondialdehyde, a break and product of lipid peroxides, with thiobarbituric acid (TBA). Trichloroacetic acid precipitates the lipoprotein in this experiment, and the amount of water-soluble malondialdehyde in the supernatant is calculated [19]. Lipid peroxidation was estimated in terms of thiobarbituric acid reactive species (TBARS). 0.1 ml of the tissue homogenate was added with 2.0 ml of the TCA–TBA– HCl reagent (15% w/v TCA, 0.375% w/v TBA and 0.25 N HCl). The contents were boiled for 15 min, cooled and centrifuged at 1000 rpm for 10 min. The absorbance of clear supernatant was read at 535 nm and malondialdehyde concentration of the sample was calculated using extinction coefficient of 1.56×105/M/cm [20].

2.8 Histopathological Assessment in $AlCl_3$ and MSG induced neurotoxicity model

At the end of the study, all the rats were sacrificed by approved anesthesia and their brains were removed and cleaned in ice

water. An instantaneous 10% buffered neutral formalin solution fixation was performed on a brain segment. Following fixation, tissues were embedded in paraffin, split into serial sections, and then stained for histological analysis using hematoxylin and eosin.

2.9 Statistical analysis

All the data were expressed in means ± SEM. The significance of differences in means between control and treated animals for different parameters was determined by One-way analysis of variance (ANOVA) followed by Dunnett's multiple comparison tests and *$p<0.001$* was considered statistically significant.

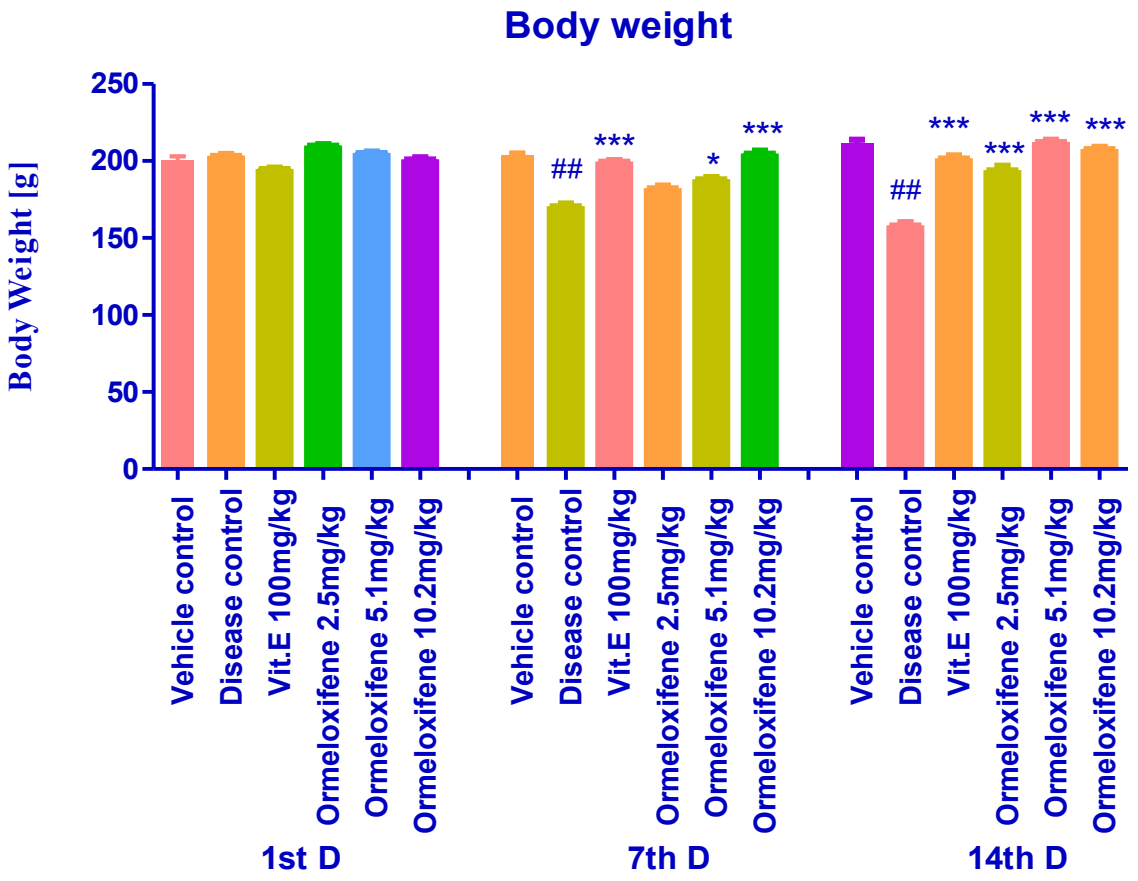
3. Results

3.1 Effect of Ormeloxifene on Neurobehavioral parameters in $AlCl_3$ induced neurotoxicity in rats

3.1.1 Effect of Ormeloxifene on Body Weight

The result of this study revealed the potential neuroprotective activity of Ormeloxifene. A significant *($p<0.001$)* decreased body weight was observed in the control group compared to the normal group. The groups treated with Ormeloxifene 2.5, 5.1 and 10.2mg/kg and vitamin E (100 mg/kg) dose showed a significant *($p<0.001$)* increased body weight compared to the control group. (Fig. 1)

Fig 1. Effect of Ormeloxifene on Body Weight in $AlCl_3$ induced neurotoxicity in rats



All the Values were expressed as mean ± SEM. The statistical significance in the mean is analyzed using one- way ANOVA followed by Tukey's multiple comparison test , *##P<0.03,*P<0.03 **P<0.01, ***P<0.001.*The disease control is compared with vehicle control, standard group and treatment groups.

3.1.2 Effect of Ormeloxifene on Neurological Scoring

The result of this study revealed the potential neuroprotective activity of Ormeloxifene. A significant *($p<0.001$)* decreased neurological scoring (slow gait, abnormal splay and minimal forward motion) was observed in the control group compared to the normal group. The groups treated with Ormeloxifene 2.5, 5.1 and 10.2mg/kg and vitamin E (100mg/kg) dose showed a significant *($p<0.001$)* increased neurological scoring compared to the control group. (Fig 2)

Fig 2. Effect of Ormeloxifene on Neurological scoring in $AlCl_3$ induced neurotoxicity in rats

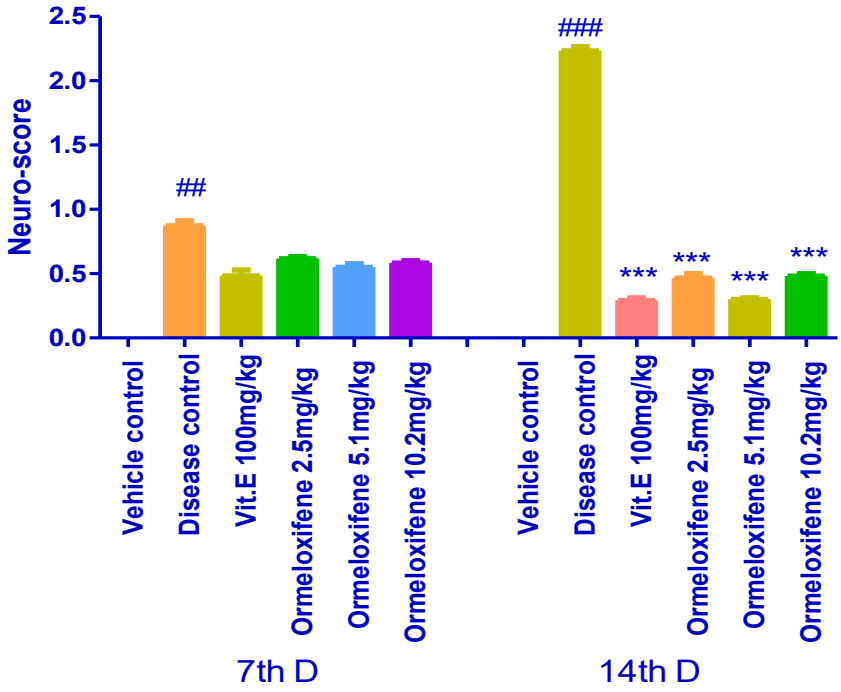


Fig 2. All the Values were expressed as mean ± SEM. The statistical significance in the mean is analyzed using one- way ANOVA followed by Tukey's multiple comparison test , *##P<0.03, ###P<0.001****P<0.001*. The disease control is compared with vehicle control, standard group and treatment groups.

3.1.3 Effect of Ormeloxifene on locomotor activity

The result of this study revealed the potential neuroprotective activity of Ormeloxifene. A significant *(p<0.001)* decreased locomotor activity was observed in the control group compared to the normal group. The groups treated with Ormeloxifene 2.5, 5.1 and 10.2mg/kg and vitamin E (100mg/kg) dose showed a significant *(p<0.001)* increased locomotor activity compared to the control group. (Fig 3)

Fig 3. Effect of Ormeloxifene on locomotor activity in $AlCl_3$ induced neurotoxicity in rats

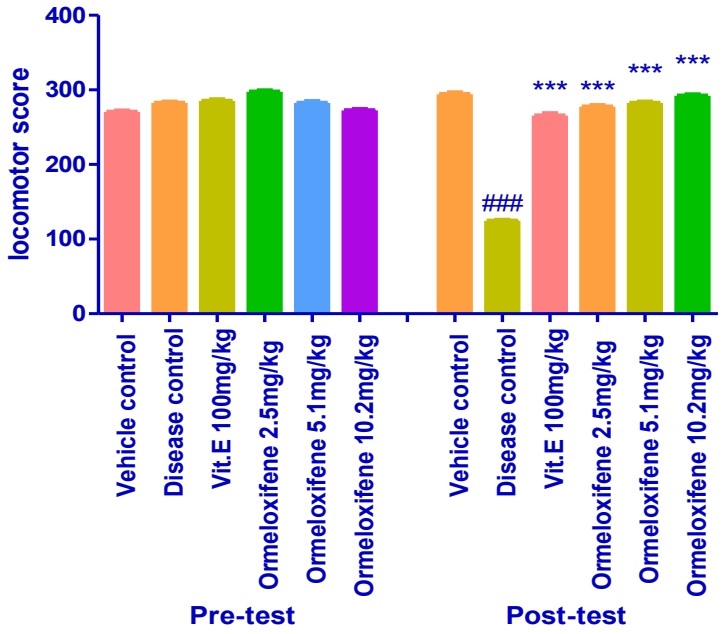


Fig 3. All the Values were expressed as mean ± SEM. The statistical significance in the mean is analyzed using one- way ANOVA followed by Tukey's multiple comparison test, *###P<0.001***P<0.001*. The disease control is compared with vehicle control, standard group and treatment groups

3.1.4 Effect of Ormeloxifene on Grip Strength

The result of this study revealed the potential neuroprotective activity of Ormeloxifene. A significant *(p<0.001)* decreased grip (poor fall of time) was observed in the control group compared to the normal group. The groups treated with Ormeloxifene 2.5, 5.1 and 10.2mg/kg and vitamin E (100mg/kg) dose showed a significant *(p<0.001)* increased grip (improved fall of time) compared to the control group. (Fig 4)

Fig 4. Effect of Ormeloxifene on Grip Strength in $AlCl_3$ induced neurotoxicity in rats

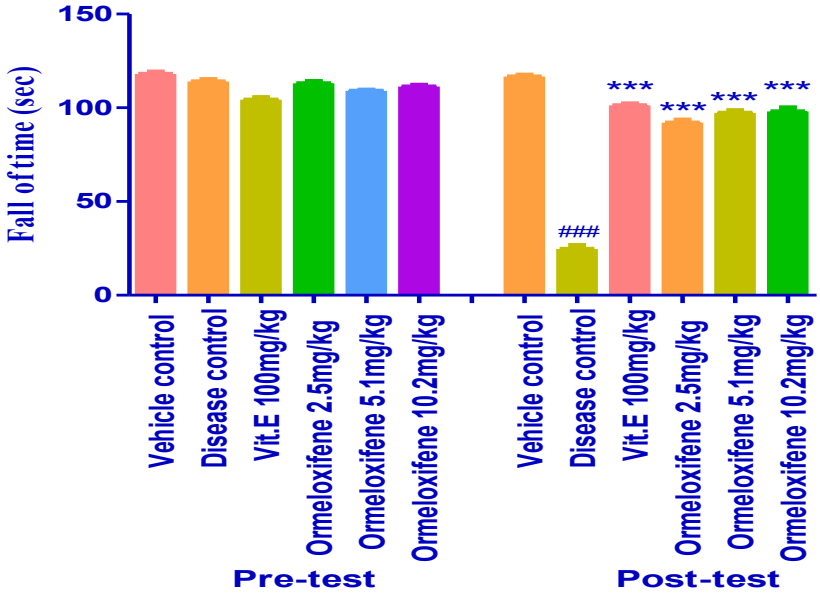


Fig 4: All the Values were expressed as mean ± SEM. The statistical significance in the mean is analyzed using one- way ANOVA followed by Tukey's multiple comparison test, *###P<0.001, *P<0.01***P<0.001*. The disease control is compared with vehicle control, standard group and treatment groups

3.1.5 Effect of Ormeloxifene on spatial memory

The result of this study revealed the potential neuroprotective activity of Ormeloxifene. A significant *(p<0.001)* decreased spatial memory (reduced time spent in novel arm) was observed in the control group compared to the normal group. The groups treated with Ormeloxifene 2.5, 5.1 and 10.2mg/kg and vitamin E (100mg/kg) dose showed a significant *(p<0.001)* increased spatial memory (increased residence time in novel arm) compared to the control group. (Fig 5)

Fig 5. Effect of Ormeloxifene on spatial memory (Y-Maze) in $AlCl_3$ induced rats

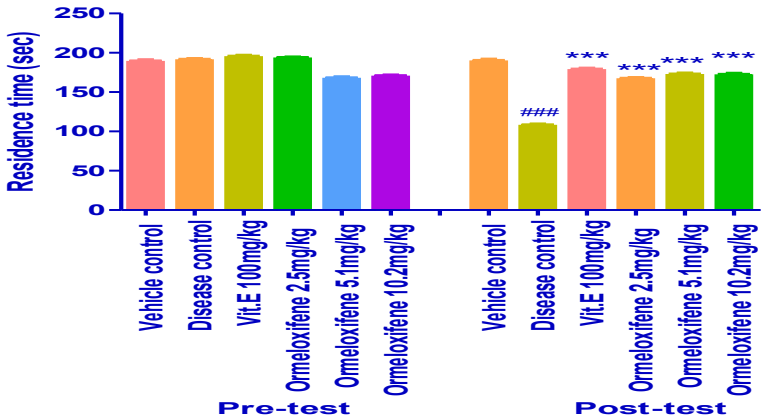


Fig no 5: All the Values were expressed as mean ± SEM. The statistical significance in the mean is analyzed using one- way

ANOVA followed by Tukey's multiple comparison test, *###P<0.001***P<0.001*. The disease control is compared with vehicle control, standard group and treatment groups

3.1.6 Effect of Ormeloxifene on Morri's water maze test in the target quadrant

The result of this study revealed the potential neuroprotective activity of Ormeloxifene. A significant *(p<0.001)* decreased spatial memory (increased escape latency & decreased time spent in target quadrant) was observed in the control group compared to the normal group. The groups treated with Ormeloxifene 2.5, 5.1 and 10.2mg/kg and vitamin E (100mg/kg) dose showed a significant *(p<0.001)* increased spatial memory spatial memory (decreased escape latency & increased time spent in target quadrant) compared to the control group. (Fig 6)

Fig 6. Effect of Ormeloxifene on Morri's water maze test in the target quadrant in $AlCl_3$ induced rats

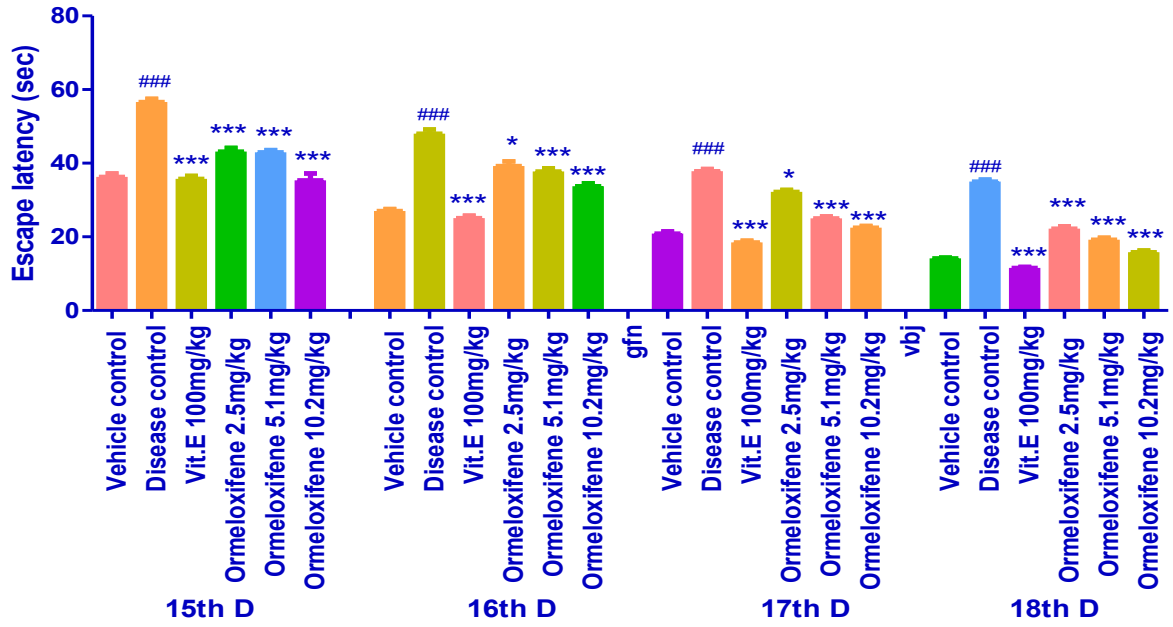


Fig no 6: All the Values were expressed as mean ± SEM. The statistical significance in the mean is analyzed using one- way ANOVA followed by Tukey's multiple comparison test, *###P<0.001***P<0.001*. The disease control is compared with vehicle control, standard group and treatment groups

3.2 Effect of Ormeloxifene on biochemical parameters in $AlCl_3$ induced neurotoxicity in rats

The result of this study revealed the potential neuroprotective activity of Ormeloxifene. A significant *(p<0.001)* increased LPO and acetylcholine Esterase and decreased GSH levels was observed in the control group compared to the normal group. The groups treated with Ormeloxifene 2.5, 5.1 and 10.2mg/kg and vitamin E (100mg/kg) dose showed a significant *(p<0.001)* increased GSH and decreased LPO and acetylcholine Esterase levels compared to the control group. (Fig 7)

Fig 7. Effect of Ormeloxifene on biochemical parameters in $AlCl_3$ induced rats

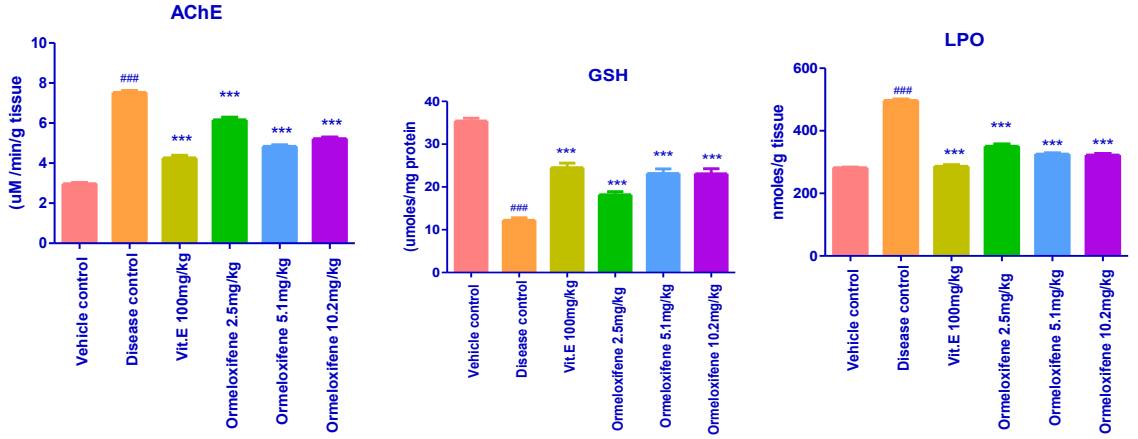


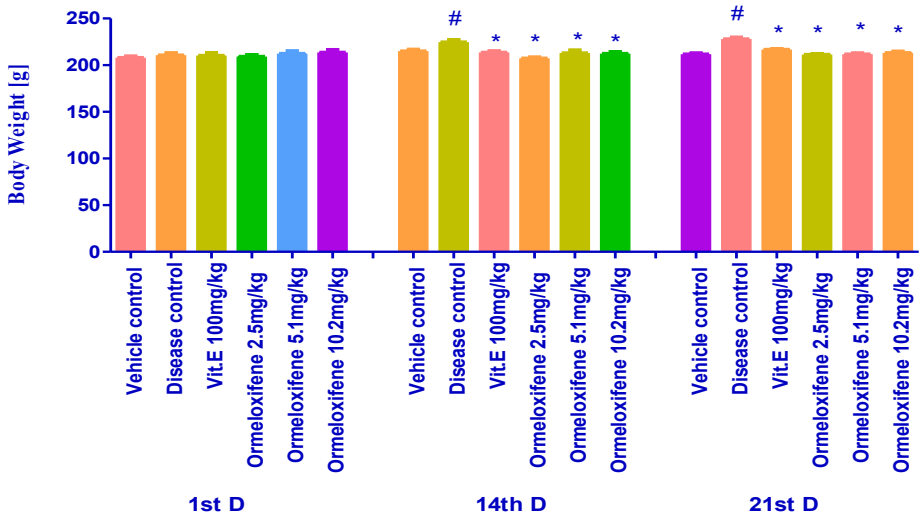
Fig 7. Values were expressed as mean ± SEM. The statistical significance in the mean is analyzed using one-way ANOVA followed by Tukey's multiple comparison test ***$P<0.001$ ###P<0.001. The disease control is compared vehicle control, standard group and treatment groups.

3.3 Effect of Ormeloxifene on Neurobehavioral parameters in MSG induced neurotoxicity in rats

3.3.1 Effect of Ormeloxifene on Body Weight

The result of this study revealed the potential neuroprotective activity of Ormeloxifene. A significant *($p<0.001$)* gradual increased body weight was observed in the control group compared to the normal group. The groups treated with Ormeloxifene 2.5, 5.1 and 10.2mg/kg and vitamin E (100mg/kg) dose showed a significant *($p<0.001$)* slight increased body weight compared to the control group. (Fig 8)

Fig 8. Effect of Ormeloxifene on Body Weight in MSG induced rats



All the Values were expressed as mean ± SEM. The statistical significance in the mean is analyzed using one- way ANOVA followed by Tukey's multiple comparison test , *#P<0.02,*P<0.03*. The disease control is compared with vehicle control, standard group and treatment groups.

3.3.2 Effect of Ormeloxifene on Neurological Scoring

The result of this study revealed the potential neuroprotective activity of Ormeloxifene. A significant *($p<0.001$)* decreased neurological scoring (slow gait, abnormal splay and minimal forward motion) was observed in the control group compared to the normal group. The groups treated with Ormeloxifene 2.5, 5.1 and 10.2mg/kg and vitamin E (100mg/kg) dose showed a significant *($p<0.001$)* increased neurological scoring compared to the control group. (Fig 9)

Fig 9. Effect of Ormeloxifene on Neurological Scoring in MSG induced rats

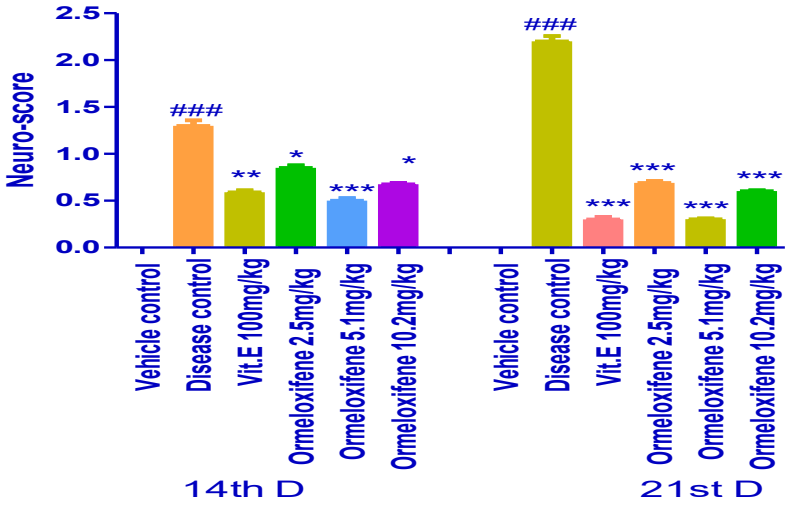


Fig 9. All the Values were expressed as mean ± SEM. The statistical significance in the mean is analyzed using one- way ANOVA followed by Tukey's multiple comparison test , *###P<0.001,*P<0.03, ***P<0.001*. The disease control is compared with vehicle control, standard group and treatment groups

3.3.3 Effect of Ormeloxifene on locomotor activity

The result of this study revealed the potential neuroprotective activity of Ormeloxifene. A significant *(p<0.001)* decreased locomotor activity was observed in the control group compared to the normal group. The groups treated with Ormeloxifene 2.5, 5.1 and 10.2mg/kg and vitamin E (100mg/kg) dose showed a significant *(p<0.001)* increased locomotor activity compared to the control group. (Fig 10)

Fig 10. Effect of Ormeloxifene on locomotor activity in MSG induced rats

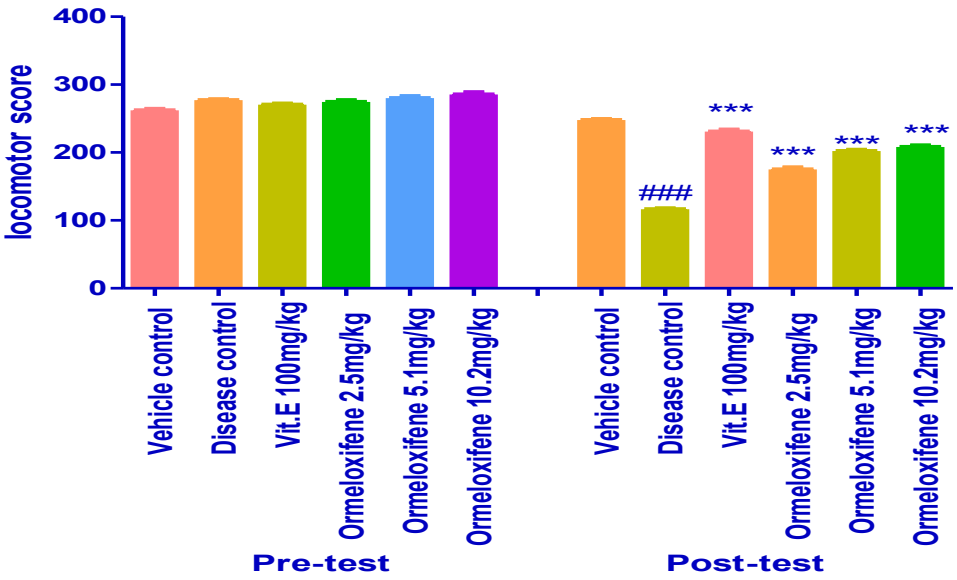


Fig 10. All the Values were expressed as mean ± SEM. The statistical significance in the mean is analyzed using one- way ANOVA followed by Tukey's multiple comparison test , *###P<0.001,***P<0.001*. The disease control is compared with vehicle control, standard group and treatment groups

3.3.4 Effect of Ormeloxifene on Grip Strength

The result of this study revealed the potential neuroprotective activity of Ormeloxifene. A significant *(p<0.001)* decreased grip (poor fall of time) was observed in the control group compared to the normal group. The groups treated with Ormeloxifene 2.5, 5.1 and 10.2mg/kg and vitamin E (100mg/kg) dose showed a significant *(p<0.001)* increased grip (improved fall of time) compared to the control group. (Fig 11)

Fig 11. Effect of Ormeloxifene on Grip Strength in MSG induced rats

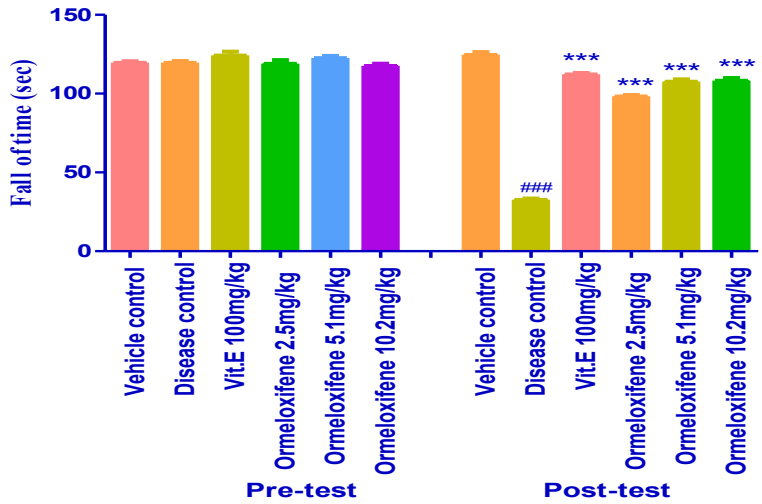


Fig 11. All the Values were expressed as mean ± SEM. The statistical significance in the mean is analyzed using one- way ANOVA followed by Tukey's multiple comparison test, *###P<0.001,***P<0.001*. The disease control is compared with vehicle control, standard group and treatment groups

3.3.5 Effect of Ormeloxifene on spatial memory

The result of this study revealed the potential neuroprotective activity of Ormeloxifene. A significant *(p<0.001)* decreased spatial memory (reduced time spent in novel arm) was observed in the control group compared to the normal group. The groups treated with Ormeloxifene 2.5, 5.1 and 10.2mg/kg and vitamin E (100mg/kg) dose showed a significant *(p<0.001)* increased spatial memory (increased residence time in novel arm) compared to the control group. (Fig 12)

Fig 12. Effect of Ormeloxifene on spatial memory (Y-Maze) in MSG induced rats

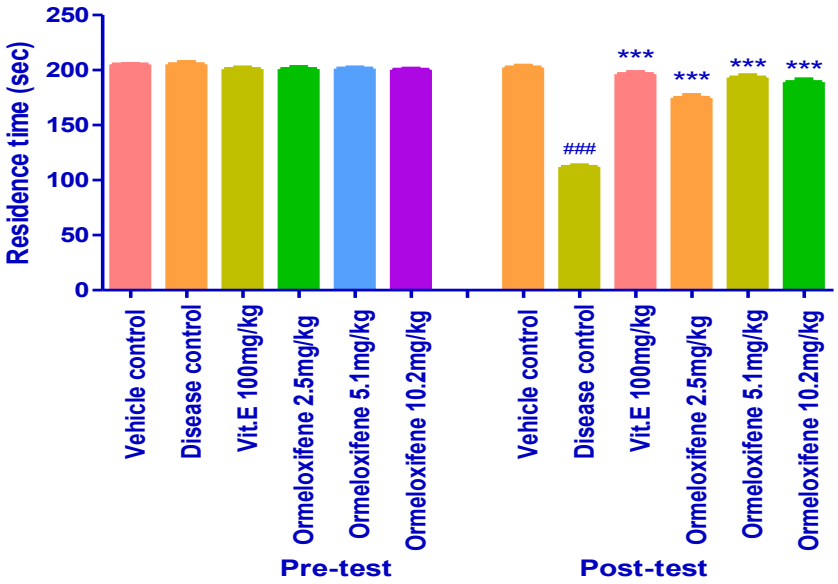


Fig 12. All the Values were expressed as mean ± SEM. The statistical significance in the mean is analyzed using one- way ANOVA followed by Tukey's multiple comparison test, *###P<0.001,***P<0.001*. The disease control is compared with vehicle control, standard group and treatment groups

3.3.6 Effect of Ormeloxifene on Morris water maze test

The result of this study revealed the potential neuroprotective activity of Ormeloxifene. A significant *(p<0.001)* decreased spatial memory (increased escape latency & decreased time spent in target quadrant) was observed in the control group compared to the normal group. The groups treated with Ormeloxifene 2.5, 5.1 and 10.2mg/kg and vitamin E (100mg/kg) dose showed a significant *(p<0.001)* increased spatial memory spatial memory (decreased escape latency & increased time spent in target quadrant) compared to the control group. (Fig 13)

Fig 13. Effect of Ormeloxifene on Morri's water maze test in the target quadrant in MSG induced rats

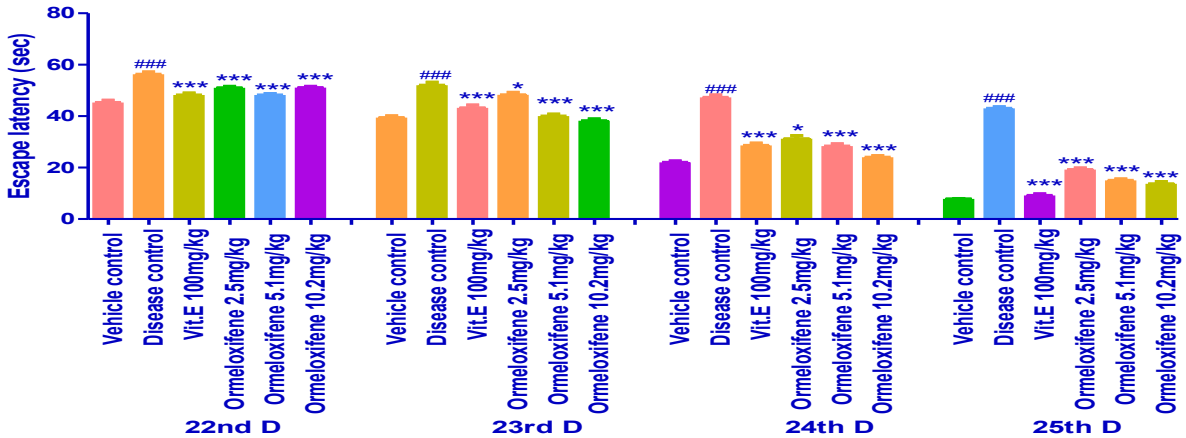


Fig 13. All the Values were expressed as mean ± SEM. The statistical significance in the mean is analyzed using one- way ANOVA followed by Tukey's multiple comparison test, *###P<0.001***P<0.001*. The disease control is compared with vehicle control, standard group and treatment groups

3.4 Effect of Ormeloxifene on biochemical parameters in MSG induced neurotoxicity in rats

The result of this study revealed the potential neuroprotective activity of Ormeloxifene. A significant *(p<0.001)* increased LPO and acetylcholine Esterase and decreased GSH levels was observed in the control group compared to the normal group. The groups treated with Ormeloxifene 2.5, 5.1 and 10.2mg/kg and vitamin E (100mg/kg) dose showed a significant *(p<0.001)* increased GSH and decreased LPO and acetylcholine Esterase levels compared to the control group. (Fig 14)

Fig 14. Effect of Ormeloxifene on biochemical parameters in MSG induced rats

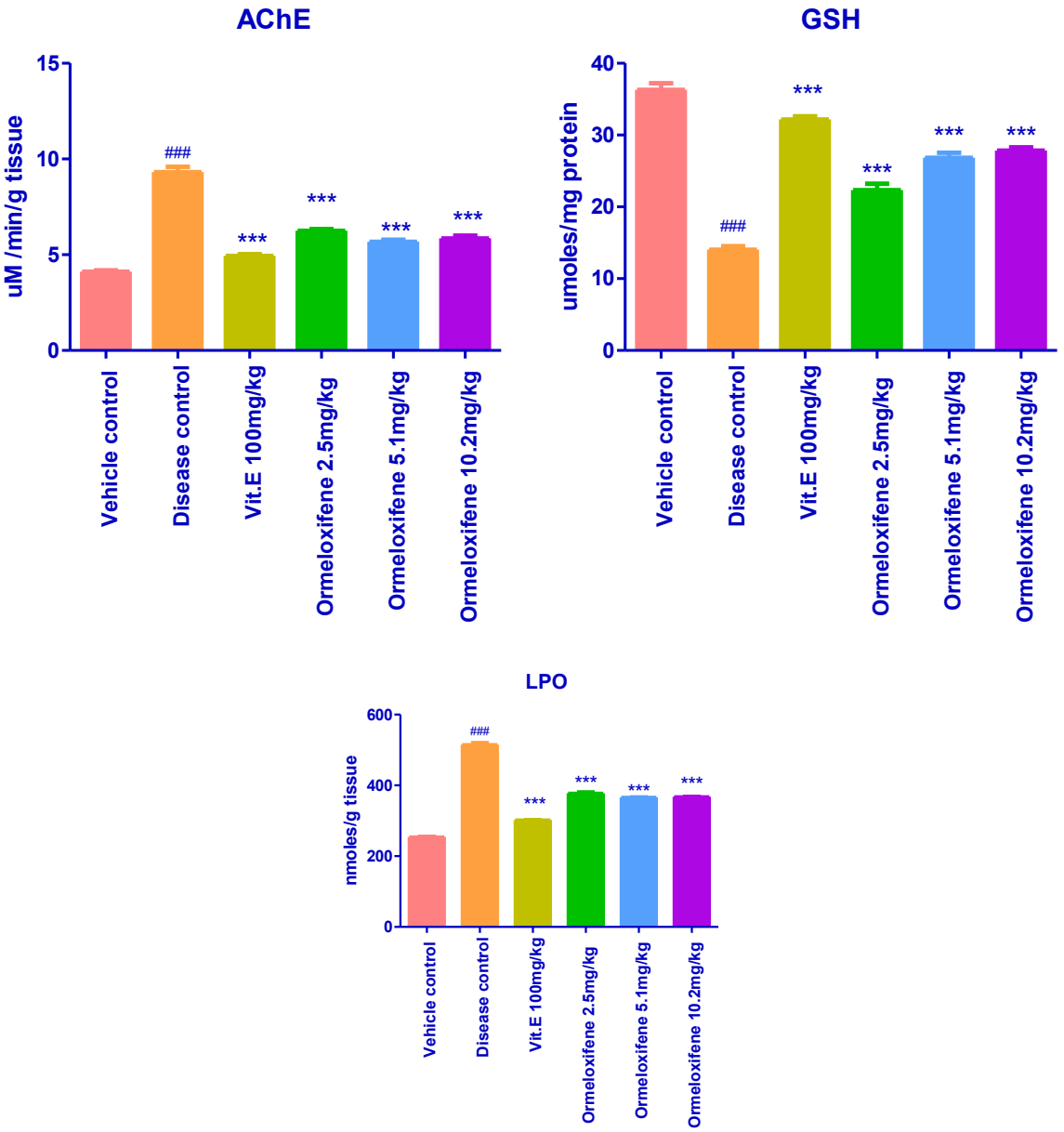


Fig 14. Values were expressed as mean ± SEM. The statistical significance in the mean is analyzed using one-way ANOVA followed by Tukey's multiple comparison test ****P<0.001* ###P<0.001. The disease control is compared vehicle control, standard group and treatment groups.

4. Discussion

$AlCl_3$ has recently been connected to several neurological conditions, including AD, as metals are known to produce neurodegeneration brought on by oxidative stress [21, 22]. After being absorbed via the blood-brain barrier (BBB), $AlCl_3$ builds up in the brain, especially in the hippocampus, which is responsible for memory and learning [23]. Several studies

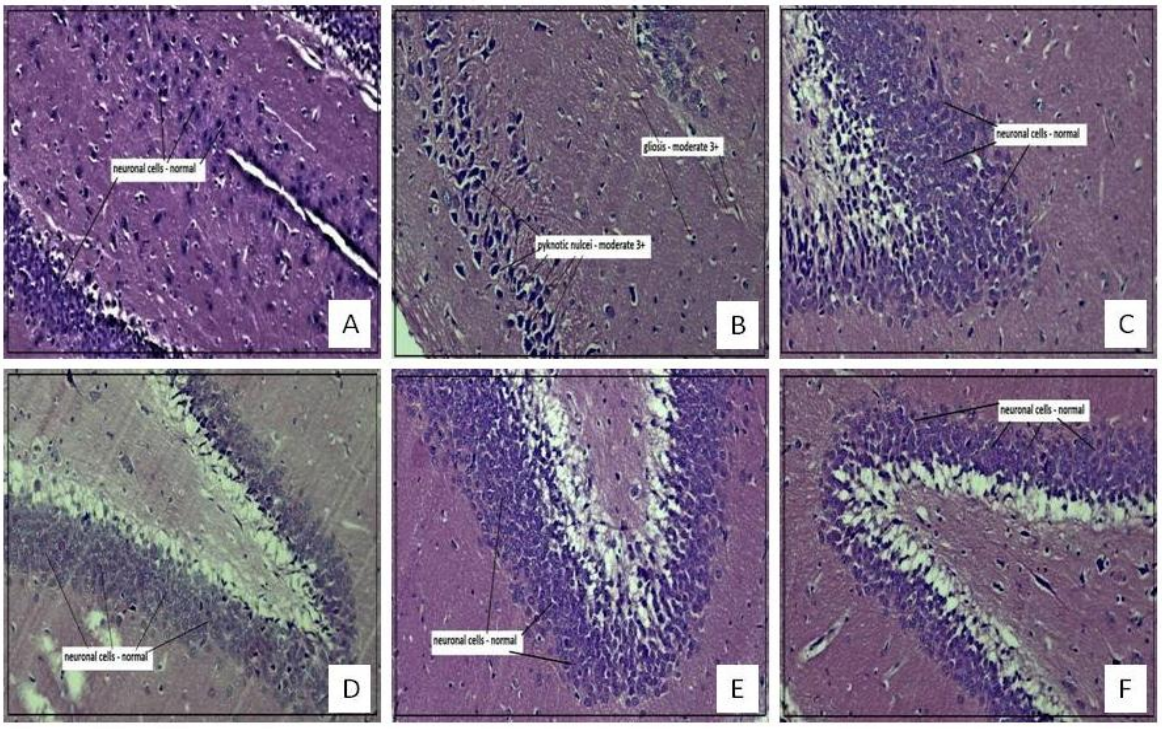
have shown that AD sufferers' brains have higher levels of Al Since there is currently no cure or treatment for AD, elucidating the precise mechanism of $AlCl_3$-induced neurotoxicity is crucial. Accordingly, previous studies on animals demonstrated that exposure to $AlCl_3$ results in neurochemical alterations in the animals, which ultimately lead to memory and learning loss [24, 25]. In this study, $AlCl_3$ administration significantly diminished behavioral parameters such as motor function, grip strength and memory as observed in the reduced performance in the rotarod, Y-maze and Morris's water maze tests, perhaps because to a malfunction in the hippocampal area. Remarkably, Ormeloxifene effectively restored motor function, grip strength and memory. The improved performance in the behavioral tests across all doses indicates that Ormeloxifene may counteract the neurotoxic effects of $AlCl_3$ by enhancing mitochondrial function and reducing oxidative stress suggesting its immense therapeutic potential.

The brain's low antioxidant levels, low mitotic rate, and high oxygen consumption make it especially susceptible to oxidative stress. Excessive oxidative damage from Al poisoning weakens the brain's antioxidant defenses and destroys neurons. [26, 27]. Accordingly, acute cognitive impairment and possibly an early indicator of the development of neurodegenerative diseases are associated with an imbalance between oxidative stress and antioxidant defenses [28]. In this study, Al administration induced oxidative stress, indicated by increased LPO levels, AchE activity and decreased secretion of antioxidant enzyme such as GSH due to mitochondrial dysfunction and neurodegeneration. Nevertheless, the rats treated with ormeloxifene shown significant decrease in AchE activity and lipid peroxidation (LPO) levels, along with an increase in glutathione (GSH) levels, which signifies improved antioxidant capacity. Since oxidative damage is linked to mitochondrial malfunction and neurodegeneration, reducing oxidative stress is essential to reducing the neuronal damage caused by $AlCl_3$. Numerous herbal compounds have been shown to inhibit oxidative stress by the antioxidant mechanism in $AlCl_3$ induced neurotoxicity in rats [29]. Hence, this data further supports the antioxidant effect of ormeloxifene as a neuroprotective agent against $AlCl_3$ induced rat model.

The memory impairment in neurodegenerative disorders is linked to failure in cholinergic transmission which affects memory & learning. The BBB can be altered by Al, a potent cholinotoxin, to mimic changes in cholinergic transmission [30]. AChE and transmembrane protein activity significantly increased in rats given $AlCl_3$ in the current study. In accordance with earlier research, ormeloxifene treatment of rats intoxicated with $AlCl_3$ decreased the activity of transmembrane proteins and AChE to have the ability to inhibit AChE [31]. This suggests that ormeloxifene provides neuroprotection by inhibiting AChE activity.

Histopathological studies reveals that rats treated with $AlCl_3$ showed severe neuronal damage, (Fig.15)

Fig 15. Effect of Ormeloxifene on Histopathological changes in $AlCl_3$ induced neurotoxicity in rats



The histopathological analysis of brain sections across different groups reveals distinct outcomes. In the normal group (Plate 1), neuroglial cells exhibit healthy architecture with no signs of damage. The control group (Plate 2) shows severe neurodegeneration caused by $AlCl_3$, including gliosis, necrosis, and apoptosis, particularly in the cortex and hippocampus. The treatment group (Plate 3) demonstrates significant recovery, with mostly normal neuronal and glial cells, though mild degenerative changes persist. Plates D, E, and F show

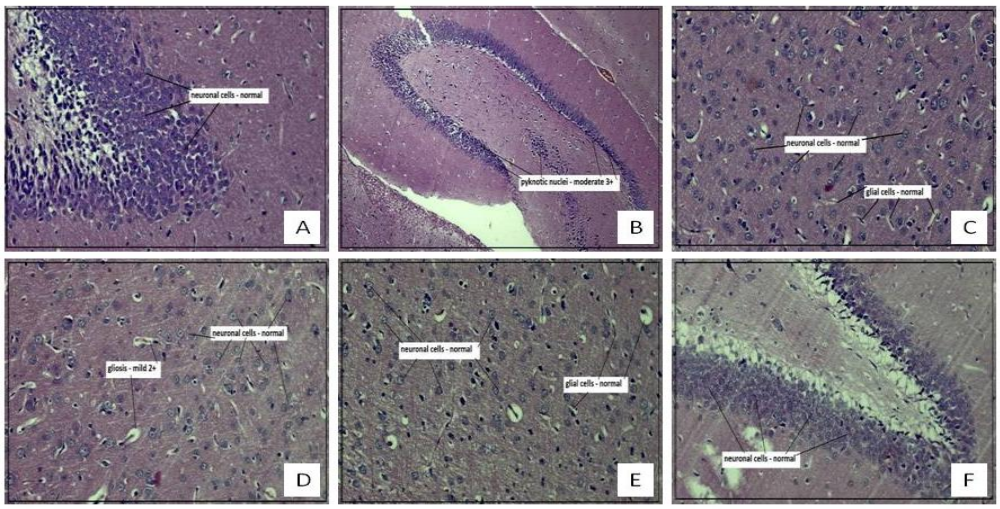
varying recovery stages, with Plate D showing mild gliosis, while Plates E and F display near-normal brain architecture.

including gliosis and apoptosis. In contrast, ormeloxifene treatment reduced these effects, with only mild neurodegenerative changes, suggesting a neuroprotective role. This effect is likely due to ormeloxifene ability to stabilize neuronal membranes, lower oxidative stress and modulate neuroinflammation. However, more investigation is required to ascertain the precise mechanism of action.

The current study's findings demonstrate that MSG administration causes excitotoxicity and cognitive deficit, as evidenced by hypoactivity manifested in markedly reduced motor function, grip strength, and memory as demonstrated by decreased performance on the rotarod, Y-maze, and Morri's water maze tests. These results were in line with that of [32, 33], who demonstrated that MSG has an excitotoxic effect on the brain with significant long-term damage of many brain areas, especially if administered in early life. In addition, Hassan et al. [34], reported that MSG intake in early life induced neurotoxicity that impairs the short-term memory and affects the exploratory behavior in rats. When MSG dissolves in water, it separates into sodium and glutamate ions, which raises plasma glutamate levels up to 17 times higher than the typical basal level and may be the cause of these excitotoxic effects [33]. Additionally, administering MSG raises the concentration of glutamate in the extracellular brain, which causes changes in motor and behavioral function [35]. This is because glutamate reacts with its receptors, causing neuronal cells to undergo apoptosis and necrosis through over-activation of glutamate receptors. Glutamate receptors promote the release of $Ca2+$ from their reserves, which over-activates the mitochondria and triggers several intracellular enzymes, including phospholipases, endonucleases, and proteases, which harm cell membranes, cytoskeletons, and DNA [36]. A further research showed that MSG administration results in a cognitive loss in rats with hypoactivity and irritability due to a lack of short-term and long-term potentiation, poor synaptic plasticity, and triggered hippocampus degradation [37]. It is also possible that MSG's excitotoxic effects stem from the oxidative stress it induces in the hippocampus, as this is a characteristic of neurodegenerative illnesses. This is demonstrated by the increased levels of antioxidant enzymes in brain regions. Due to the brain's limited antioxidant capacity and rapid metabolic activity, it can readily impact the brain. Released free radicals cause peroxidation of cell membranes and DNA, causing cell damage and apoptosis [38]. The presence of these free radicals activates signaling pathways of inflammation and cell damage and may cause disruption of the blood–brain barrier by affecting the endothelial basement membrane [33]. Another factor contributing to excitotoxicity is the production of free radicals. When glutamate receptors are stimulated by calcium influx, free radicals are produced from mitochondria [39].

On contrast, the results of the current study indicated that administration of ormeloxifene also demonstrated a protective effect against MSG-induced neurotoxicity. Rats treated with ormeloxifene showed significant improvements in locomotor activity, grip strength and memory retention, comparable to the effects seen in the $AlCl_3$ model. The reduced escape latency in the Morris water maze and improved time spent in the target quadrant during the probe trial suggest that ormeloxifene can effectively enhance spatial memory in MSG-treated rats. Biochemical analysis further confirmed the protective effects of ormeloxifene in the MSG model. The AchE activity was significantly reduced in ormeloxifene-treated groups, along with an increase in GSH levels, indicating a reduction in oxidative stress. Additionally, LPO levels were decreased, which correlates with reduced lipid membrane damage and neuronal preservation. These findings suggest that ormeloxifene can attenuate MSG-induced excitotoxicity and oxidative damage by stabilizing cellular structures and promoting antioxidant defenses. Histopathological results also supported the neuroprotective role of ormeloxifene, with the treated rats showing only mild gliosis and inflammation compared to the severe congestion and pyknotic nuclei observed in disease control animals (Fig. 16).

Fig 16. Effect of Ormeloxifene on Histopathological changes in MSG induced neurotoxicity in rats



The histopathological analysis of brain sections across different groups reveals distinct outcomes. Plate A displayed intact neuroglial and neuronal cells with normal vesicular nuclei and cytoplasm. In Plate B, MSG-induced neurotoxicity caused moderate gliosis, apoptosis, and severe congestion in the hippocampal region, with pyknotic nuclei and dense inflammation. Plate C showed mostly normal glial and neuronal cells with slight inflammation. Plate D revealed normal neurons with scattered cells, mild inflammation, and mild gliosis, indicating early degeneration. Both Plates E and F exhibited normal neuroglial and neuronal cells, with no significant damage or degeneration.

This indicates that ormeloxifene may have potential therapeutic value in protecting against glutamate-induced excitotoxic damage and reducing neuroinflammation.

Oxidative stress is a key contributor to neurodegenerative diseases, as mimicked by the $AlCl_3$ and MSG models in this study. Ormeloxifene' s ability to reduce oxidative stress markers, enhance antioxidant defenses, and improve mitochondrial function positions it as a promising neuroprotective candidate. The study suggests that oxidative stress is not only a consequence but also a driver of neurodegeneration. Ormeloxifene' s protective effects on motor and cognitive functions highlight its potential to target early neurodegenerative processes, preserving neuronal integrity and function.

5. Conclusion

In conclusion, ormeloxifene demonstrates remarkable neuroprotective potential against neurotoxicity induced by both aluminum chloride ($AlCl_3$) and monosodium glutamate (MSG) in rats. The results highlight ormeloxifene' s capacity to significantly reduce oxidative stress, inhibit excitotoxic damage, and attenuate neuroinflammation, leading to notable improvements in motor and cognitive functions. These protective effects are corroborated by both biochemical and histopathological evidence, positioning ormeloxifene as a promising therapeutic candidate for neurodegenerative disorders where oxidative stress and excitotoxicity are pivotal contributors. Further exploration of its underlying mechanisms and clinical relevance could unveil new pathways for neuroprotection and broaden its applicability in the treatment of such conditions.

Abbreviations

AchE – Acetylcholinesterase

$AlCl_3$ – Aluminium Chloride

AD – Alzheimer's Disease

GSH - Glutathione

LDL – Low Density Lipoprotein

LPO – Lipid Peroxidation

MSG – Monosodium Glutamate

PD – Parkinson's Disease

Declarations:

Ethics Approval and Consent to participate

A consent was obtained from the Institutional Animal Ethical Committee approved the experiment protocol by the number GCP/IAEC/DOP/2022-2023/80

Consent for Publication

Not applicable

Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available but are available from the corresponding author on reasonable request.

Funding

Not Applicable

Competing Interest

Not Applicable.

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