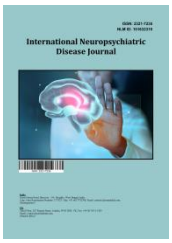

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Unravelling Tamoxifen's Neuroprotective Effects against Manganese-Induced Neurodegeneration, Cognitive Impairment and Hippocampal Damage Via the Nrf-2 Pathway in Adult Male Wistar Rats

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Authors' contributions

This work was carried out in collaboration among all authors. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Neurodegenerative diseases pose a serious threat to public health. Manganese (Mn) is a metal necessary for biological systems; however, it can become hazardous at high concentrations, causing neurological damage. This study explores tamoxifen's potential to protect against Mn-induced neurotoxicity in adult male Wistar rats.

Methodology: Forty (40) adult male Wistar Rats weighing between 170g-220g were used and divided into four groups (A-D) (n=10). Manganese (Mn) and Tamoxifen (TMX) dosages were prepared and administered daily for 45 days through oral gavage. Group A received normal saline as a placebo; Group B received 200 mg/kgbwt of Mn only; Group C received 100 mg/kgbwt of TMX and 200 mg/kgbwt of Mn; Group D received 100 mg/kgbwt of TMX only. At the end of the experiment, behavioral tests were carried out, and animals were sacrificed; the brain was then excised, cleaned, and washed with saline for analysis.

Results: After the 45 days of treatment the Mn-only group exhibited clear signs of neurobehavioral toxicity. On the other hand, the TMX-treated rats exhibited significantly improved exploratory behavior and locomotor activity indicating its effect in reverting Mn neurotoxicity. The histological assessments further revealed that TMX preserved hippocampal morphology, reducing glial cell distortion and vacuolation caused by Mn exposure, while the immunohistochemical analysis showed activated Nrf-2 expression in TMX-treated groups.

Conclusion: These results suggest that TMX has significant neuroprotective effects and could be a potential therapeutic agent for neurodegenerative diseases.

Keywords: Tamoxifen; manganese; alzheimer's disease; neurodegenerative diseases; neurotoxicity; Hippocampus.

1. INTRODUCTION

One of the leading causes of aging-related cognitive decline in people is dementia, which almost always results in total reliance on caregivers. Globally, there are already approximately 55 million dementia patients, and by 2050, that number is predicted to quadruple [1]. "The countries with the lowest socio-demographic index (SDI) are anticipated to have the most considerable percentage change in the overall number of dementia cases between 2019 and 2050. The increase in dementia cases across the board is mostly due to aging and population growth" [2].

"Diseases that impact the central and peripheral nervous systems are referred to as neurological disorders (NDDs). Numerous conditions, including nervous system damage, ischemia, oxidative and ER cellular stress, inflammation, aberrant protein deposition in neural tissue, autoimmune-mediated neuronal loss, and viral or prion infections, can result in NDDs" [3,4]. "Alzheimer's disease (AD), Parkinson's disease (PD), amyotrophic lateral sclerosis, diminished movement ability and attention problems, memory loss or personality changes, and weakness and cognitive decline are some of the characteristics of NDDs of the brain. With high disability-adjusted life years (DALY)—a measurement of the number of years of healthy life lost owing to illness—NDDs exhibit a significant impact on quality of life" [5,6]. "NDDs are second only to headache disorders in terms of prevalence, and as life expectancies rise and the population grows, so too will the incidence of NDDs like PD and AD globally" [7]. "The current NDD treatments provide momentary symptom alleviation, as a result, there is a great need to find new treatments and neuroprotective substances to stop or slow the development of NDDs" [8].

Manganese (Mn) is a naturally occurring strong metal currently the fifth most common metal and the twelfth most abundant element in its entirety [9]. Manganese (Mn) is involved in several biological procedures, with significant positions in glucose regulation. For several enzymes, including superoxide dismutase (SOD) and glutamine synthetase (GS), Mn is a significant co-factor in the central nervous system (CNS) [10]. Excessive exposure to Mn, however, is neurotoxic, leading to a neurodegenerative disease that affects cortical constructions and basal ganglia, particularly globuspallidus, striatum, and substantianigra pars reticulata [11,12]. The largest metal concentrations are found in legumes, rice, nuts and whole fruits. Mn can also be discovered in seafood, seeds, chocolate, tea, green leafy vegetables, spices,

soybeans, and some fruits like pineapple and acai [13]. Cytotoxicity connected with increased Mn dose has been reported to contribute to dystonic-related neurological disturbance, similar to those frequently found in idiopathic Parkinson's illness (PD) [14,15]. Childhood Mn intake is also correlated with parenteral nutrition, frequently accompanied by increased concentrations of Mn [16]. Several transport mechanisms, including divalent iron transporter 1 (DMT-1) and transferrin/transferrin binding scheme (Tf / TfR), regulate Mn concentration in the CNS. It remains to be determined, however, which processes control Mn flow during development [17].

Tamoxifen is a derivative of triphenylethylene, a first-generation SERM and non-steroidal analog of estradiol [18]. Tamoxifen is primarily used as a breast cancer treatment [19], although several studies have also demonstrated tamoxifen's beneficial effects on the brain [20, 21]. It was also used for the Albright syndrome and according to recent research, tamoxifen may pass the blood-brain barrier and cause a reaction in the brain comparable to that of estradiol [22]. The idea that tamoxifen affects the central nervous system is supported by a number of research studies conducted so far [23, 24]. It has been determined how tamoxifen affects a number of brain disorders, such as traumatic brain injury, cerebral ischemia, cerebrovascular stroke, subarachnoid hemorrhage, and SCI [25, 26].

“Tamoxifen's protective effect on mitochondrial activity may account for some of the drug's effects on these disorders” [27], it was also reported to reduces reactive astrocytes after brain injury [28]. “Previous studies have reported distinct routes and mechanisms of action by which tamoxifen exerts its neuroprotective action on various diseases. Serious side effects include a tiny increase in uterine cancer danger, depression, issues with sight, and pulmonary embolism. Common side effects include times of irregularity, weight reduction, and hot flashes” [29].

The range of potential pathways through which tamoxifen may exhibit neuroprotective properties has been steadily increasing and it offers a potential therapy option due to its advantageous impact on maintaining brain function, which has been shown both in vivo and in vitro of different brain diseases. This also ensures that the substance will not be harmful when used to treat certain diseases, the application and elucidation of the compound's mechanisms of action, along with the timely identification of aspects pertaining to its metabolism and degradation, are required [30-32]. Hence, this paper focuses on the Neuroprotective efficacy of tamoxifen on manganese-induced neurodegenerative disease in Wistar rats.

2. MATERIALS AND METHODS

2.1 Chemicals

Tamoxifen and Manganese were procured from Sigma Chemical Corporation (St. Louis, MO, USA)/ Sigma-Aldrich, Hamburg, Germany, and were dissolved in distilled water. All other chemicals used in the study were of analytical grade.

2.2 Animals and Experimental Design

Forty (40) adult male Wistar rats (between 170g-220g) were purchased from the breeding stock of the Faculty of Agriculture and Agricultural Technology, Federal University of Technology, Akure. The rats were housed in the Laboratory Animal house, Department of Human Anatomy, Federal University of Technology, Akure. They were maintained with constant 12 h/12 h dark and light cycle at room temperature (22 ± 3 ºC) throughout the study. Full aeration was enhanced by wire guaze cage properly partitioned into four chambers and roomy enough to allow for proper ventilation and free movement within it. The floor of the cage was lined with carpet pieces and sprayed with coarse sawdust which served as a cushion. The rats were fed with growers’ marsh (pellets), purchased from a feed store at Agro feeds, FUTA, Akure, and water ad libitum and acclimatized for 7 days.

2.2.1 Experimental design

The animals were divided into four groups (n= 10). Mn dosage and TMX dosage (100mg) were prepared freshly each day for administration. The groups were as follows:

Group A (control) received normal saline as a placebo.

Group B received 200mg/kg Body Weight (BW) of Mn only.

Group C received 100mg/kg BW of TMX and 200mg/kg BW of Mn

Group D received 100mg/kg BW of TMX only.

All administration was done via oral gavage once daily and the experiment spanned 45 days. At the end of the experiment, behavioral experiments were carried out, animals were sacrificed through cervical dislocation. The brain was excised, cleaned, and washed with saline (0.9% sodium chloride).

2.3 Behavioral Parameters

"Prior to the commencement of the experiment, the rats underwent neurobehavioral training using Morris Water Maze (MWM), Open field test (OFT), and Y maze tests for spatial memory and working memory and Open field test" [33,34].

2.3.1 Morris water maze test

"This test was carried out to assess the spatial learning and memory of the rats. A pool of water measuring about 100 cm in diameter and 30 cm in depth was used. An escape platform about an inch deep from the surface of the water was placed in one of the quadrants outside of which was a visual cue. The animals were trained 3 times before the commencement of the experiment. During the training, each rat was placed in each of the other three quadrants for a maximum period of 60 seconds to find the escape platform at intervals of 25 minutes between quadrants until the escape latency period was reduced to less than 25 seconds. During the test, the pool was colored, and the animals were placed in each of the three quadrants different from the escape platform quadrant at an interval of 25 minutes between quadrants. The time taken to find the escape platform was recorded as the escape latency period" [24].

2.3.2 Y-maze

"The rats underwent neurobehavioral training 3 times after the acclimatization period before the commencement of the experiment and behavioral testing at the end of the last administration. The Y-maze was carried out in a quiet dimly lit room between the hours of 9 am and 3 pm. All behavioral tests were videotaped and later scored by an independent observer who was unaware of the experimental protocol. The animals were placed in a Y-maze whose arms measured 75 cm in length and 15 cm in breadth with an angle of 120° between the arms" [24]. The rats are placed on a predetermined start arm and were allowed to explore the maze for duration of 5 minutes. Arm entry (hind limbs completely in the arm) is scored. The percentage of spontaneous alternation is calculated as: [correct alternation/ (total number of arm entries – 2)] × 100.

2.3.3 Open Field Test (OFT)

The open field apparatus was constructed using plywood measuring 100 cm by 100 cm and a height of 50 cm. The floor was divided into square grids each measuring 25 cm in length with a blue marker and a center square of the same length was drawn using a red marker. Each rat was picked by the tail and dropped in the center square and allowed to explore for 5 minutes while the video was captured by a camera from above the apparatus. The two behavioral patterns assessed are the number of lines crossed and rearing frequency. The number of lines crossed was the frequency with which the rat crossed one of the grid lines with all four paws; the rearing frequency was the number of times the rat stood on its hind limbs.

2.4 Tissue Collection and Processing

"After the behavioral tests were concluded, the rats were subjected to cervical dislocation, and the brain tissues were immediately excised and dissected into two hemispheres. All the right hemispheres were fixed in 4% paraformaldehyde for histological processing, while the left hemispheres were rinsed three times in 0.25 M sucrose for five minutes and stored in 30% sucrose at 4°C. Paraffin wax sections were obtained for histological analysis. The hippocampus was excised from the fixed brain and dehydrated in ascending grades of alcohol (50%, 70%, 90%, and 100%). The tissues were then cleared in xylene twice for 15 min each. Infiltration and embedding were done with paraffin wax in a Leica hot air oven at 56°C with tissues eventually embedded in paraffin wax at similar orientations. Tissue sections were obtained serially using a rotary microtome (Leica RM2245) and then mounted on glass slides. Sections were taken at 30 µm for the Hematoxylin and Eosin staining process using the method of Pearse. The slides were analyzed using a Leica®DM5000B microscope and photographed with a Leica EC3 digital camera" [77].

2.5 Hematoxylin and Eosin Staining

Brain sections were stained using the method of Pearse (1975) as modified by Fischer et al.

(2008). This method was carried out to demonstrate the general cytoarchitecture of the prefrontal cortex. “Slides containing paraffin sections were placed in a slide holder and deparaffinization and rehydration of sections were done in the following reagents respectively: thrice for 3 minutes in xylene, thrice for 3 minutes 1:1 xylene with 100% ethanol, once for 3 minutes in 95% ethanol, once in 80% ethanol for 3 minutes and then once in deionized H2O for 5 minutes, while excess water was blotted from the slide holder before taking them into hematoxylin. Subsequently, hematoxylin staining was done with the following procedure: once for 3 minutes with hematoxylin, once in tap water for 5 minutes (to allow the stain to develop); slides were dipped 12 times in acid ethanol, rinsed twice with tap water for 1 minute, then rinsed once in deionized water for 2 minutes and left overnight. Excess water was blotted from the slide holder before putting into eosin” [77].

For eosin staining and dehydration, the following procedures were followed: slides were placed once for 30 seconds in eosin (up to 45 seconds for older batches of eosin) then thrice for 5 minutes in 95% ethanol, followed by thrice for 5 minutes in 100% ethanol (excess ethanol was blotted before putting into xylene) and then thrice for 15 minutes in xylene. Following these, the slides were covered and slipped using a distrene plasticizer in xylene (DPX) as mountant (one drop of DPX was placed on the slide using a glass rod). Coverslips were angled to let them fall gently on the slide. Thereafter, the slides were dried overnight in the hood.

2.5.1 Congo red staining technique

Congo red dye was used to stain for amyloid plaque in the brain sections by adopting the method of Pearse [35] as modified by Fischer et al. [36]. The slides were analyzed using a Leica®DM5000B microscope, and photomicrographs were captured with a Leica EC3 digital camera.

2.5.2 Immunoperoxidase technique for astrocyte expression

Trypsin (catalog ID ab970, Abcam, Cambridge, MA, USA) was used in an enzymatic antigen retrieval approach, with the pH adjusted to 7.8 using 1 M NaOH and held at 4°C to create the working solution (0.05%). Hydrogen peroxide suppressed endogenous peroxidase, and 5% bovine serum albumin (BSA) was used to reduce unintended protein reactions. The diluted primary antibody (anti-glial fibrillary acidic protein) was applied to each slide and incubated at 4°C overnight. After washing, the slides were incubated with a secondary antibody and color was intensified using 3,3-diaminobenzidine [37] The slides were then dehydrated, cleaned in ethanol and xylene, counterstained with hematoxylin, and mounted with DPX. Following behavioral tests, the rats were sacrificed, and their brains were removed. The left hemispheres were stored in 30% sucrose, while the right hemispheres were fixed in 4% paraformaldehyde for histological processing. Sections were dehydrated, cleaned, embedded in paraffin wax, and cut into serial pieces for examination using a Leica EC3 digital camera and Leica®DM5000B microscope.

2.5.3 Immunohistochemical analysis on nrf-2 expression

The tissue was deparaffinized and rehydrated, immersed in xylene for 10 min, excess liquid was removed, and then dipped in anhydrous ethanol for 3 min. The slides were immersed in 85% ethanol for 3 min rinsed in de-ionized water, and then rinsed in PBS buffer for 3 min. After deparaffinization and rehydration, antigen retrieval was done, and 1× ethylenediaminetetraacetic acid antigen repair was added to tissues after which slides were removed and rinsed with PBS for 3 min (Protocol for immunohistochemistry kit).

2.5.4 Image analysis and cell count

The sections were photographed with a digital brightfield microscope. Non-overlapping micrographs were created at x400 magnification and used in Image J program for image analysis (NIH, USA). GFAP reactive cells were counted using the Image J program's Cell Counter function to assess positive immunoreactive cells, whilst Nrf2 immunoreactivity was quantified using the ImmunoRatio function, which separates and measures the percentage of DAB (positive immunoreactivity) using digital color deconvolution.

2.6 Biochemical Analysis

2.6.1 Superoxide dismutase (SOD) assay

“The brain tissues were placed in 0.25 M sucrose solution and homogenized. Tissue homogenate was collected in a 5 ml sample bottle and centrifuged at 3,000 rpm for 15 minutes using a

centrifuge (Model 90-1; Jiangsu Jinyi Instrument Tech, Jiangsu, China). The supernatant was collected with Pasteur pipettes into sample bottles and placed in a freezer at –4°C. SOD was using the spectrophotometric technique" [38]. "The reaction mixture (3 ml) contained 2.95 ml carbonate buffer, 0.02 ml of homogenate, and 0.03 ml of 2 mM SOD substrate in 0.005 N HCl, used to initiate the reaction. The reference cuvette contained 2.95 ml buffer, 0.03 ml of substrate, and 0.02 ml of water. The absorbance was read at regular intervals of 1 minute for 5 minutes at 480 nm. Values are expressed in U/mg of protein" [77].

2.6.2 Catalase (CAT) assay

CAT activity was analyzed using the protocols of Clairborne [39], in a solution containing 50mM phosphate buffer, 19mM hydrogen peroxide and tissue homogenates. The reaction was ended by the addition of dichromate/ acetic acid solution. Values are expressed as µmole of H2O2 consumed/mg protein/min.

2.6.3 Reduced Glutathione Level (GSH)

GSH was assayed using the protocols of Jollow et al. [40], in a solution containing tissue homogenates, 4% sulfosalicylic acid, and subsequently DTNB. Values are expressed in nmol/mg of protein.

2.6.4 Lipid Peroxidation (LPO) level

LPO was quantified as Malondialdehyde (MDA), using the protocols of Farombi et al. [41]. The reaction contained tissue homogenates, 5% (w/v) butylated hydroxytoluene (BHT), 10% TCA, and 0.75% TBA in 0.1 mol/L of HCl. MDA was calculated by using the following equation: R¼1.56_105 L/mol/cm, where R is the extinction coefficient. Values are expressed in nmol/mg of protein or U/mg protein.

2.6.5 Determination of Nitric Oxide (NO) level (nitrite)

Nitric oxide measured as nitrite was determined according to the method of Moshage et al. [42]. Briefly, 2 ml of 10 mM of sodium nitroprusside is prepared in 0.5 ml of phosphate buffer saline (pH 7.4). Next, 0.5 ml of sample extract is added and incubated at 25°C. After 150 min of incubation, 0.5 ml of Griess reagent (1% sulfanilamide, 2% H3PO4, and 0.1% naphthyl ethylenediamine dihydrochloride) is added to 0.5 ml of incubated solution. The reaction mixture is re-incubated for 30 min at room temperature. The rate of absorbance is measured at 546 nm, and the inhibition percentage is calculated. NO inhibition= [(A546 Control−A546 Sample)/A546 Control] ×100. Values are expressed in µM/g.

2.6.6 Brain monoamine neurotransmitter analysis

Monoamine neurotransmitter levels (DA, 5-HT, and NE) were analyzed by weighing wet tissue and homogenizing it in 5 mL HCl-butanol for about a minute. The sample was centrifuged at 2000 rpm for 10 minutes, and 1 mL of the supernatant was mixed with heptane and HCl, then shaken vigorously and centrifuged again to separate the phases. The aqueous phase was used for the neurotransmitter assays, with all steps performed at 0°C [43].

For dopamine and norepinephrine: the aqueous phase was mixed with HCl, EDTA/sodium acetate buffer, iodine solution, and Na2SO3, then heated to 100°C before reading the spectra on a spectrofluorometer.

For serotonin: the aqueous extract was treated with OPT reagent, heated to 100°C, and then analyzed in the spectrofluorometer (model Jasco-FP-6500, Japan).

2.7 Statistical Analysis

Statistical analysis was performed using Graph Prism® software (version 3.05 for Windows, GraphPad Software, San Diego California, USA) by one-way analysis of variance (ANOVA) with Tukey's multiple comparisons test. The data were reported as means ± SEM, while differences between means at $p < 0.05$ were considered significant.

3. RESULTS

3.1 Effect of Tamoxifen on the Behavioral Parameters in Mn-Induced Neurodegeneration in Normal and Experimental Rats

The animal were tested in the Morris Water Maze (MWM), Open field and Y-Maze tests to assess learning and memory (both short- and long-term). The results revealed that the rats in the TMX-treated group (group D) had the highest number of rearing per five minutes (Fig. 1(C)), only slightly higher than the control group (group A), but significantly higher ($p < 0.05$) than that of the

Mn only group (group B) and the combination group of TMX + Mn (group C). The Mn-treated group (group B) had the lowest number, however, with the combination-treated group (group C) exhibiting a significantly higher number ($p < 0.05$). The control and TMX-only groups (groups A and D) exhibited a similar number of lines crossed, significantly higher than the other groups. The negative control group (group B) had the least number of lines crossed (Fig. 1(D)), significantly lower ($p < 0.05$) than the combination group (group C). (Fig. 1(A)) of the data shows that the Mn-treated group had the most extended escape latency length ($p < 0.05$), which was much higher than that of the control group; when compared to the control and combination-treated groups (Mn + TMX), the TMX-treated group showed the shortest length of escape latency ($p < 0.05$) (Fig. 1(A)).

3.2 Effects of TMX on brain Antioxidant enzymes (GSH, SOD, and CAT) on Mn-Treated Rats

The evaluation of antioxidant levels in this study revealed that group B receiving Mn treatment had the lowest expression of antioxidant enzymes, catalase (CAT), superoxide dismutase (SOD), and glutathione (GSH), while group D receiving only TMX had the highest expression (Fig. 2). The control group (group A) had almost similar antioxidant enzyme levels with the TMX-only group (group D) and was only slightly lower. In contrast, the rat group to which a combination of Mn and TMX (group C) was administered had a significantly higher expression ($p < 0.05$) of antioxidant enzymes in comparison with the Mn-only group of rats (Fig. 2).

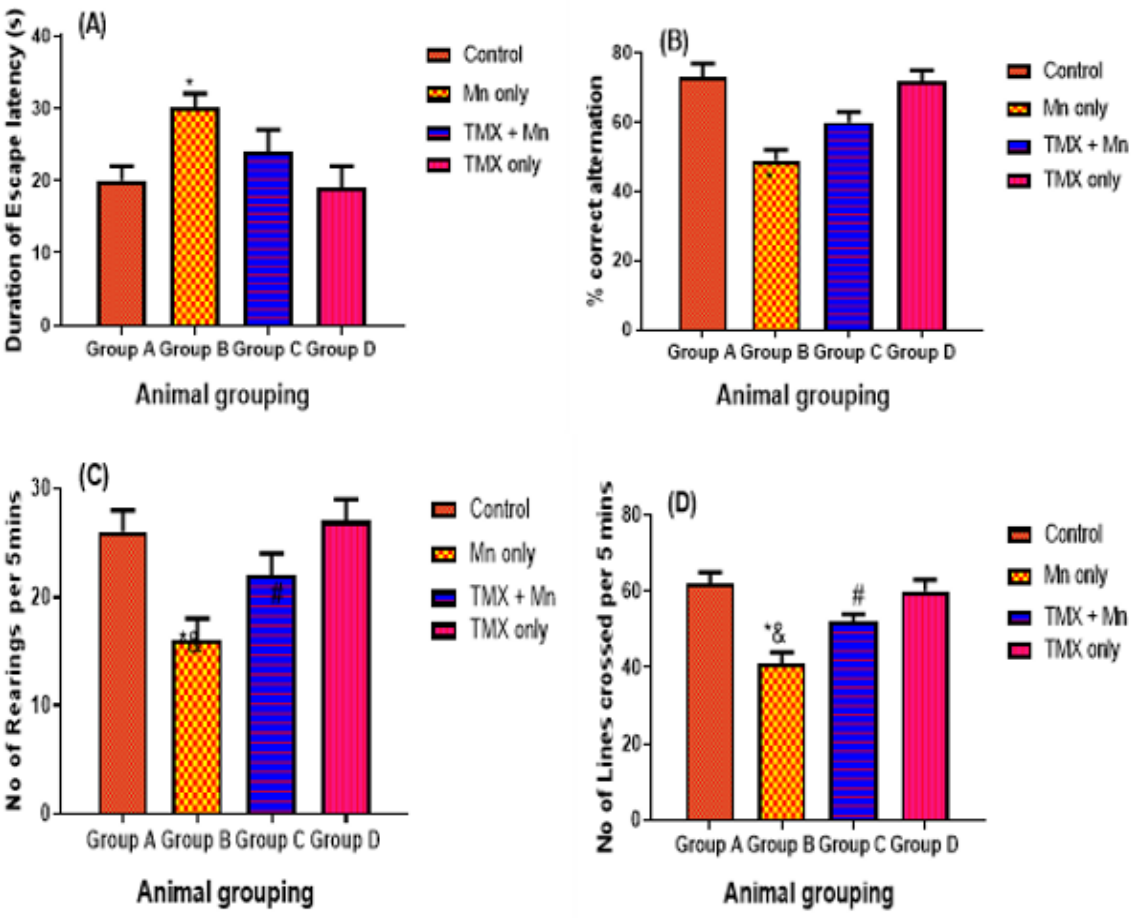

Fig. 1. Tamoxifen effects on Neurobehavioral stress tests in Mn-induced neurotoxicity in normal and treated rats

*Values expressed as Mean ± SEM, n=8; *Significantly different from the normal control group (Group A). #Significantly different from normal and Mn controls (Groups A and B) (P < 0.05), One–way ANOVA*
**: p < 0.05 as compared to group A and D; #: p < 0.05 as compared to group A and D*

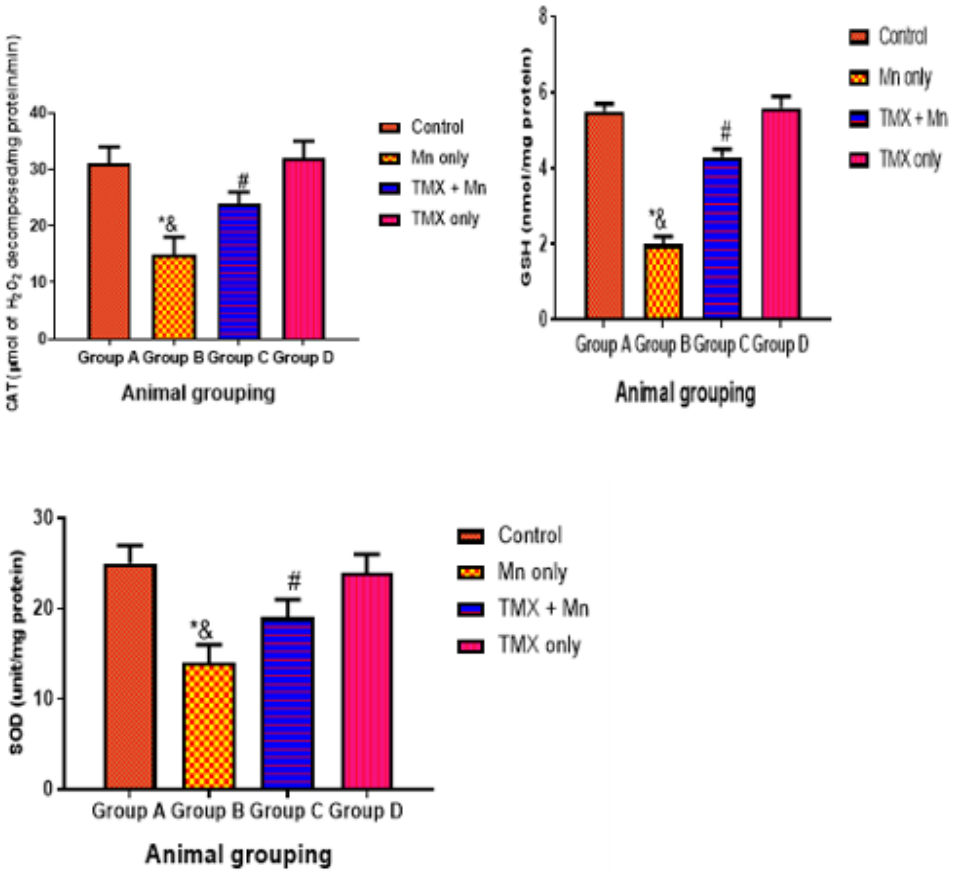

Fig. 2. Tamoxifen effects on brain Antioxidant enzymes in Mn-induced neurotoxicity in normal and treated rats

*Values expressed as Mean ± SEM, n=8; *Significantly different from the normal control group (Group A). #Significantly different from normal and Mn controls (Groups A and B) (P < 0.05), One–way ANOVA*
**: p < 0.05 as compared to group A and D; &: p < 0.05 as compared to group C; #: p < 0.05 as compared to groups A and D*

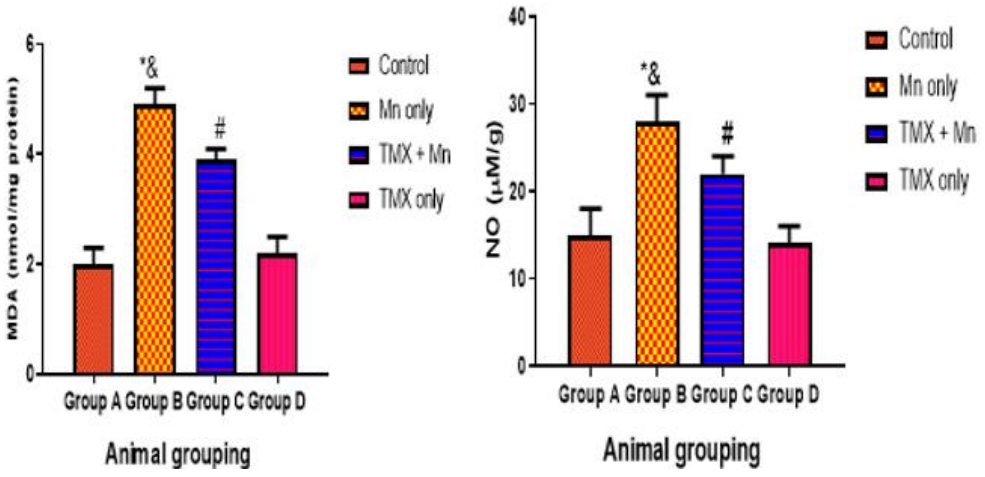

Fig. 3. Tamoxifen effects on brain oxidative markers in Mn-induced neurotoxicity in normal and treated rats

*Values are expressed as Mean ± SEM, n=8; *Significantly different from the normal control group (Group A). #Significantly different from normal and Mn controls (Groups A and B) (P < 0.05), One–way ANOVA*
**: p < 0.05 as compared to group A and D; &: p < 0.05 as compared to group C; #: p < 0.05 as compared to groups A and D*

3.3 Effects of TMX on Brain Oxidative Markers (MDA and NO) on Mn Treated Rats

The results of the evaluation of oxidative stress markers malondialdehyde (MDA) and nitrous oxide (NO) showed that their levels were highest in the group of rats to which only Mn was administered (group B) and lowest in the control (group A) and TMX only (group D) treated groups which had almost similar levels (Fig. 3). Compared to the control and TMX-only groups, the group that received a combination of TMX and Mn treatment (group C) displayed notably elevated levels of oxidative stress indicators ($p < 0.05$). However, it was significantly lower ($p < 0.05$) than the Mn-only treated group (Fig. 3(A&B)).

3.4 Effects of Tamoxifen on brain Monoamine Neurotransmitters in Mn-Treated Rats

The results of the evaluation of monoamine neurotransmitter (dopamine, norepinephrine, and serotonin) levels showed that the group to which Mn-only was administered (group B) had the lowest levels of serotonin and dopamine. The control group (group A) had the highest levels of serotonin and dopamine, almost similar to but slightly higher than the levels exhibited in the TMX-only group (group D) (Fig. 4). The group to which a combination of Mn and TMX was administered (group C) showed significantly lower serotonin and dopamine levels ($p < 0.05$) in comparison with the TMX-only (group D) and control groups, but the neurotransmitter levels were higher than the Mn only treated group (group B) (Fig. 4). There was, however, a significant increase in norepinephrine levels in the Mn-treated group in comparison with the other groups (Fig. 4). The control group exhibited the least norepinephrine level, with the level in the TMX-only group being only slightly higher (Fig. 4).

3.5 Effects of Tamoxifen on Hippocampal Morphology of Mn-Treated Rats

The results of the histological assessment showed normal hippocampal morphology with numerous glial cells in the control group (group A) (Fig. 5 & 6 (A)); few pyramidal cells with distorted glial cells between the inner pyramidal layer and outer marginal layer and vacuolated neuropils in the rat group treated with Mn only (group B) (Fig. 5 & 6 (B)); in the group to which a combination of TMX and Mn was administered (group C), there was preserved proliferation of glial cells interspersed between the inner pyramidal layer and outer marginal layer and a lesser occurrence of vacuolated neuropils than the negative control group (Fig. 5 & 6(C)); in the group to which TMX only was administered (group D), there was normal orientation of glial cells similar to the control (Fig. 5 & 6(D)).

Fig. 6(A) further shows the normal orientation of neuronal cells in the control group; Fig. 6(B) the neuronal degeneration of the Mn group with amyloid plaques deposited; Fig. 6(C) the normal histology and neuronal cell orientation with reduced amyloid plaques of the combination group receiving Mn and TMX; and Fig. 6(D) shows the neuronal cell orientation of the TMX only group similar to that of the control group.

3.6 Immunohistochemical Assessment of Tamoxifen effects on the Hippocampus of Mn-Treated Rats (nrf-2)

The results of the immunohistochemical evaluation in this study demonstrated that the hippocampus (Fig. 7) from the rat group that received only Mn group was where Nrf-2 was mildly expressed and the number of viable neuronal cells, indicating that Mn notably damaged and reduced cells in the hippocampal regions of the rat brains. The other group exhibited significantly less Nrf-2 immunoreactivity in comparison with the control (group A) and TMX-treated groups (group B), with the Mn group exhibiting the most miniature expressions in both the hippocampal region (Fig. 7). The control rat group exhibited normal cell orientation and reduced levels of beta-amyloid deposition, the same as all the other rat groups except the Mn group.

The results further showed that in the rat group treated with Mn only, there was increased beta-amyloid plaque formation and decreased Nrf2 expression with the quantity of viable neuronal cells, in the hippocampus (Fig. 7(B)), with this group showing the highest levels. All other groups showed lower levels of beta-amyloid deposition and normal cell orientation in the control group (Fig. 7(A)); which indicates that Mn severely damaged and decreased the number of cells in the hippocampus regions of the rat brains.

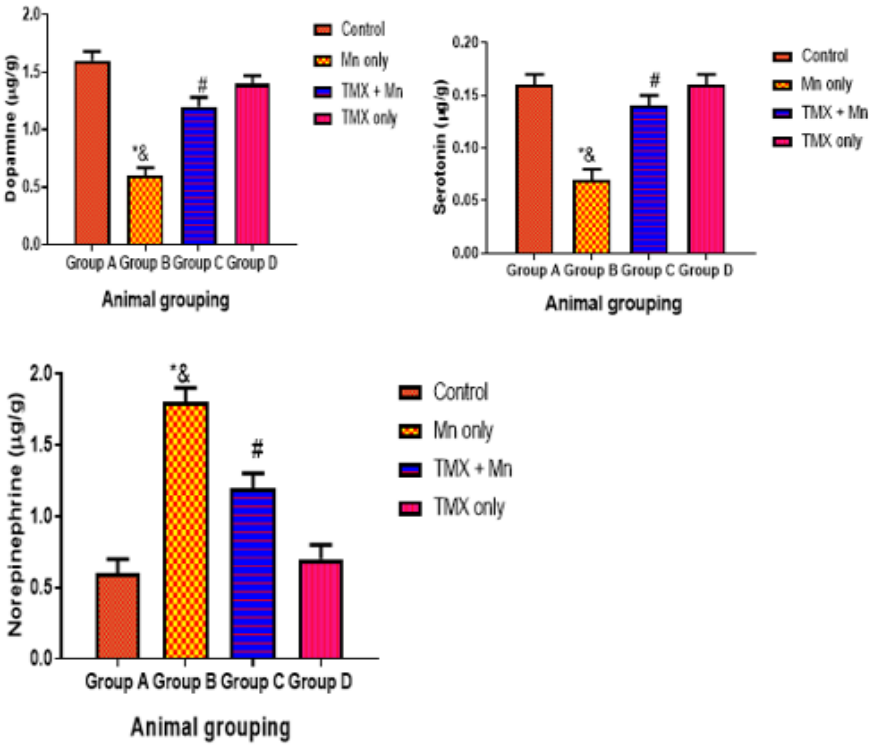

Fig. 4. Tamoxifen effects on brain monoamine neurotransmitters in Mn-induced neurotoxicity in normal and treated rats

*Values expressed as Mean ± SEM, n=8; *Significantly different from the normal control group (Group A). #Significantly different from normal and Mn controls (Groups A and B) (P < 0.05), One–way ANOVA*
**: p < 0.05 as compared to group A and D; &: p < 0.05 as compared to group C; #: p < 0.05 as compared to groups A and D*

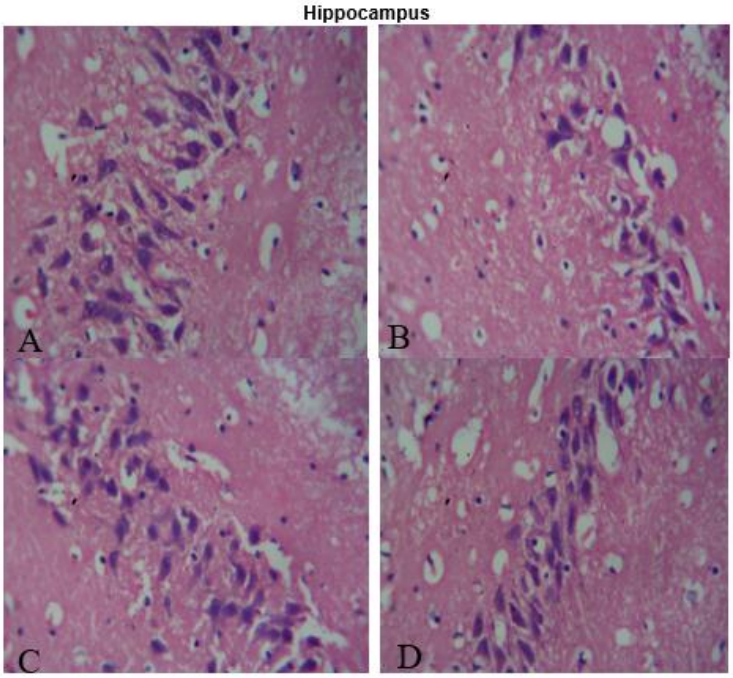

Fig. 5. Tamoxifen effects on hippocampal morphological parameters (H & E) Hippocampus

Stains: H&E, Magnification: X400, scale bar=50µm

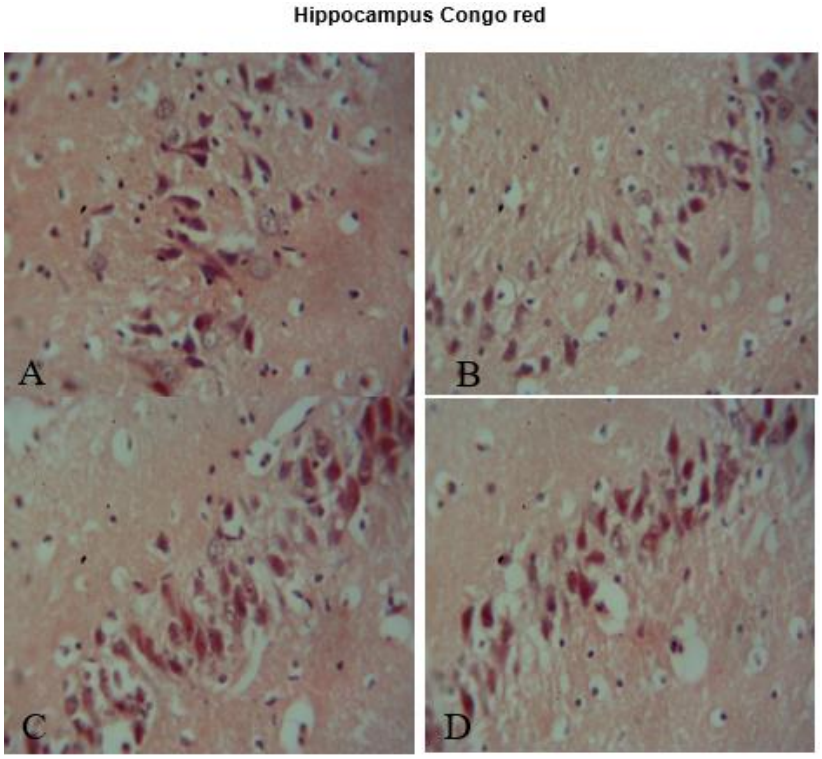

Fig. 6. Tamoxifen effects on the hippocampal morphological parameters (Congo Red)
Stains: H&E, Magnification: X400, scale bar=50µm

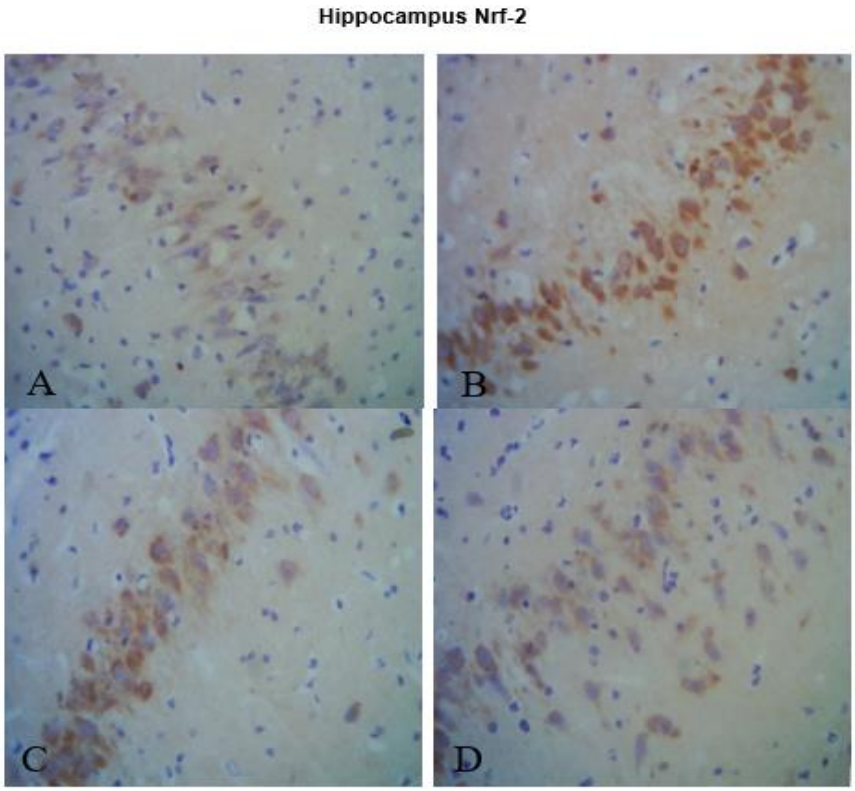

Fig. 7. Tamoxifen effects on NRF-2 positive cells Expression in the Hippocampus of Mn-treated Rats

4. DISCUSSION

Novel neuroprotective medicines are desperately needed for the rapidly increasing number of people with neurodegenerative diseases (NDD) [43]. This current study evaluated the neuroprotective efficacy of tamoxifen (TMX) on the neurobehavioral, histology, biochemical, immunohistochemical, and histological alterations in mitigating manganese (Mn)-induced neurodegenerative effects in adult male Wistar Rats. In line with earlier studies on TMX's antioxidative and neuroprotective properties, the results demonstrate important insights into the possible therapeutic effects of TMX in neurodegenerative disorders [45,46].

Exposure to Mn levels beyond the recommended daily consumption (2.3 mg for males and 1.8 mg for women) may cause manganism, an illness characterized by degeneration of the dopaminergic system and a complicated behavioral syndrome involving difficulties with motor function [47]. This study results showed that in comparison with the control group, oral treatment of Mn in the experimental groups caused neurodegeneration in the hippocampus of the treated rats. In the Mn group only, there were few deformed glial cells, indicating that the oral injection of Mn may have affected the rats' brains. This is consistent with the growing evidence that Mn is a neurotoxin that affects hereditary dystonia/parkinsonism and environmental exposure [48]. Moreover, Mn-treated rats demonstrated reduced latencies compared to control groups when their motor coordination was evaluated, supporting Mn-induced decreased motor activity [49]. These findings are in line with earlier studies that have been published in the literature [50], which demonstrate the preferential Mn buildup in basal ganglia in a number of rodents and nonhuman monkey models.

Learning and working memory were substantially impaired, as evidenced by the Y-maze test and open-field data. The open field test was used to compute the escape latency duration to evaluate spatial learning and memory, and the Y-maze was used to estimate percent correct alternation in order to examine working and cognitive memory [51]. We observed that the Y-Maze and Open Field Test results shows that treatment with TMX improves exploratory and locomotor activities. Specifically, the TMX-treated group showed the highest number of rearings, and lines crossed, comparable to the control group, and significantly higher than the Mn-only group. This suggests that TMX can potentially reverse Mn-induced deficits in motor functions and locomotor activity. These findings are consistent with previous studies that have demonstrated the protective effects of TMX on motor and cognitive functions in various models of neurodegeneration [52,53]. The Y-Maze Test results further showed that TMX-treated rats showed improved correct alternation percentages, suggesting better spatial memory and cognitive function [54].

The genesis of various NDDs is linked to several variables; nonetheless, an important feature that all NDDs have in common is the increase in brain oxidative stress [55]. Previous studies have demonstrated the important role of antioxidant enzymes in protecting neurons from oxidative stress. Reducing cellular damage, SOD catalyzes the dismutation of superoxide radicals into oxygen and hydrogen peroxide, which is subsequently broken down into water and oxygen by CAT [56]. Major intracellular antioxidant GSH keeps the redox equilibrium of cells in check by neutralizing reactive oxygen species (ROS). Major intracellular antioxidant GSH ensures that the balance of redox status of the cells is maintained through the elimination of reactive oxygen species (ROS) [57]. The overexpression of several enzymes highlights TMX's potential as a neuroprotective drug against Mn-induced oxidative stress. The antioxidant enzyme analysis showed that TMX significantly increased the levels of CAT, SOD, and GSH in the brain. Consequently, the TMX-only group exhibited the highest levels of these enzymes, followed by the combination-treated group, with the Mn-only group showing the lowest levels. Thus, treatment with TMX can potentially exert a strong antioxidative effect, potentially neutralizing oxidative stress. This is in line with previous research highlighting the role of TMX in enhancing antioxidant defenses and reducing oxidative damage in neurodegenerative disease models [58,59].

Oxidative stress plays a pivotal role in the pathogenesis of neurodegenerative diseases, including those induced by manganese (Mn) toxicity. The Mn-induced neurobehavioral alterations were associated with increased oxidative stress. The evaluation of lipid peroxidation and oxidative stress markers [60], malondialdehyde (MDA), and nitrous oxide (NO), further supports the antioxidative role of TMX. The Mn-only treated group exhibited the highest levels of these markers, indicating severe

oxidative stress. In contrast, the TMX-treated groups showed significantly lower levels of MDA and NO, comparable to the control group. This reduction in oxidative markers is following a previous study that also reported that TMX can effectively attenuate oxidative stress, a critical factor in neurodegenerative processes [61,62]. MDA a byproduct of polyunsaturated fatty acid peroxidation, serves as a reliable marker for oxidative stress [63] and elevated MDA levels are associated with neuronal damage and neurodegenerative conditions. Similarly, NO, a critical signaling molecule, can contribute to neurotoxicity when produced in excess, leading to the formation of peroxynitrite, a potent oxidant [64]. This ability of TMX to lower MDA and NO levels further indicates its efficacy in protecting neuronal integrity and function [65]. TMX has been further reported to modulate oxidative stress pathways, enhancing the expression of antioxidant enzymes and reducing ROS production [66].

We observed through the analysis of monoamine neurotransmitters that TMX-treated rats had higher levels of dopamine and serotonin compared to Mn-only treated rats, while norepinephrine levels were significantly lower. This is consistent with the report that TMX may help maintain neurotransmitter balance, which is crucial for normal cognitive and motor functions [67]. These results are also consistent with the previous findings that TMX can modulate neurotransmitter systems and improve neuropsychiatric symptoms [68,69]. The preservation of dopamine and serotonin levels by TMX could explain the improved neurobehavioral outcomes observed in this study [70].

Chronic neuroinflammation is a defining feature of neurodegenerative illnesses, and it has been proposed that Nrf2 is a crucial regulator that the brain needs to protect against inflammation [71]. According to several studies [72], Nrf2 has emerged as a significant therapeutic target of NDs because of its important role in shielding neurons and eventually preserving the life of injured neurons [73]. This present study showed the neuroprotective effects of TMX observed align with its known antioxidative properties. It has further been shown to activate the nuclear factor erythroid 2–related factor 2 (Nrf2) pathway, a key regulator of cellular antioxidant responses [74]. The upregulation of Nrf2 and subsequent increase in antioxidant enzyme expression could explain the enhanced antioxidative capacity observed in TMX- significant neuroprotection against Mn-induced oxidative damage. This is in line with studies that have shown Nrf2 plays a critical role in maintaining cellular redox homeostasis by regulating treated rats. From this study TMX, by modulating Nrf2 and antioxidant enzyme activity, offers the expression of antioxidant and detoxification enzymes [75]. The immunohistochemical analysis further revealed activated Nrf-2 expression in TMX-treated rats, indicating reduced oxidative stress response activation, which correlates with the improved antioxidant enzyme activity observed [76]. This further supports the antioxidative and neuroprotective effects of TMX, as Nrf-2 plays a key role in cellular defense against oxidative stress.

5. CONCLUSION

Our findings in this work demonstrate that Tamoxifen, a therapeutic drug, can be used to treat neurological problems, as it improved behavioral outcomes, enhanced antioxidant defenses, reduced oxidative stress, restored neurotransmitter balance, preserved hippocampal morphology, and modulated stress response markers in our study. These findings highlight the potential of TMX as a therapeutic agent against Mn-induced neurodegenerative changes under in vivo conditions using multiple mechanisms in an animal model. However, as future studies will depend heavily on the development of even more specialized drugs, further research is required to identify additional markers associated with Mn-induced neurotoxicity and the possible modulatory role of tamoxifen.

DISCLAIMER (ARTIFICIAL INTELLIGENCE)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc) and text-to-image generators have been used during writing or editing of this manuscript.

CONSENT

It is not applicable.

ETHICAL APPROVAL

The processes of protocols using the experimental animals were following the Guide for the Care and Use of Laboratory Animals and approved by the Health Research Ethics

Committee of the College of Medicine, University of Lagos.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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