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Biochemical and Histopathological Evidence of Metronidazole-Induced Neurotoxicity in Rats

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A – research concept and design; B – collection and/or assembly of data; C – data analysis and interpretation; D – writing the article; E – critical revision of the article; F – final approval of article.

Abstract

Background: Metronidazole-induced neurotoxicity is a recurring challenge in pharmacotherapy of susceptible infections, however pathological evidence and biomolecular involvement needs unravelling.

Objectives: this study aims to gather neuropathologic evidence and determine biomolecular involvement in metronidazole-induced neurotoxicity.

Methods: Twenty rats were assigned to 2 groups (n=10). Group 1 was given 5 mL/kg 0.5% Tween-80®, group 2 received 50 mg/kg metronidazole. The treatments were administered via oral gavage on a daily basis. On the fifteenth day, 5 rats from each group were sacrificed under halothane anesthesia, blood and the brains were excised for toxicological analysis. The remaining animals were treated for 28 days and euthanized on the 29th day and the same parameters assessed.

Results: Histological distortions and hemorrhage occurred in the granular layer, loss of Purkinje fibres and congestion were seen in the Purkinje layer and over expression of neuron specific enolase occurred in the cerebellum of metronidazole treated rats. Reduced superoxide dismutase activity, increased malondialdehyde (brain homogenate) and reduced serum brain derived neurotrophic factor concentrations were recorded in metronidazole treated rats.

Conclusion: Histological distortions in the granular and Purkinje layers of the cerebellar cortex, increased oxidative stress and reduced BDNF concentrations in treated rats are manifestations of metronidazole-induced neurotoxicity.

Keywords: Neurotoxicity, Purkinje cells, Oxidative stress, Metronidazole, Neurotrophic factor, Cerebellum.

INTRODUCTION

Metronidazole is an antibacterial agent with an active nitroimidazole moiety. It possesses clinical effectiveness in protozoal and anaerobic bacterial infections. It's efficacy and usefulness cuts across all facets of healthcare for treatment of simple infections. Metronidazole contributes a predominant part in treatment of chronic diseases due to suspected and confirmed anaerobic bacterial infections (Chin and Hughes, 2018). As a result of its affordability in readily available dosage forms like tablets, suspension

and intravenous solution, coupled with its rapid antibacterial action and low resistance, metronidazole is adjudged to be the benchmark drug for anaerobic infections like *Clostridium difficile*-associated disease, antibiotic-associated diarrhea, and Crohn's disease (Chin and Hughes, 2018).

Although metronidazole is well tolerated independent of the route of administration, adverse effects often occur with its use. This is because metronidazole has good cellular, central nervous system, and cerebrospinal fluid penetration, thereby resulting in appreciable therapeutic efficacy even in anaerobic brain abscess infections (Kim *et al*., 2007). The undesirable consequences of metronidazole are often tolerable and principally manifest as gastrointestinal disturbances like abdominal cramps, nausea, and constipation (Chin and Hughes, 2018). A plethora of neuropsychiatric effects, including insomnia, dizziness, excitation, confusion, depression-like effects, encephalopathy, cerebellar dysfunction, seizures, vertigo are also reported (Bahn *et al.,* 2010). The reasons for the neuropsychiatric effects are not well understood even though several hypotheses have been proposed by various researchers, such as DNA fragmentation and oxidative stress (Kuriyama *et al.,* 2011). This study aims to assess some histopathological and biochemical changes involved in metronidazole-induced neurotoxicity.

METHODOLOGY

Materials and methods

Metronidazole powder (442-32-2 t1503-25g Sigma, St. Louis, MO, USA) was bought from Merck chemicals and reagents, Lagos, Nigeria, other chemicals used were of analytical grade and from reputable manufacturers.

Experimental Animals

For this study, 20 apparently healthy albino rats with an average weight of 130.5 g were housed in four different cages, with separate cages for males and females (a maximum of 5 rats per cage) to avoid overcrowding. The animals were maintained on normal rodent feed (Topfeeds, Calabar, Nigeria) and had free access to drinking water. Ethical approval was obtained from the institutional animal and ethics committee (NDU/PHARM/AEC/56). All animals were handled in accordance with standard protocols (National Research Council, 2011) and ARRIVE 2.0 guidelines for handling of animals (https://arriveguidelines.org/arrive-guidelines/sample-size).

Toxicological evaluation of 50 mg/kg daily metronidazole for 14 and 26 days respectively

Twenty rats were assigned to 2 groups (n=10). Group 1 was given 5 mL/kg 0.5% Tween-80®, group 2 received 50 mg/kg metronidazole. The treatments were administered via oral gavage on a daily basis. On the fifteenth day, 5 rats from each group were sacrificed under halothane anesthesia, blood and the brains were excised for toxicological analysis. The remaining animals were treated for 28 days and euthanized on the 29th day and the same parameters were assessed.

Histological assessment

The excised brains were scrutinized for visible injury and fixed in 10% formal-saline for 48 hours after which they were processed, sectioned (5 µm thick) using a rotary microtome (Leica RM 2125) and stained following the methods described by Zebedee *et al.*, (2022). Thereafter, photomicrographs were taken with the aid of a Motic™ 9.0 megapixels microscope camera at x400 magnification.

Immuno-histochemical staining

Previously sectioned 5 µm cerebellar cortex slices were de-waxed, hydrated in distilled water and prepared for staining as previously described by Krenacs *et al.*, (2010). The micropolymer HRP/DAB detection kit (Abcam ab80436) was used for immuno-histochemical staining following manufacturer's instructions. After staining and fixing the tissue sections on permanent slides, the slides were washed four times and then stained with Iyiola-Avwioro hematoxylin for two minutes. The slides were then blued in tap water for 3 minutes, treated with alcohol, xylene and mounted with resinous mountant followed by counter staining with Iyiola-Avwioro hematoxylin for two minutes (Iyiola and Avwioro, 2011) and photomicrographs taken (Motic™ 9.0 megapixels, x 400 magnification).

Estimation of brain derived neurotrophic factor (BDNF) following treatment with metronidazole (50 mg/kg) for 14 and 28 days respectively.

The estimation of plasma BDNF was carried out using ChemiKine™ BDNF sandwich ELISA kit (Millipore, USA) as described by Kozisek *et al*. (2008). Following

the treatment, blood samples were collected from anesthetized animals using 2 mL syringes and transferred into EDTA-laced sample tubes. The blood was spun (3000 *g* for 15 minutes) in a centrifuge to obtain plasma. Aliquots (100 µL) of BDNF standards (7.82–500 pg/mL recombinant human BDNF) and the diluted (1:20) samples were added in duplicate to rabbit anti-human BDNF pre-coated 96-well plates. Each plate was covered with the cover strip and incubated for 24 hours at 8°C. The plates were then washed four times (2 minutes per session) in 250 µL of wash-buffer, incubated in 100 µL of the diluted biotinylated mouse anti-BDNF monoclonal antibody for 3 hour and washed 4 times in wash buffer. A quantity (100 µL) of streptavidin-HRP complex was transferred into each well and incubated for 1 hour. Each well was then incubated with 100 µL of 3,3′,5,5′-tetramethylebenzidine (TMB)/ enhancer substrate at room temperature for 15 minutes, the reaction was stopped with 1 M HCl and the absorbance was immediately measured on an ELISA reader (MicroPlate Reader 550, Bio-Rad Laboratories, Denmark) at 450 nm.

Effect of daily oral metronidazole treatment (14 and 28 days) on superoxide dismutase (SOD) activity in rat brain homogenates

Brain tissues (n=5) were homogenized in ice-cold phosphate-buffered saline (PBS) and centrifuged at 3000 *g* for 15 minutes at 4°C. The resultant supernatant was used to assay the activities of superoxide dismutase (SOD) and lipid peroxidation (Zhang *et al*., 2020). Activity of SOD was determined using ab65354 (Abcam, USA) kit. The manufacturer's instructions were strictly followed in the preparation of reagents for the assays. Previously prepared brain tissue supernatant samples were diluted 1:10 using double distilled water and allowed to equilibrate with other reagents at room temperature prior to commencement of the assay. Reaction wells (sample, blank 1, blank 2, and blank 3) were set up using the multi-well plate enclosed in the assay kit. A volume (20 µL) of supernatant sample was added to the sample wells and blank 2 wells, while 20 µL of double distilled water was added to blank 1 and blank 3. An aliquot (200 µL) of working reagent was added to all reaction wells, then 20 µL dilution buffer was added to blank 2 and blank 3, and 20 µL SOD enzyme added to the sample wells and blank 1. All the reaction wells were properly mixed and incubated at 37°C for 20 minutes and the optical density read off at 450 nm (MicroPlate Reader 550, Bio-Rad Laboratories, Denmark). All assays were performed in duplicates and SOD activity calculated as percentage inhibition of xanthine oxidase which is responsible for the formazan coloration in the reaction using Equation 1.

Estimation of lipid peroxidation levels in rat brain homogenates following daily oral metronidazole (50 mg/kg) treatment for 14 and 28 days respectively.

The degree of lipid peroxidation was ascertained by measuring malondialdehyde (MDA) levels (Spickett *et al*., 2010). MDA levels in brain tissue supernatant was assayed by the thiobarbituric acid (TBA) method. The MDA standard was prepared by hydrolyzing 1,1,3,3-tetraethoxypropane (16.4 µL) stock solution in 50 mL 0.2 mM HCl and heating the mixture at 100°C for 1 hour. The standard curve for estimation of total MDA was plotted using concentrations of 1, 2, 3, 5, 7, and 10 µM obtained from serial dilution of 10 mM MDA standard. Briefly, 1 mL of brain tissue homogenate from each rat was incubated with 1 mL 0.37 % TBA in 50 mM NaOH and 1 mL of 2.8 % trichloroacetic acid and boiled for 20 minutes to yield a colored MDA–TBA adduct. The resultant adduct was then purified by centrifuging at 1,500 *g* for 10 minutes, the supernatant was collected into micro-tubes for measurement of absorbance at 532 nm (Varian Cary 50 UV–Visible spectrophotometer) against a blank and compared with the standard curve. All the results were extrapolated against the standard curve and recorded as µM MDA/g of tissue. All the assays were conducted in duplicate and average values recorded.

Statistical analysis and data presentation

Results are presented as mean ± standard error of mean (SEM) and "n" represents the number of animals per group. Statistical analysis was done using one-way ANOVA followed by Dunnet's post hoc test for multiple comparisons (GraphPad Prism 6 Software, San Diego California USA). Correlations for protein analysis were calculated by Pearson's test. Statistical differences between compared data were considered significant at $p<0.05$.

$$\boldsymbol{SOD\ activity} = \frac{(\boldsymbol{A_{Blank\,1} - A_{Blank\,3}}) - (\boldsymbol{A_{sample} - A_{Blank\,2}})}{\boldsymbol{A_{Blank\,1} - A_{Blank\,3}}} \boldsymbol{x100\%} \ \ldots\ldots\ldots\ldots Equation\ 1$$

Where A is absorbance at 450 nm

RESULTS AND DISCUSSION

Histological assessment of cerebellar cortex slices in metronidazole treated rats reveals histological distortions.

In the rat cerebellum several histological distortions were seen in the metronidazole treated rats. There was extensive hemorrhage but mild vacuolation of the neuropil in the granular cell layer of the cerebellum in the 14-days treated group (Figure 1B). In the 28-days treated group, multifocal intramyelinic oedema, neuronal pyknosis, moderate multifocal gliosis and widespread vacuolation (spongiosis) of the neuropil occurred (Figure 1C).

In the 14-days treated group (Figure 2B) multifocal loss of cells in the Purkinje cell layer occurred with a few Purkinje cells showing eosinophilic necrosis, pyknotic granular cells and capillary congestion. These same effects are seen in Figure 2C which represents the 28-days treated group but some ghost cells, several pyknotic granular cells and capillary congestions are also present.

Histological assessment of organs reveals the effects of a chemical or biological substance administered to the test subject (Yuet-Ping *et al.*, 2013). In the rat cerebellum several histological distortions including extensive hemorrhage, moderate multifocal gliosis and widespread vacuolation (spongiosis) of the neuropil occurred in the granular cell layer which is in agreement with some other reports (Agarwal *et al*., 2016). Multifocal loss of Purkinje cells, with a few Purkinje cells showing eosinophilic necrosis, pyknotic granular cells and capillary congestion occurred in the Purkinje layer of the treated rats are in consonant with some previous reports (Agarwal *et al*., 2016).

Experimental studies have substantiated that cerebellar damage induces deficiencies in motor learning in man and laboratory animals. One renowned laboratory model is the vestibulo-ocular reflex (VOR) which allows the maintenance of gaze on a fixed object when the head is turned around or rotated (Nguyen-Vu *et al*., 2013). The cerebellum is thought to be the seat of cognition in the human brain and it is known to have over 40 billion neurons. It receives impulse from several sensory systems emanating from various parts of the brain and spinal cord and integrates these sensory impulses into cognition and motor activity (Markousis-Mavrogenis *et al*., 2022). The cerebellar cortex is organized into three separate bands, the granular (bottom), the Purkinje (middle) and the molecular layer with flattened dendritic trees of Purkinje fibres accompanied with a huge array of parallel fibres sticking into the Purkinje cell dendritic trees at right angles makes up the top layer.

The Purkinje cells are principal neurons in the cerebellar cortex whereupon the excitatory (glutamatergic) parallel fibres (granule cell axons), climbing fibres and glutamatergic inputs from mossy fibre pathway ultimately converge (Bastian, 2011). Inhibitory efferent cerebellar (GABAergic) inputs which descend into the deep cerebellar nuclei (dentate, interpositus and fastigial nuclei) also receive inputs from purkinje fibres (Nguyen-Vu *et al*., 2013). Acquisition of instructive signals controlling the induction of cerebellum-dependent learning is thought to be controlled by Purkinje fibres and climbing neural fibres supplying the cerebellum (Nguyen-Vu *et al*., 2013). The granular cell layer is replete with different types of neuronal cells including mossy fibers which convey the major (excitatory) inputs to the cerebellar cortex, granule cells onto which the mossy fibers converge to form synapses and golgi cells which serve as feedback regulators of granule cells as they supply inhibitory inputs to the granule cells (Court and Wade, 2022). Granule cells have minimal soma with few dendrites and provide specialized inputs based on their location and localized mossy fiber input, and thousands of granule cells converge onto a single Purkinje cell (Court and Wade, 2022). These granule cell-purkinje fibre synapses are known to influence cerebellum-dependent learning (Court and Wade, 2022).

Furthermore, previous research have reported notable functional interactions amongst the cerebellum, the prefrontal cortex and the hippocampus (Onuki *et al.,* 2015) and these brain structures are known to modulate cardinal cognitive functions encompassing attention and memory and their malfunction has been associated with cognitive deficits consistent with neuropsychiatric disorders (Bast *et al.*, 2017). Cortico-hippocampal neural activity is believed to be controlled by GABAergic inhibition and neural disinhibition in these regions has emerged as a pathophysiological feature in some neuropsychiatric disorders including cognitive dysfunction (Millan *et al*., 2012). The cerebellum and the hippocampus are known to have a two-way influence on each other in terms of spatial learning and memory (Bast *et al.*, 2017 Disruption of spatio-temporal control of neural activity in a particular brain region and its outgrowths may interfere with neural information processing inside the region and efferent regions (Bast *et al.*, 2017). Disruption of Purkinje fibres which control the outflow of the cerebellum or lesions in the cerebellum can affect the hippocampal response due to their dual influence on each other (Bast *et al.*, 2017). Disruption of Purkinje and granullar cell layers in the metronidazole treated rats may cause neuropsychiatric

effects contributing to metronidazole-induced neurotxicity. This is in consonance with that of other researchers who proposed that purkinje cell damage produced negative cerebellum-dependent motor and cognitive functions (Bastian, 2011).

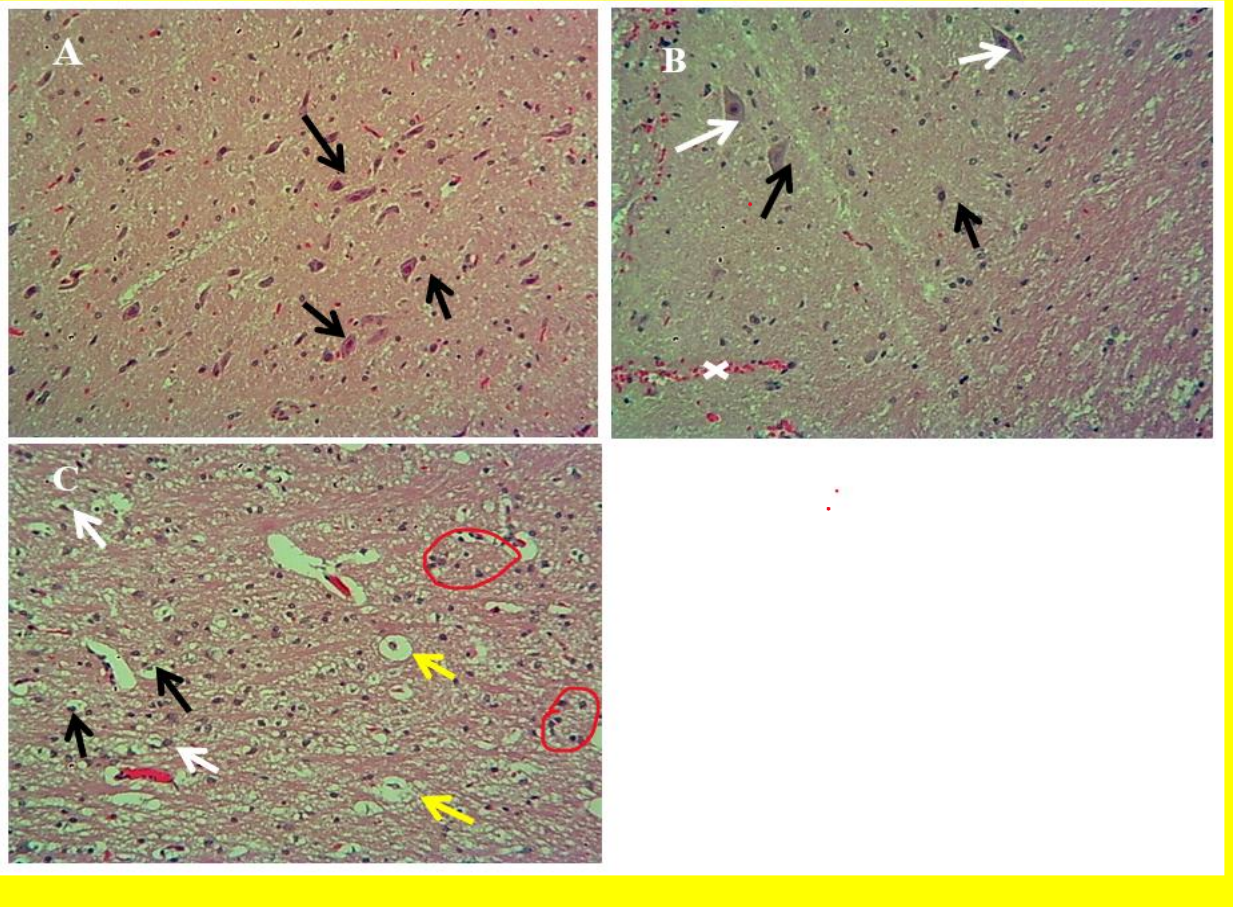

Figure 1: Photomicrographs of cerebellum (granular cell layer) of treated rats. Plate A: control showing normal cellular and parenchymal architecture with arrows pointing to normal granular cells; Plate B: granular layer of a 50 mg/kg p.o. treated (x14 days) rat showing locally extensive areas of cerebral hemorrhage and mild vacuolation (white x) of the neuropil and a few ghost granular cells (black arrows), together with normal cells (white arrows); and Plate C: Granular cell layer of a 50 mg/kg p.o. treated rat (x28 days) showing multifocal intramyelinic oedema (black arrows), neuronal pyknosis (white arrows), moderate, multifocal gliosis (red ellipse), and widespread vacuolation (spongiosis) of the neuropil (yellow arrows). H&E x400.

C

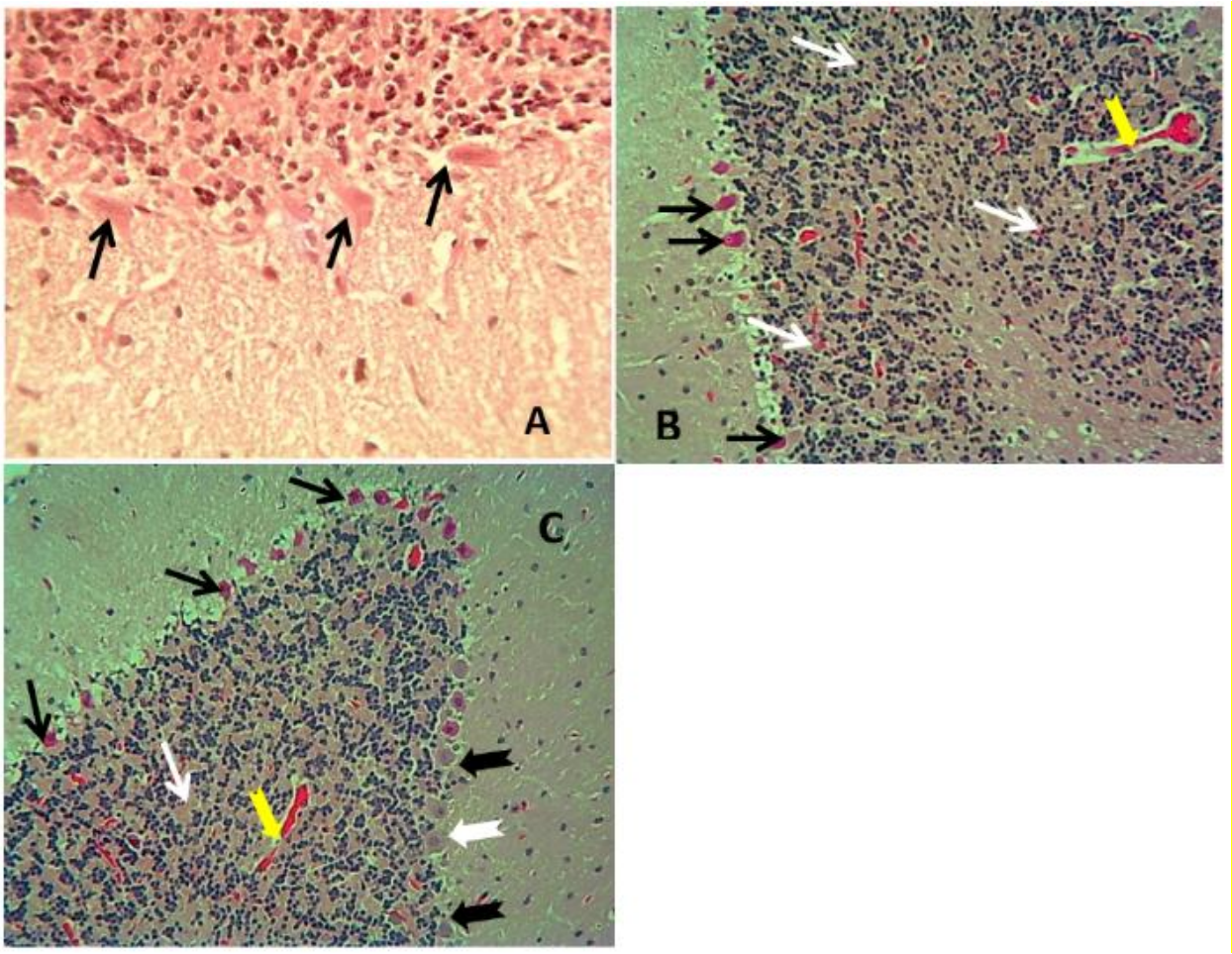

Figure 2: Photomicrographs of cerebellum (Purkinje cell layer) of treated rats. Plate A: control showing intact Purkinje cell layer with Purkinje cells (black arrows); Plate B: multifocal loss of cells in a rat treated with metronidazole 50 mg/kg p.o. for 14 days, a few evident Purkinje cells show eosinophilic necrosis (black arrows), pyknotic granular cells (white arrows), and capillary congestion (notched yellow arrows); Plate C: several Purkinje cells show eosinophilic necrosis (black arrows), some ghost cells (notched black arrow), one relatively normal Purkinje cell (notched white arrow), several pyknotic granular cells (white arrow), and capillary congestion (yellow notched arrow) all in a rat treated with 50 mg/kg metronidazole for 28 days. H&E, x400.

Immuno-histochemistry of cerebellar cortical slices of rats treated with metronidazole revealed a progressive degeneration.

Neuron specific enolase (NSE) staining in the cerebellar cortices of rats treated with metronidazole showed that the control rat (Figure 3A) was negative, i.e., did not pick up the stain. For the cortex of the rat treated with metronidazole for 14 days, there was moderate brownish cytoplasmic staining for NSE (Figure 3B). There was expression of NSE indicated by copious brownish cytoplasmic staining in the 28-days treated rat cerebellar cortical slice (Figure 3C).
Neurons are important components in the nervous system and are necessary for the maintenance of brain homeostasis. Immunohistochemical staining is useful in detecting neuronal damage and brain injury. Neuron-specific enolase (NSE) is a glycolytic enzyme specifically expressed in neurons and has been used as a marker for neuronal damage in brain injury (Rahmy and Hassona, 2004). Neuron specific enolase can be evaluated both in serum and on postmortem tissues, and the level of expression is indicative of the extent of damage (Woertgen *et al.*, 2001). In this study we report a progressive NSE staining in the cortical slices of metronidazole treated rats. The results from this study suggests that prolonged administration of metronidazole may cause neurotoxicity due to neuronal degeneration.

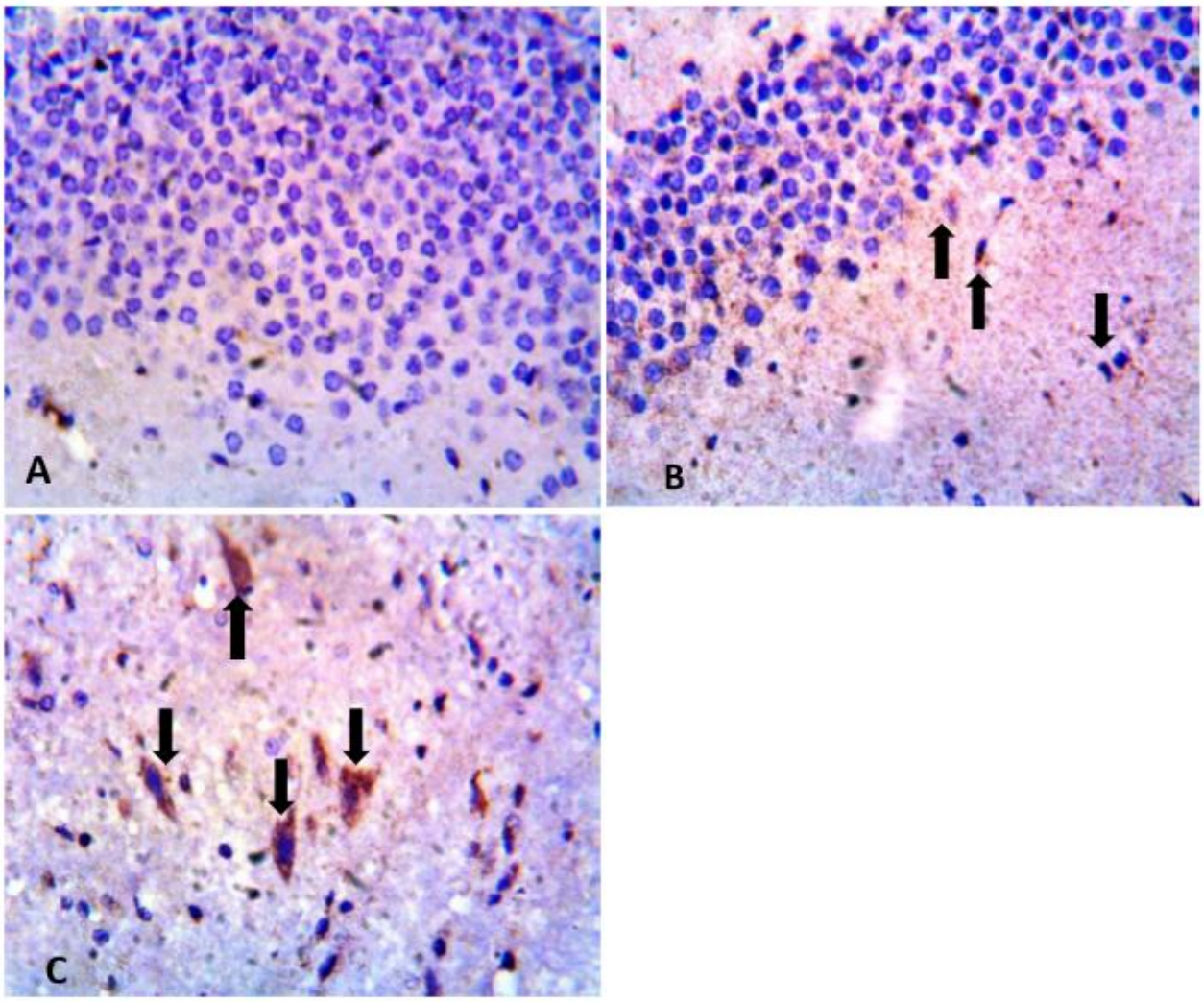

Figure 3: Immuno-histochemical staining for neuron specific enolase (NSE) in cerebellar cortex slices (X400) of rats following 28 days daily oral treatment with metronidazole (50 mg/kg). Plate A: control group with normal stain devoid of NSE pigments. Plate B: moderate brownish cytoplasmic deposits (black arrows) for NSE in the 14 days treated group. Plate C: showing expression of NSE (black arrows) indicating cerebellar injury in the 28 days treated rat.

Brain derived neurotrophic factor (BDNF) concentration in metronidazole treated rats reduced following treatment.

The results for estimation of plasma BDNF in treated rats are presented in Figure 4. In the 14-days treated rats (Figure 4A), BDNF levels were not different from control but in the 28-days treated rats (Figure 4B), BDBF levels reduced significantly ($P<0.01$) compared to control.

Brain-derived neurotrophic factor (BDNF) is a freely accessible neurotrophic factor in diseased or healthy brain (Lu *et al.*, 2005). BDNF is present in remarkable quantity in several brain regions including peripheral organs such as thymus, spleen, heart, gut and over 70% of circulating BDNF is stored in the platelets. Blood and plasma concentrations of BDNF are reflective of the concentration in the brain (Klein *et al*., 2011). BDNF plays vital roles including regulation of neurotransmitter synthesis, neuronal plasticity, survival and maintenance in several brain regions.

In humans, BDNF is recognized as a useful biological marker for neurotoxicity and many other neuropsychiatric disorders (Chen *et al*., 2017). Several brain disorders cause a decrease in serum and brain BDNF concentrations and persons with neuropsychiatric or neurodegenerative conditions often present with reduced blood and brain BDNF concentrations. Reduced BDNF concentrations have been reported in major depressive disorder, bipolar disorder, schizophrenia, Alzheimer's disease,

Parkinson's disease and epilepsy (Lima Giacobbo *et al*., 2019). In this study, BDNF concentrations were reduced in the 28-days metronidazole treated rats and can be seen as an evidence and contributory factor to metronidazole-induced neurotoxicity.

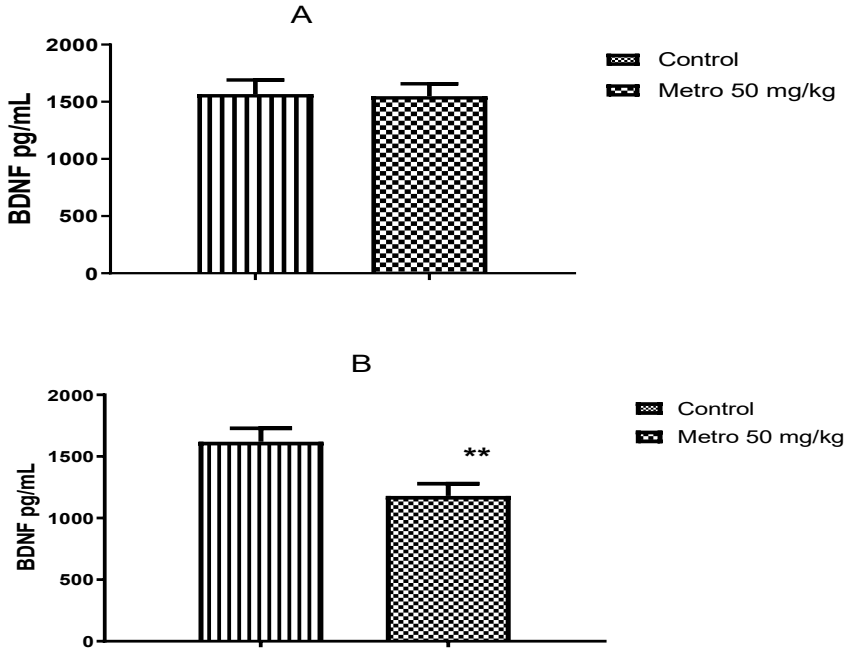

Figure 4: Plasma brain derived neurotrophic factor (BDNF) concentration of rats. Effects on rats that received 50 mg/kg of metronidazole (Metro) for 14 days (A) or 28 days (B). $^{**}P<0.01$ compared to control, n=5.

Metronidazole reduces superoxide dismutase activity and increased lipid peroxidation in treated rats.

Administration of metronidazole (50 mg/kg) for 14 days had no significant effect on the activity of superoxide dismutase (SOD) in rats (Figures 5A). However, administration of metronidazole for 28 days significantly (*P<0.01)* inhibited SOD activity (Figure 5B).

The degree of lipid peroxidation as reflected in the concentration of malondialdehyde (MDA) is shown in Figure 6. MDA concentration increased significantly (*P<0.*001) after 14- or 28-days treatment with 50 mg/kg of metronidazole, the compared to control.

Oxidative stress can be assessed in humans and animals using various techniques which measure different parameters including lipid peroxidation, SOD activity, ROS estimation, NO estimation and some others. The brain is overtly susceptible to oxidative stress owing to its exceptional oxygen consumption and abundance of polyunsaturated biomolecules which are also prone to oxidative reactions (Schieber and Chandel, 2014). Redox active agents are replete in the brain including antioxidants (ascorbic acid, glutathione (GSH), lipoic acid, homocysteine, retinol, a-tocopherol, carotenoids and several others); enzymes (superoxide dismutase, glutathione peroxidases, catalase); oxidizing agents such as reactive oxygen species (ROS) e.g., hydrogen peroxide, the free radicals e.g., hydroxyl (OH^-), nitrosium, nitroxyl, and superoxide (O_2^-) and reactive nitrogen species (RNS) e.g., NO^-, which reacts with oxygen to form the highly toxic peroxynitrite radical (Schieber and Chandel, 2014). These endogenous agents are genereted under physiologic conditions or under oxidative stress and can be induced by many drugs including metronidazole (Dingsdag and Hunter, 2018). The effects of these redox agents on neurotoxicity depends on several factors including their concentration, the brain region and the types of neurons affected (Calvo *et al.*, 2016).

The mechanism of action of metronidazole involves the formation of nitro anion radicals (RNS) which can be reduced to the corresponding nitroso, hydronitroxide, and amine compounds (Ceruelos *et al*., 2019). Destruction of lipids in terms of peroxidation often commences when a free radical species (ROS/RNS) binds to and extracts a hydrogen molecule from methylene groups (CH_2) in polyunsaturated biomolecules which results in the formation of a lipid radical. The lipid radical then interacts with molecular oxygen to form a lipid peroxyl radical. The resulting lipid peroxyl radical passes through a cyclisation reaction to produce

endoperoxides and malondialdehyde (MDA), the destructive end product of lipid peroxidation which causes injury to proteins and DNA (Calvo *et al.*, 2016). The increase in MDA levels and reduced SOD activity in the metronidazole treated rats may be due to the RNS resulting from the metabolism of metronidazole. This finding is in agreement with some other researchers (Dingsdag and Hunter, 2018). By implication, increased lipid peroxidation presenting as increased MDA levels and reduced SOD activity can serve as evidence of oxidative stress which is a well-known contributor to the pathophysiology of neurotoxicity. Results from this study suggests that oxidative stress may be a contributing factor to metronidazole-induced neurotoxicity.

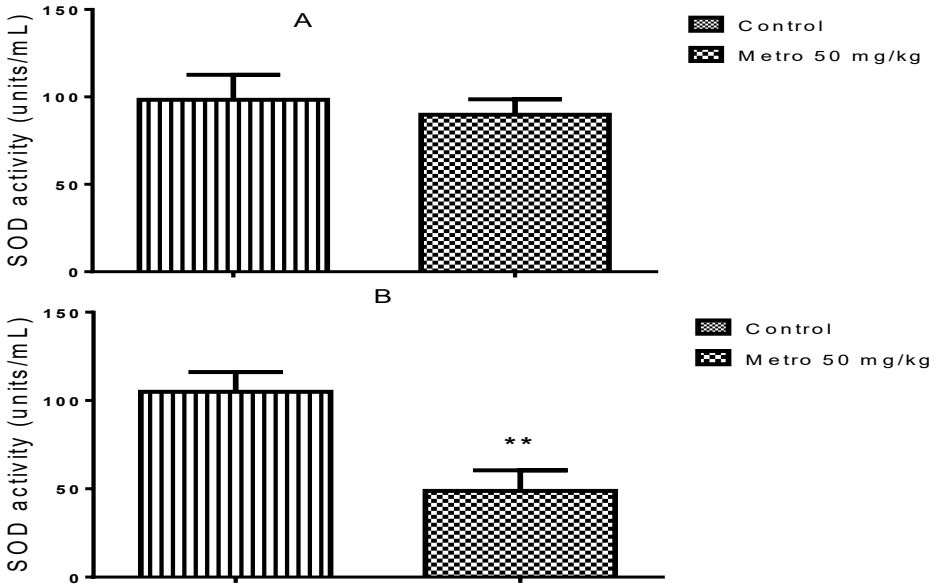

Figure 5: Superoxide dismutase (SOD) activity in brain homogenates of 50 mg/kg oral metronidazole treated rats (A, 14 days; B, 28 days). $^{**}P<0.01$ compared to control, n=5.

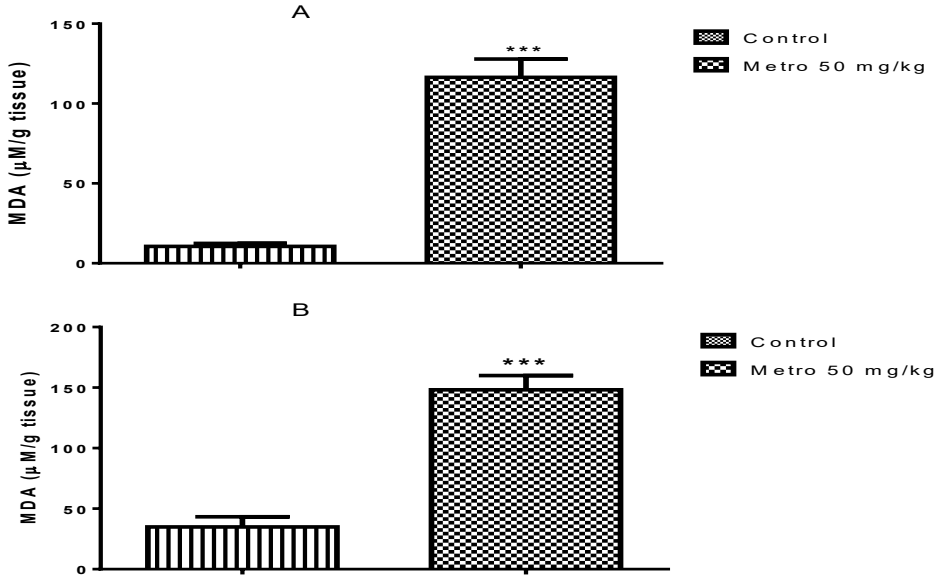

Figure 6: Metronidazole (50 mg/kg) treatment for 14 or 28 days resulted in elevated MDA concentration in brain tissue homogenates of treated rats. $^{***}P<0.001$ versus control, n=5.

CONCLUSION

Histology is an important aspect of drug toxicity studies and provides information about anatomic or molecular effects of a drug on specific organs. Histological distortions on brain structures, depletion of Purkinje fibres and proliferation of neuron specific enolase which signify neuronal cell degeneration were seen in this study. Biological molecules can serve diagnostic functions or as biomarkers of disease progression. Reduced BDNF concentration in metronidazole treated rats is reported in this study. An imbalance in redox activity induced by drug molecules often give rise to drug-induced oxidative stress. Impaired house-keeping function of redox agents expressed as reduction in SOD activity and increased lipid peroxidation in metronidazole treated animals is reported in this study. Hence, it is concluded that metronidazole induced histological distortions in the granular and Purkinje layers of the cerebellar cortex, reduced the concentration of BDNF, reduced SOD activity and increased MDA concentration in brain homogenates of treated rats, and these effects may contribute to metronidazole-induced neurotoxicity.

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