



Evaluation of Chronic Administration of Metronidazole on the Morphological and Biochemical Parameters on the Cerebral Cortex of Adult Wistar Rats

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Authors' contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Metronidazole has been implicated in diverse neurologic syndromes such as; cerebellar syndrome, encephalopathy, seizures, optic neuropathy, autonomic neuropathy and peripheral neuropathy. Metronidazole is an antibiotic drug used to treat infections of the reproductive system, gastrointestinal tract, skin, heart, bone joint, lung, blood, nervous system and sexually transmitted diseases (STDs). This study therefore, investigated the effects of metronidazole on the cerebral cortex of adult wistar rats.

Thirty-two (32) adult wistar rats of average weight of 180g of both sexes were distributed into four groups of eight (8) animals per group. Group A was the control group while group B, C and D rats were treated with 250 mg/kg, 500 mg/kg and 1000 mg/kg of metronidazole respectively. Metronidazole was administered orally on daily basis to the animals for 28 days. The weights of the rats were taken weekly using a weighing scale. On the 28th day of the treatment, the animals were sacrificed by cervical dislocation. The skulls were excised and the brains were harvested, weighed immediately using a sensitive weighing balance and then fixed in 10% formolcalcium for routine histological techniques and the other parts were processed for biochemical analysis of Malondialdehyde (MDA), Nitric Oxide (NO) and Succinate dehydrogenase (SDH).

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The results showed that there was a significant increase in the body weights of wistar rats in A and B while the mean body weights of the wistar rats reduced significantly in group C and D. The brain weights in group B and C increased insignificantly while brain weight in group D increased significantly when compared with group A. The biochemical analysis showed significant increase ($P < 0.05$) in the level of MDA, NO and SDH in group B, C and D as compared with group A. Histological study of the cerebral cortex revealed conspicuous degenerative changes in group B while group C and D showed increased degenerative cerebral cortical layers with peripheral and central degenerative changes.

The study concluded that metronidazole exhibited a neurodegenerative effect on the cerebral cortex of the wistar rats investigated. It is recommended that other studies should be carried out to corroborate these findings.

Keywords: *Metronidazole; cerebral cortex; neuronal degeneration; malondialdehyde (MDA); nitric oxide (NO); succinate dehydrogenase (SDH).*

1. INTRODUCTION

Metronidazole (MTZ) is a synthetic 5-nitroimidazole antibiotic which is widely useful in the treatment of infections caused by anaerobic bacteria [1,2]. Initially, metronidazole was used to treat vaginal trichomoniasis and it has great significant clinical effects. It is widely used to treat and prevent oral anaerobic infections. It has been used oftentimes in hospitals to prevent and treat brain, respiratory, pelvic, skin, soft tissue, joint, and peritoneal infections, cardiomyitis, and septicemia that are caused by anaerobic bacteria. It is also used to treat parasitic infections such as amebiasis, and also gastrointestinal infections as well as trichomoniasis and giardiasis [2].

Metronidazole is primarily used for treating pelvic inflammatory disease, bacterial vaginosis, pseudomembranous colitis, rosacea (topical), fungating wounds (topical), intra-abdominal infections, aspiration pneumonia, lung abscess, periodontitis amoebiasis, oral infections, giardiasis and trichomoniasis. It is also used to treat infections such as *Peptostreptococcus*, *Bacteroides*, *Fusobacterium*, *Prevotella* and *Clostridium* species which are caused by susceptible anaerobic organisms [3]. It is mostly used along with other drugs to exterminate *Helicobacter pylori* and also to prevent infection in people that are recovering from surgery. [3].

Metronidazole is bitter and the liquid suspension contains metronidazole benzoate. It may be unsuitable in people with diarrhea or feeding tubes in the jejunum or duodenum because the constituent of the liquid suspension may require hydrolysis in the gastrointestinal tract (Dickman, 2012) [4]. It is metabolized in the liver [5]. Approximately 30–60% of an oral or IV dose is

metabolized in the liver by hydroxylation, side-chain oxidation, and glucuronide conjugation [6]. Metronidazole began to be used commercially in France in 1960 [7]. It is referred to as the safest and most effective medicines needed in a health system by the World Health Organization referred and is also on the World Health Organization's List of Essential Medicines. [8].

The cerebral cortex is the part of the brain that functions to make human beings unique. Distinctly human traits including higher thought, language, and human consciousness as well as the ability to think, reason and imagine all originate in the cerebral cortex. [9]. The cerebral cortex is the largest site of neural integration in the central nervous system. [10]. There are between 14 and 16 billion neurons in the cerebral cortex. These are organized into cortical columns and minicolumns of neurons that make up the layers of the cortex [11]. The cerebral cortex can be divided into four sections, which are known as lobes. The frontal lobe, parietal lobe, occipital lobe, and temporal lobe have been associated with different functions ranging from reasoning to auditory perception. [12]. The cerebral cortex is the largest site of neural integration in the central nervous system. [10].

In most mammals, apart from small mammals that have small brains, the cerebral cortex is folded, providing a greater surface area in the confined volume of the cranium. Apart from minimizing brain and cranial volume, cortical folding is crucial for the wiring of the brain and its functional organization. In mammals with a small brain there is no folding and the cortex is smooth. [13,14]. Without doubt, metronidazole is very effective in treating bacterial and parasitic infections but when it is taken in high dosage, it

can produce diverse neurologic syndromes such as; cerebellar syndrome, encephalopathy, seizures, optic neuropathy, autonomic neuropathy and peripheral neuropathy [15]. The central nervous system side effects connected to metronidazole toxicosis have been reported in humans and animals including dogs, rats and rats. Cerebellar syndrome following prolonged exposure to metronidazole have also been reported [16].

A study previously conducted reported the harmful effects of metronidazole on the Germard Leydig cells following penetration into the blood-testis barrier [17]. Ligha *et al* [18] also reported that administration of metronidazole for 8 weeks MTZ administration (200 or 400 mg/kg) caused a harmful effect on the testes of male rats [18]. Another previous study has shown clearly that metronidazole crosses the blood-brain barrier and accumulates within the hippocampus, olfactory bulb and cerebellum [19].

2. MATERIALS AND METHODS

2.1 Care of the Animals

A total number of thirty-two (32) adult wistar rats of average weight of 180g of both sexes were used for the experiment. The animals were purchased in Ogbomoso. The cages where the animals were kept were cleaned and disinfected before the animals were moved into them. The rats were fed with feed mill and water ad libitum and they received human care according to the criteria outline in the guide for the Animal care and use in research prepared by the Institution Animal Care and Use Committee. After three weeks period of acclimatization, the animals were distributed into four groups according to their body weights.

2.2 Experimental Grouping

The adult wistar rats were grouped into four (4) different groups of eight (8) animals each labelled from A to D. Group A contains the control animals while the Group B, C and D contain the treated animals. The animals in group A were given standard laboratory mouse chow and distilled water while the animals in group B, C and D received 250 mg/kg, 500 mg/kg and 1000 mg/kg of metronidazole respectively for 4weeks alongside with their feed and water. The route of administration of the drug to the animals was through oral route of administration.

2.3 Weight of the Animals

The weights of the experimental animals were monitored and weighed with a sensitive weighing scale and the values were recorded. The body weights were taken during acclimatization periods and during administration so as to monitor and study the effect of administration of metronidazole on the weight of the adult wistar rats.

2.4 Acclimatization Period

The rats were acclimatized for three (3) weeks beginning from 29th January to 17th February, 2020. During the acclimatization period, the rats were kept in their cages and fed with their feed mill which was purchase at Glory Vet in Isale General area, Ogbomoso. During acclimatization two rats died in the control group.

2.5 Drug Administration

The name of the drug administered is Metronidazole and it was purchased from Akol Pharmacy, a NAFDAC certified pharmacy located behind LAUTECH Teaching Hospital, General Area, Ogbomoso. Metronidazole was given to the animals at 7:00am daily before feeding them for a period of 28 consecutive days. The route of administration of the drug to the animals was through oral route of administration and this was done by using a calibrated syringe with a cannular attached to its tip to draw the required volume of the solution. Bead was attached to the tip of the cannular to prevent injury to the wistar rats. The cannular was passed through the diastema of the rats and lowered down to the oesophagus of the rats, the solution was expelled from the tip of the cannular which flows to the stomach of the rat where proper reabsorption and metabolism of the drug will take place.

2.6 Sacrificing of the Animals

The animals were sacrificed 24hours after the last administration of metronidazole through cervical dislocation so as to keep them in a near living state while still breathing. The head of the animals were cut off from their bodies and then the brains were harvested. The brains were fixed in 10% formol calcium and stored for tissue preparation. While the brains were being harvested, the weight of the organs were taken and the bottles which the organs were stored were labelled according to the groups in which the animal belong and then marked for identification.

2.7 Photomicrography

The digital micrographs of the cerebral cortex sections were obtained to show the morphological changes that occurred in the treated groups as compared to the control group. The photomicrographs were taken at the Department of Anatomy, Ladoké Akintola University of Technology, Ogbomoshó, Oyo State, using digital Am scope (MD 900) photomicroscope.

2.8 Statistical Analysis

All data were expressed as Mean \pm SEM. The statistical analysis of the results obtained in this study was evaluated and tested for significance using Analysis of Variance (ANOVA). If P-value of ANOVA was less than 0.05 ($P < 0.05$), then result was significant. If P-value of the ANOVA was greater than 0.05 ($P > 0.05$), then that means that the result is not significant.

2.9 Enzyme Assay

2.9.1 Determination of malondialdehyde (MDA)

Malondialdehyde (MDA) level was estimated in the respective homogenate samples obtained from each group by employing the procedure of Vashney and Kale (Vashney and Kale, 1990). Lipid peroxidation was carried out by measuring the Thiobarbituric Acid – Reactive (TBAR) products. The method was based on formation of pink colored product when 2-thiobarbituric acid (TBA) reacts with MDA in acidic medium for 30min to form thiobarbituric acid reactive substances (TBARS). The absorbance of the resultant pink product was measured spectrophotometrically at 534nm.

2.9.2 Determination of nitric oxide (NO)

Under acidic condition nitrite ion (NO_2^-) reacts with sulphanilamide to form a diazonium salt, which combines with N-(1-naphthyl)-ethylene diamine dihydrochloride (NEDD) dihydrochloride to form a bright coloured pinkish red azo dye. The colour product is directly proportional to the amount of nitrite present in the sample (Thayer and Huffaker 1980; Green *et al.*, 1982).

2.9.3 Determination of succinate dehydrogenase (SDH)

The spectrophotometer used in the assay was a Kontron Uvikon 860 and the cuvettes had a 1-cm

light path. The reaction mixture was composed of 100 mmol/liter triethanolamine (HCl), pH 8.3, 0.5 mmol/liter EDTA, 2 mmol/liter KCN, 2 mmol/liter INT, 12 g/liter Cremophor EL, and 20 mmol/liter succinate previously adjusted at pH 7.4. The reaction was started by mixing 990 μl of the reagent with 10 μl of mitochondrial suspension. Color development at 30°C was recorded for 6 minutes at 500nm. Blanks were carried out in the absence of substrate (succinate), and the obtained rate was subtracted from the sample rates. Considering that the molar absorbance of the INT-formazan at 500nm is 19300 (Gella *et al.*, 1981), the following formula was deduced for the calculation of the catalytic concentration: $\text{U/ml} = \Delta A / \text{min} \times 5.18$ (Munujos *et al.*, 1993).

3. RESULTS

3.1 Body Weight Analysis

The body weights of Wistar rats in the control group (A) increased from mean value of 130.3 \pm 12.86 at week 0 to 147.0 \pm 6.71 at week 4 denoting 16.5% weight gain.

Group B which received a low dose of metronidazole (250 mg/kg) increased in body weights of the wistar rats from mean value of 183 \pm 4.66 at week 0 to 191.8 \pm 3.17 at week 4 which denote 8% weight gain.

The animals in group C which received a medium dose of metronidazole (500 mg/kg) showed a decrease in body weights of the wistar rats from mean value of 212 \pm 5.3 at week 0 to 206.5 \pm 6.5 at week 4 denoting 5.5% weight loss.

Group D animals which received a high dose of metronidazole (1000 mg/kg) showed a significant decrease in body weights of the wistar rats from mean value of 245.5 \pm 4.8 at week 0 to 214.6 \pm 11.41 at week 4 which denote 30.9% weight loss.

Table 2 shows that the mean brain weight of the animals in group A was 1.49 \pm 0.02 with the relative brain weight of 1.01% which increased insignificantly to 1.55 \pm 0.06 with the relative brain weight of 0.81% in group B and also increased insignificantly to 1.57 \pm 0.03 with relative brain weight of 0.76% in group C and finally increased significantly to 1.65 \pm 0.05* with relative brain weight of 0.77% in group D.

MDA : Malondialdehyde

NO : Nitric Oxide

SDH : Succinate dehydrogenase

Table 1. Showing the mean ± S.E.M of the body weight of wistar rats before and during administration

Period	Group a	Group b	Group c	Group d
Week 0	130.3 ± 12.86	183.0 ± 4.66**	212.0 ± 5.31***	245.5 ± 4.87***
Week 1	113.7 ± 11.32	171.5 ± 4.29***	198.8 ± 4.96***	231.5 ± 3.96***
Week 2	135.7 ± 7.07	185.5 ± 3.70***	212.5 ± 5.90***	248.3 ± 5.12***
Week 3	142.3 ± 8.94	188.3 ± 2.43***	206.3 ± 4.33***	229.5 ± 8.53***
Week 4	147.0 ± 6.71	191.8 ± 3.17***	206.5 ± 6.50***	214.6 ± 11.41***

Significance: $P < 0.05$, value greater than 0.05 were considered insignificant while values lesser than 0.05 are considered significant (*). Values were expressed as mean ± Standard error of mean

Table 2. Showing the mean ± S.E.M of Brain of wistar rats after treatment

Groups	Brain weight (Mean ± S.E.M) (g)	Relative brain weight (%)
A	1.49 ± 0.02	1.01
B	1.55 ± 0.06	0.81
C	1.57 ± 0.03	0.76
D	1.65 ± 0.05*	0.77

Significance: $P < 0.05$, value greater than 0.05 were considered insignificant while values less than 0.05 are considered significant (*). Values were expressed as mean ± Standard error of mean.

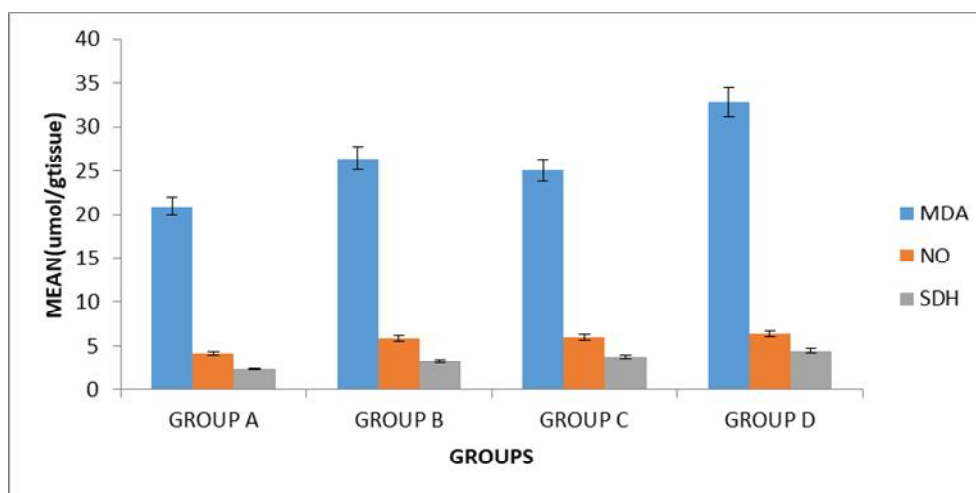


Fig. 1. Histogram showing the changes in level of MDA, NO and SDH after treatment

Table 3. showing the effect of metronidazole on the levels of MDA, NO and SDH in the brain

Groups	MDA (nmol/g tissue)	NO (µmol/g tissue)	SDH (µmol/g tissue)
A	20.94 ± 0.26	4.114 ± 0.24	2.319 ± 0.43
B	26.43 ± 1.87*	5.871 ± 0.22***	3.259 ± 0.42
C	25.09 ± 0.80***	5.943 ± 0.42**	3.704 ± 0.25*
D	32.81 ± 1.42***	6.357 ± 0.29***	4.467 ± 0.26**

Significance: $P < 0.05$, value greater than 0.05 were considered insignificant while values less than 0.05 are considered significant (*). Values were expressed as mean ± Standard error of mean

Table 3 revealed significant increase ($P < 0.05$) in the level of MDA in the treated groups when compared with the control from 20.94 ± 0.26 in group A to 26.43 ± 1.87 in group B, 25.09 ± 0.80 in group C and 32.81 ± 1.41 in group D.

The level of NO increased significantly ($P < 0.05$) in the treated groups compared with the control, it increased significantly in group A from 4.114 ± 0.24 to 5.871 ± 0.22 in group B, 5.943 ± 0.42 in group C and 6.357 ± 0.29 in group D.

The level of SDH increased significantly ($P < 0.05$) in the treated groups compared with the control, it increased from 2.319 ± 0.43 in group A to 3.259 ± 0.42 in group B, 3.704 ± 0.25 in group C and 4.467 ± 0.26 in group D.

The graph above showed significant increase ($P < 0.05$) in the level of MDA in group B, C and D when compared with group A.

Group B, C and D showed a significant increase ($P < 0.05$) in the level of NO as compared with Group A.

Group B, C and D showed a significant increase ($P < 0.05$) in the level of SDH as compared with group A.

3.2 Histological Observation

Representative micrographs of H&E staining showing the general and magnified cytoarchitecture of the cerebral cortex in Wistar rats in group A (control), Group B (Treated with 250 mg/kg of metronidazole for 28days), Group C (Treated with 500 mg/kg of metronidazole for 28days), Group D (Treated with 1000 mg/kg of metronidazole for 28days). Magnification: X100 and X400 respectively.

Normal histological features of the cortex are observed in group A treatment; this was

characterized by large pyramidal as well as granule neurons, the pyramidal cells are characterized with long axons that extend well from the soma to adjacent neurons within the neuropil. Apical and basal dendrites extend from the well delineated soma of the pyramidal neurons in this group. Perineural space surrounding these cells appears intact, with intact nuclear and cytoplasmic content, both pyramidal and granular cells appear darkly stained with no signs of diffused content with distinct layering.

Group B treatment caused conspicuous degenerative changes in the cortex that was characterized by peripheral and central degenerative changes, clustered pyknotic pyramidal and granule neurons that appear with fragmented cytoplasm and condensed nuclei. Wide Perineural spaces can be seen surrounding degenerating neurons, axons and dendrites are scarcely appreciable around neurons in this group, neuronal population appear scarcely appreciable in this group (red arrow).

Group C and D show increase degenerative cerebral cortical layers with peripheral and central degenerative changes. The pyramidal and granule neurons appeared degenerated with increased perineural spaces and the cortical tissue appeared distorted.

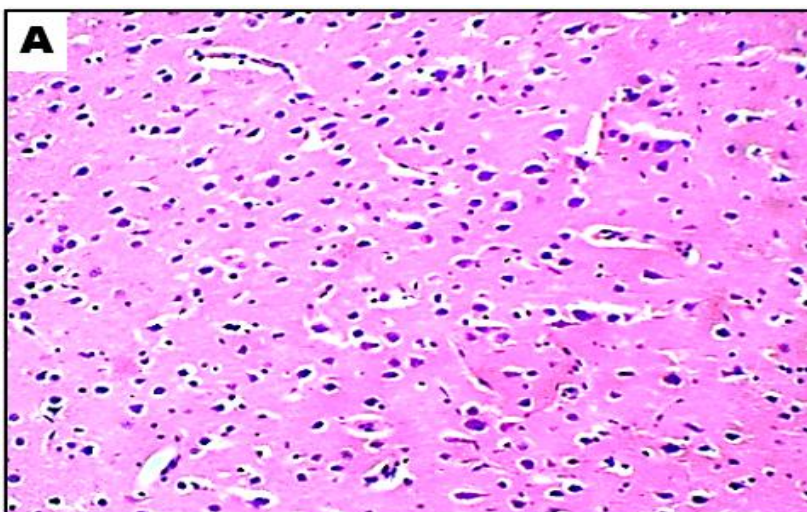


Plate 1A. Photomicrograph of the cerebral cortex of adult wistar rat in group A- control X100 (H&E)

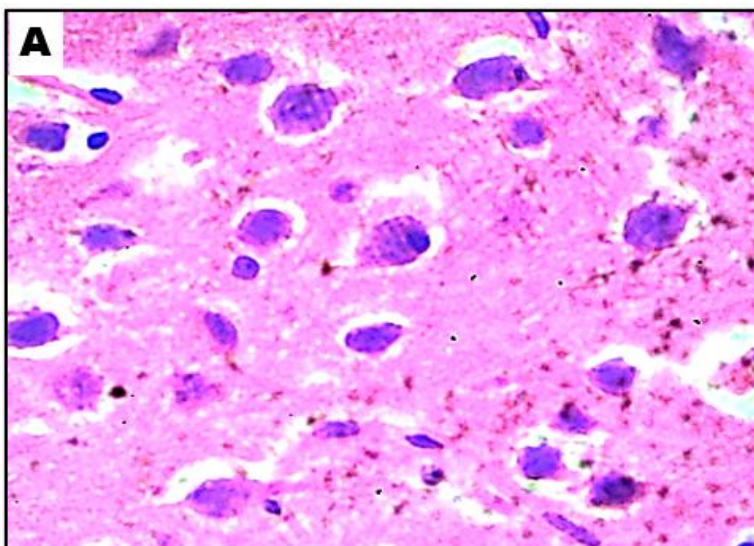


Plate 1. Photomicrograph of the cerebral cortex of adult wistar rat in group A- control X400 (H&E)

3.3 Group A (Control)

PLATE A: Photomicrograph of control group showing a normal histological features of the cortex. H&E staining show cortex micromorphological presentations in Wistar rats in the control group. The cortical layers appeared normal with conspicuous pyramidal neurons in the parenchyma. The External pyramidal layer (III), Internal granular layer (IV), are all demonstrated.

3.4 Group B: Treated with 250 Mg/Kg of Metronidazole

Photomicrograph of group B treated with 250 mg/kg of metronidazole for 28 days. It showed conspicuous degenerative changes. Wide

Perineural spaces can be seen surrounding degenerating neurons. The External pyramidal layer (III), Internal granular layer (IV), are demonstrated. Red arrow degenerating and unhealthy with loss of cytoplasmic and nuclear materials.

3.5 Group C: Treated with 500 Mg/Kg of Metronidazole

Photomicrograph of group C treated with 500 mg/kg of metronidazole for 28 days. It showed increase degenerative cerebral cortical layers with peripheral and central degenerative changes. External pyramidal layer (III), Internal granular layer (IV) are well demonstrated. Red arrow- degenerating and unhealthy cells with loss of cytoplasmic and nuclear materials.

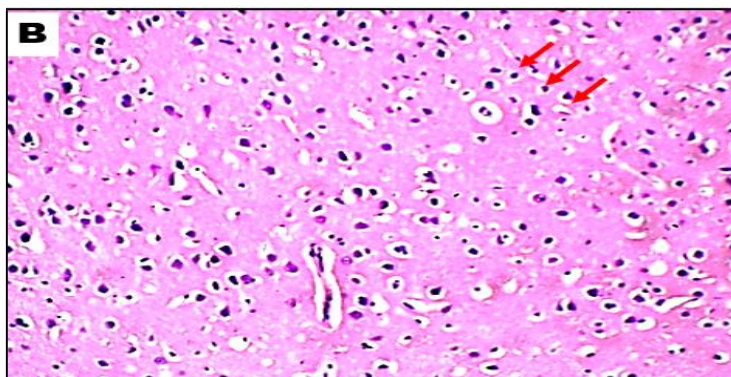


Plate 2A. Photomicrograph of the cerebral cortex of adult wistar rat in group B (experimental X100) (H&E)

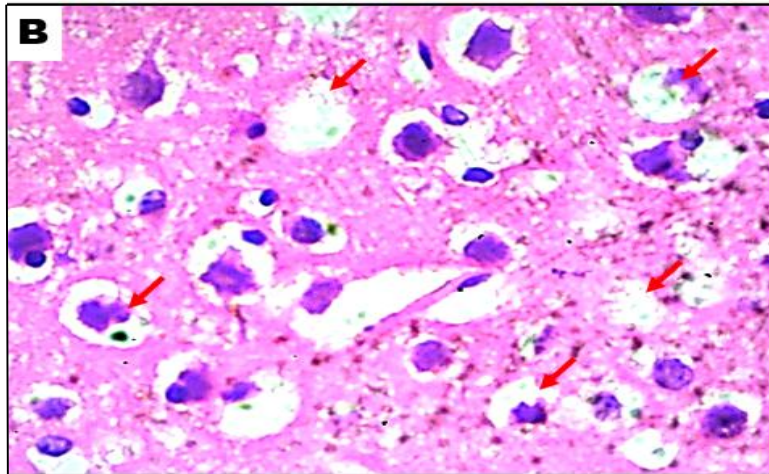


Plate 2B. Photomicrograph of the cerebral cortex of adult wistar rat in group B (experimental X400) (H&E)

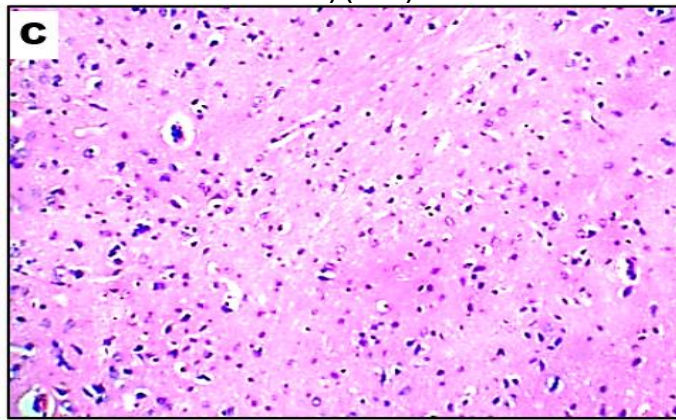


Plate 3A. Photomicrograph of the cerebral cortex of adult wistar rat in group C (experimental X100) (H&E)

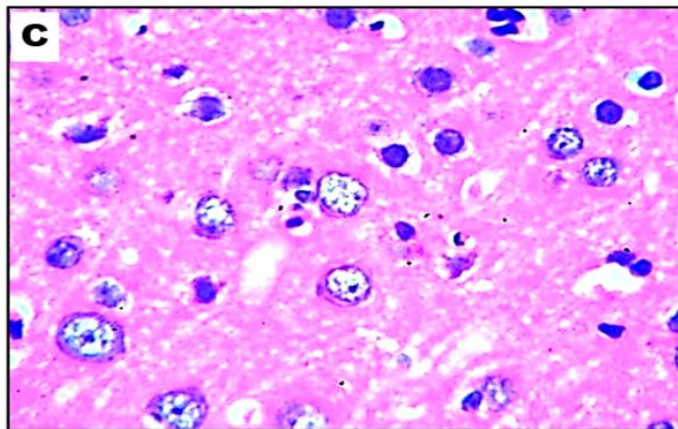


Plate 3B. Photomicrograph of the cerebral cortex of adult wistar rat in group C (experimental X400) (H&E)

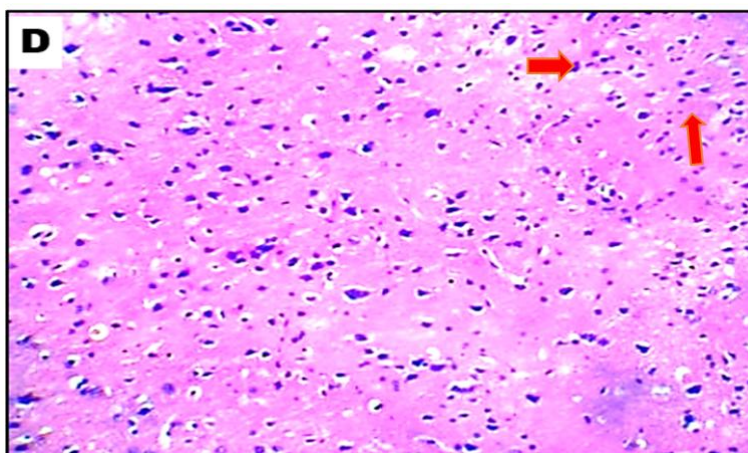


Plate 4A. Photomicrograph of the cerebral cortex of adult wistar rat in group D (experimental X100) (H&E)

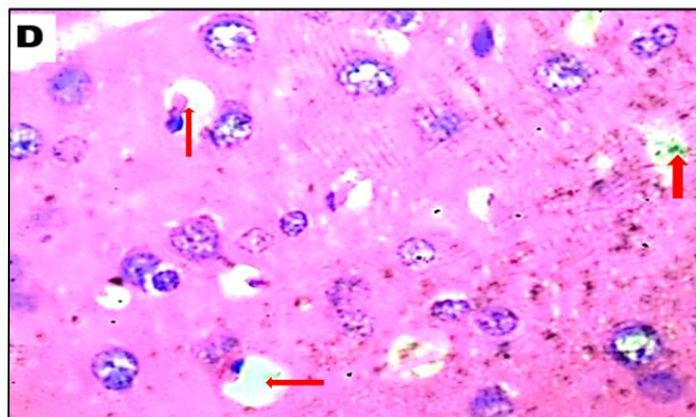


Plate 4B. Photomicrograph of the cerebral cortex of adult wistar rat in group D (experimental X400) (H&E)

3.6 Group D: Treated with 1000 Mg/Kg of Metronidazole

Photomicrograph of group D treated with 1000 mg/kg of metronidazole for 28 days. It showed increase degenerative cerebral cortical layers with peripheral and central degenerative changes. External pyramidal layer (III), Internal granular layer (IV) are well demonstrated. Red arrow- degenerating and unhealthy cells with loss of cytoplasmic and nuclear materials.

4. DISCUSSION

This study investigated the chronic administration of metronidazole on the morphology and biochemical studies on the cerebral cortex of adult wistar rat. Metronidazole (MTZ) is a

nitroimidazole antibiotic which is useful in the treatment of infections caused by anaerobic bacteria [1,2]. Previously, several studies on the oral administration of metronidazole have shown cerebellar dysfunction, visual impairment, vestibulotoxicity, cochleotoxicity, ataxic gait, dysarthria, seizures, neuropathy and central nervous system toxicity [20,21,22].

The result of this study revealed that the administration of metronidazole has some effects on the cerebral cortex of the adult wistar rats. The result of body weights analysis showed a significant increase ($P < 0.05$) in the final body weights of rats in group A while there was an insignificant increase ($P > 0.05$) in the final total body weights of rats in group B. There was also a significant reduction ($P < 0.05$) in body weights

of rats in group C and D which received 500mg/kg and 1000mg/kg of metronidazole respectively as compared with group A and B.

The loss in the body weights in this study may be due to the dose difference and duration of administration of metronidazole. Also, it could be as a result of anorexia (loss of appetite) which was noticed in the animals and supported by previous report of investigation as well [23]. A research was also carried out where the final weight of the animals treated with metronidazole decreased when compared with their initial weights, that is, there was a significant decrease in the mean final weight ($P < 0.05$) [24]. This report was also supported by another previous work carried out which showed a significant reduction in the body weights of rats treated with 200 mg/kg and 400 mg/kg of metronidazole compared to their control group [25].

The study also showed an insignificant increase in the brain weights of rats in groups (B and C) treated with 250 mg/kg and 500 mg/kg metronidazole respectively compared with the control group while group D which was treated with 1000 mg/kg of metronidazole showed a significant increase in the brain weight. In addition, a previous research showed that there was significant ($p < 0.05$) increase in the absolute brain weight gain in Olusosun (OSL) and Aba Elu (AEL) treated rats [26]. Another study showed that increase brain weight gain observed in mice exposed to landfill leachates for 7 days was the most obviously affected organ compared to other viscera examined [27].

Malondialdehyde (MDA) is a compound that describes the activity of free radicals in cells so that it is used as one of the indications of oxidative stress caused by free radicals [28]. Another study reinforces this statement by stating that the mediator Malondialdehyde (MDA) is a final product of fat peroxidation which is used as a biological biomarker of fat peroxidation and can describe the degree of oxidative stress [29].

The results of biochemical parameters investigated shows a significant increase ($P < 0.05$) in level of MDA (Malondialdehyde) in group B, C and D that received 250 mg/kg, 500 mg/kg and 1000 mg/kg of metronidazole respectively as compared with group A, that was, the control group and this finding is supported by the report of a study previously carried out which showed significant increase in MDA in the metronidazole treated animals in comparison

with the control group [30]. A study was carried out which showed that the metronidazole treated mice resulted into an expanded action of MDA which raised Lipid peroxidation (LPO) level and that suggested later harm of the body cells [18].

A study was also carried out on protective effect of selenium on gentamicin-induced oxidative stress and nephrotoxicity in rats and it was observed that gentamicin (GM) treatment caused enhanced generation of Reactive oxygen species (ROS) and accelerates the Lipid peroxidation (LPO) of bio-membranes in the renal parenchyma and consequently increased the tissue levels of MDA [31]. Additionally, another study conducted on the level of Malondialdehyde in cerebellum of rats treated with Methotrexate demonstrated that Methotrexate (MTX) caused a significant increase in MDA level in the cerebellum of wistar rat [32].

Another study on fish liver sample also indicated that MDA level was increased in all metronidazole exposed fish liver sample. The amount of MDA in 0.5 and 2.5 mg/L groups was significantly higher than that of the control ($P < 0.05$), while that in 0.1 mg/L MTZ showed only a slight elevation. [33].

Nitric oxide (NO) has basically dual action in biological systems. Low optimal concentrations regulate a lot of physiological processes however overproduction of nitric oxide plays role in pathogenesis of many diseases [34]. The level of NO (nitric Oxide) in group B, C and D that received 250 mg/kg, 500 mg/kg and 1000 mg/kg of metronidazole respectively also increased significantly ($P < 0.05$) as compared with group A. This result is supported by a previous study which indicated that administration of Doxorubicin (DOX) exhibited pronounced increase in nitric oxide (NO) and cytosolic calcium by 85 and 76% respectively as compared to normal group [35].

Succinate dehydrogenase (SDH) is the smallest member of mitochondrial respiratory chain and it ties tricarboxylic acid (TCA) cycle and the respiratory chain [36]. SDH is a membrane-bound dehydrogenase linked to the respiratory chain and a member of the tricarboxylic acid (TCA) cycle or Krebs cycle or citric acid cycle [37]. Its activity is modulated by several activators and inhibitors. The level of SDH (Succinate dehydrogenase) in group B, C and D that received 250 mg/kg, 500 mg/kg and 1000 mg/kg of metronidazole respectively increased

significantly ($P < 0.05$) as compared with group A. The result from this study is in agreement with the report from a previous work that shows the analysis of SDH activity in young and old B6xCBA/F1 mice treated with control solution (CS) or 3-Nitropropionic acid (3NP) (20 mg/kg/day). Post-hoc analysis showed that old mice presented an increase in SDH activity independently of the treatment. Regarding basal activity of SDH in old mice, it was observed that its activity was significantly higher when compared to young mice. [38].

Furthermore, findings from other studies showed that mice with 12–14 months of age have an increase in SDH activity when treated with 3-Nitropropionic acid (3NP) [39]. The results indicated that an increase in SDH activity in old mice could act as a compensatory mechanism to counteract the increase in oxidative stress related to the aging process, which would decrease the vulnerability of the behavioral alterations induced by 3NP [38]. Similarly, other previous study showed that a ten to twenty percent increase in succinate dehydrogenase activity found in homogenates of fresh heart tissue from rats which had received ethanol continuously for six months compared with tissue from control rats which had not been given ethanol [40].

Microscopic examination shows normal histological features of the cerebral cortex are observed in groups A and in groups C and D which received 500 mg/kg and 1000 mg/kg of metronidazole respectively. Group B treatment which received 250 mg/kg of metronidazole showed conspicuous degenerative changes in the cortex. Normal histological features of the cortex are observed in group A treatment; this is characterized by large pyramidal as well as granule neurons, the pyramidal cells were characterized with long axons that extend well from the soma to adjacent neurons within the neuropil.

Group B treatment at magnification X100 caused conspicuous degenerative changes in the cortex that was characterized by peripheral and central degenerative changes, clustered pyknotic pyramidal and granule neurons that appear with fragmented cytoplasm and condensed nuclei. Wide Perineural spaces can be seen surrounding degenerating neurons. Group C and D at magnification X400 show increase degenerative cerebral cortical layers with peripheral and central degenerative changes. The pyramidal

and granule neurons appeared degenerated with increased perineural spaces and the cortical tissue appeared distorted. This result was supported by a study that show rupture of the meninges membrane, vacuolation the molecular and granular layers, Glial cells hypertrophy and Degeneration of nerve fibers in the Brain section for female white rat in pregnant stage 20-day treatment with metronidazole medicine (500 mg/kg). Brain section for female white rat in pregnant stage 20-day treatment the metronidazole medicine (1000 mg/kg) show Pia matre damaged, Degeneration of nerve fibers, vacuolation around nerve cells and supporting glial cells. [41].

A study was also carried out which show that the treatment of rabbits with metronidazole in a dose of 20 and 40 mg/kg, and through microscopic histological examination of the brain and sciatic nerve, led to cavitations in the brain, spongy changes and degeneration of nerve bundles in the sciatic nerve with cell degeneration and the loss of Burkinji cells with severe congestion in the brain with loss of the covering Myeloma of the sciatic nerve axons [42].

This result was also supported in a previous research which showed that the cerebral cortex of group B rats that were exposed to 135 mg/kg of metronidazole showed satellite perineuronal oligodendroglia surrounding small shrunken neurons with contracted dense nuclei and condensed cytoplasm while the microscopic changes of group C rats which consist of congestion of cerebral and cerebellar blood vessels and hemorrhage. Satellitosis and neuronophagia were also observed in the treated groups. Moreover, mild mononuclear perivascular cuffing, focal and diffuse glial proliferations were present. Cerebellar degeneration was clear and represented by selective necrosis of Purkinje cells and depletion in granule cells layer [16].

5. CONCLUSION

The study concluded that the findings of this study showed that administration of high dosage of metronidazole induced neurodegenerative changes in the cerebral cortex of the adult wistar rats investigated.

ETHICAL APPROVAL

Animal Ethic committee approval has been collected and preserved by the author(s)

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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