



## Original Research Article

# The effect of metronidazole on the histology of the cerebellum and pituitary gland in female wistar rats

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## ABSTRACT

Metronidazole (MTZ) has been reported to cause neurotoxicity and this has great public health importance. This is an experimental study designed to evaluate the effects of metronidazole on the histology of the cerebellum and pituitary gland in female wistar rats. Twenty (20) adult female wistar rats weighing between 170-260g were divided into four groups (A-D) comprising of five (5) rats each. Group A (the control), was given normal rat feed with water, while group B, C, and D received 50mg/kg, 200mg/kg and 400mg/kg body weight of MTZ orally on daily basis using intubation method for a period of twenty eight (28) days respectively. Thereafter, the experimental animals were sacrificed and their respective cerebellum and pituitary gland harvested for histological examination using haematoxylin and eosin (H and E) method. The histological examination of the cerebellum of the experimental animals in group A (control) revealed a normal histological limits, showing the cortex, prominent purkinje cells and granular layer. However, the rats in group B showed mild congestion of cerebellar blood vessels; group C showed mild displacement of the purkinje cells and the granular layer while group D revealed the displacement of the purkinje cells and the granular layer. Furthermore, the pituitary gland of the control rats (group A) showed normal histological limits. There was no significant change in the histology of the pituitary gland of the rats in group B (50mg/kg body weight of MTZ) but in the group C animals, the pars nervosa displayed the pituicytes with mild necrosis while those in group D showed that there was a reduction in the presence of pituicytes and mild congestion of the blood vessels. Thus, metronidazole has an adverse effect on the cerebellum and pituitary gland.

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## 1. Introduction

Metronidazole (MTZ) is a nitroimidazole antibiotic which is useful in the treatment of infections caused by anaerobic bacteria.<sup>1,2</sup> Since metronidazole (MTZ) is supposed to penetrate extensively into central nervous system (CNS), it has been described in literature as being responsible for both peripheral and central neurotoxicity, especially after

a prolonged use of metronidazole (MTZ).<sup>3,4</sup> Peripheral neuropathy appears to be the most common adverse effect of metronidazole use,<sup>5</sup> although it varies in the central nervous system ranging from seizures to encephalopathy and cerebellar syndrome.<sup>6,7</sup> However, the pathogenesis of metronidazole neurotoxicity is currently unknown although a number of hypotheses have been proposed in this respect in an attempt to unravel the mechanism behind metronidazole neurotoxicity. Proposed

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mechanisms include binding of metronidazole to neural RNA to inhibit protein synthesis, modulation of inhibitory neurotransmitters F-aminobutyric acid receptor within the cerebellar and vestibular, reversible mitochondrial dysfunction, and vasogenic and cytotoxic edema.<sup>8,9</sup> Oda<sup>10</sup> showed earlier in his study that metronidazole caused marked histopathological alterations in the brain, liver, kidney, testes and epididymis in rats. Also, a number of studies have reported Cerebellar dysfunction, visual impairment, vestibulotoxicity, cochleotoxicity, ataxic gait, dysarthria, and seizures following metronidazole use.<sup>11,12</sup> Therefore, this study was designed to investigate the effect of metronidazole on the histology of the cerebellum and pituitary gland in female adult wistar rats.

## 2. Materials and Methods

### 2.1. Experimental site

This study was carried out in the animal house of the Department of Human Anatomy, College of Health Sciences and Technology, Nnamdi Azikiwe University Nnewi Campus, Anambra State, Nigeria.

### 2.2. Collection, Identification and Preparation

Metronidazole by M&B (200mg) with expiration date of 2018 was purchased from a pharmaceutical store at Awka, Anambra State, Nigeria in the month of May, 2015. It was ground to fine powder and dissolved in 20ml of water 15 minutes before administration daily for proper dissolution. The animal feed used for this study was the normal grower's mash, product of premier feed mills company limited located in Sapele, Delta State, Nigeria.

### 2.3. Experimental animals and study design

Twenty (20) adult female wistar rats weighing between 170-260g were used for the experiment. The rats were bought from Nkwo Market Nnewi, Nigeria. They were housed in four standard cages and were divided into 4 groups (A to D). The animals were provided with feed and water ad libitum. Prior to the commencement of the experiment, the animals were pre-conditioned for a period of two weeks following which the substrate for the test was administered to the experimental animals for a period of 28 days; the entire experimental study lasted for a period of 6 weeks. The animal care and handling was conducted in compliance with the National Regulations for Animal Research. The group A, served as the Control group while group B, C, and D served as the Test groups respectively. Metronidazole was administered to the animals as follows:

Group A served as control group and received distill water and feed.

Group B received 50mg/kg body weight/ day of metronidazole.

Group C received 200mg/kg body weight/ day of metronidazole.

Group D received 400mg/kg body weight/ day of metronidazole.

All administration was done using syringe and oral cannula. The administration lasted for 28 days using intubation method following which the animals were sacrificed after 24 hours of administration and they were anesthetized by chloroform inhalation and the brain was harvested.

### 2.4. Extraction of organs and Histopathological Examination

At the end of the experiment and following the sacrificing of the experimental animals, the pituitary gland and cerebellum of experimental animal groups were harvested and placed immediately in a fixative with 10% Buffered neutral formalin. Thereafter, the well fixed tissues were processed, sectioned and stained following standard procedure.<sup>13</sup> The histological evaluation of the effect of metronidazole on the cerebellum and pituitary gland of female wistar rats were done by microscopic examination of haematoxylin and eosin (H&E) stained sections.

### 2.5. Ethical Consideration

The University Ethical Committee reviewed the protocols, which were consistent with International Animal Welfare Guidelines.

### 2.6. Statistical Analysis

The data obtained was presented as mean $\pm$ SD and the mean values of test groups were compared by Students t-test using Statistical package for social sciences (SPSS) (Version 23) software. Statistical significance was tested at  $P < 0.05$ .

## 3. Results

The mean weight of the control animals did not differ significantly when the initial and final mean weights were compared ( $p=0.070$ ). However, there was a statistically significant decrease in the mean final weight of the experimental animals in the groups B, C and D, when compared with their initial weight respectively ( $p < 0.05$ ). See Table 1.

### 3.1. Histopathological Findings

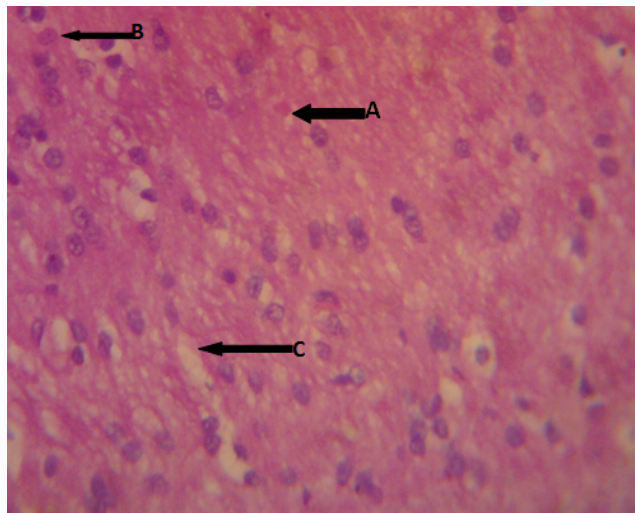
The result of the microscopic examination of the cells of the pituitary gland of the rats in group A (control) showed normal chromophobes, acidophils, basophils, pars distalis and pars nervosa. See Figures 1, 2 and 3 shows the histology of the pituitary gland of the rats in group B (treated with 50mg/kg body weight of metronidazole). There was no significant change. Figure 4 shows the histology of the

**Table 1:** Comparison of mean values of initial and final body weight in experimental animal groups studied (Mean± SD).

Experimental groups	Initial weight (g)	Final weight (g)	t-value	P-value
Group A	206.00±16.00	224.00±10.30	-2.449	0.070
Group B	234.00±11.22	212.60±10.20	3.773	0.020
Group C	218.00±11.14	200.00±8.37	2.714	0.040
Group D	240.00±15.49	208.00±10.68	5.488	0.005

\* Data were considered significant at  $P < 0.05$ .

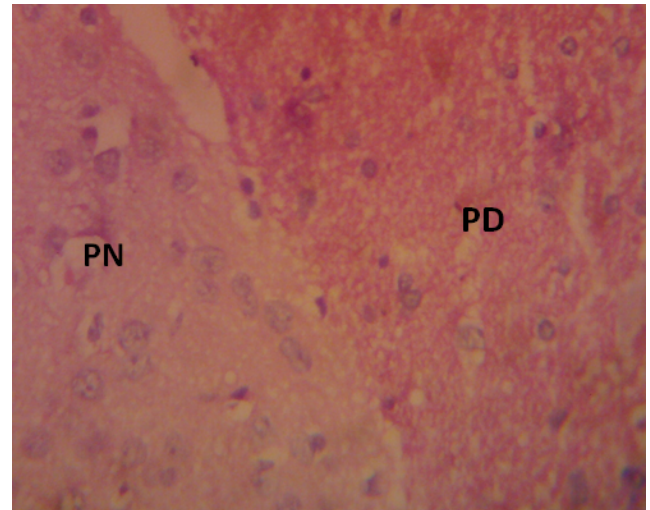
pituitary gland of the rats in group C (treated with 200mg/kg body weight of MTZ). The pars nervosa of the pituitary gland displays the pituicytes with mild necrosis. Figure 5 shows the histology of the pituitary gland of the rats in group D (treated with 400mg/kg body weight of MTZ), there was reduction in the presence of pituicytes and mild congestion of the blood vessel. Figure 6 represents the histology of the cerebellum of the rats in the control group, showing the cortex, prominent purkinje cells and granular layer. Figure 7 shows the histology of the cerebellum of the rats in group B. It showed mild congestion of the cerebellar blood vessels. Figure 8 represents the histology of the cerebellum of the rats in group C. It showed mild displacement of the purkinje cells with congestion. Figure 9 shows the histology of the cerebellum of the rats in group D. It showed displacement of the purkinje cells and the granular layer.



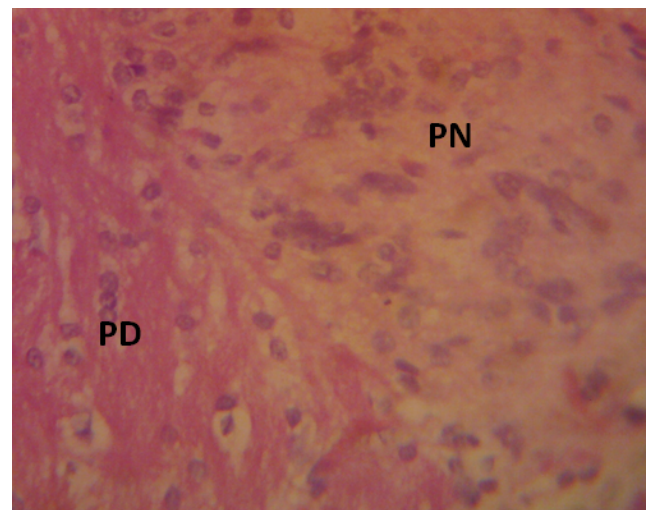
**Fig. 1:** H&E Section of the control group showing the micrograph of the normal pituitary gland. Chromophobes (C), Acidophils (A), and Basophils (B) are all normal in structure. MAGNIFICATION=X400

#### 4. Discussion

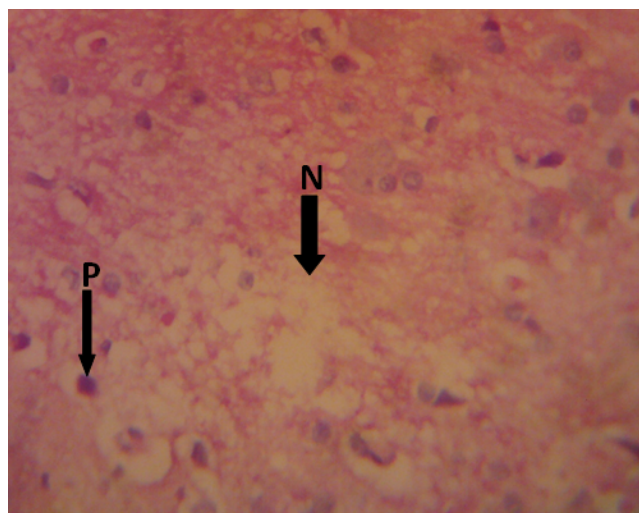
Metronidazole is a nitroimidazole antibiotic which is useful in the treatment of infections caused by anaerobic bacteria.<sup>1,2</sup> Previously, several animal studies on the oral administration of metronidazole has shown increased incidence of tumor in the lungs, liver, testes, mammary



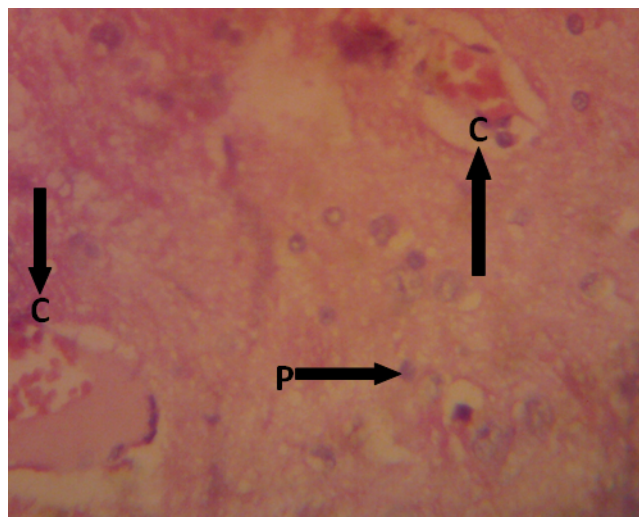
**Fig. 2:** H&E section of the pituitary gland of the control group showing the pars distalis (PD) and pars nervosa (PN). MAGNIFICATION=X400.



**Fig. 3:** H&E Section of the pituitary gland of the test Group B Showing no significant changes compared to the control group. MAGNIFICATION=X400



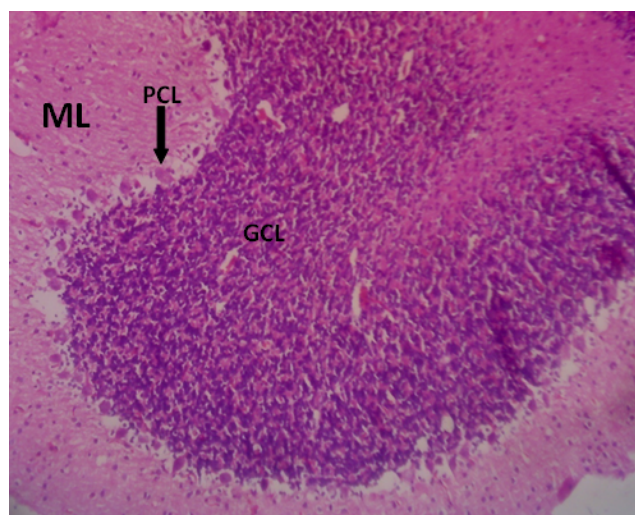
**Fig. 4:** H&E Section of the test Group C showing light micrograph of the pars nervosa, the pituitary gland displaying pituicytes (P) with mild necrosis (N). MAGNIFICATION=X400.



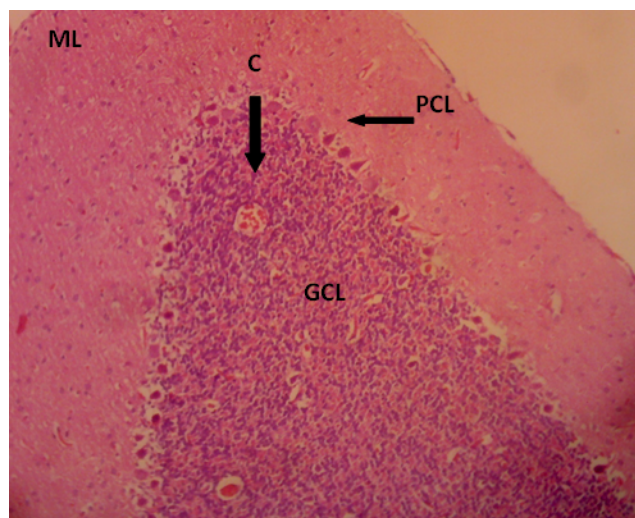
**Fig. 5:** H&E Section of the test group D. The photomicrograph displays reduction in the presence of pituicytes (P) and mild congestion of blood vessels (C). MAGNIFICATION=X400

gland and pituitary gland of certain rodent species.<sup>14,15</sup> Other studies also show that high dose or long term systematic treatment with metronidazole is associated with the development of leukopodia, neuropathy and / or central nervous system toxicity.<sup>14</sup>

In this study, observation of the body weight difference in experimental groups revealed a gradual increase in the mean weight of the experimental animals in group A (control group). This could have been due to physiological effect arising from the substance experimental animals were exposed which was basically water and feed only. However, there was a statistically significant decrease in the final



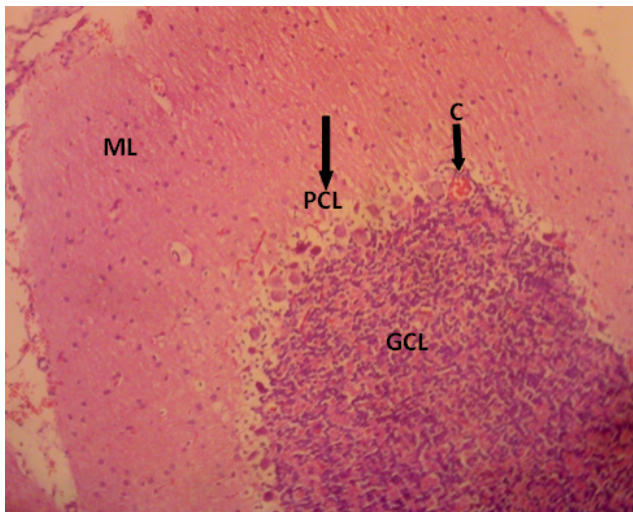
**Fig. 6:** H&E Section of the cerebellum of the control group displaying the photomicrograph of the normal cerebellum showing its cortex, prominent purkinje cells (PCL) and granular layer (GCL). MAGNIFICATION=X100



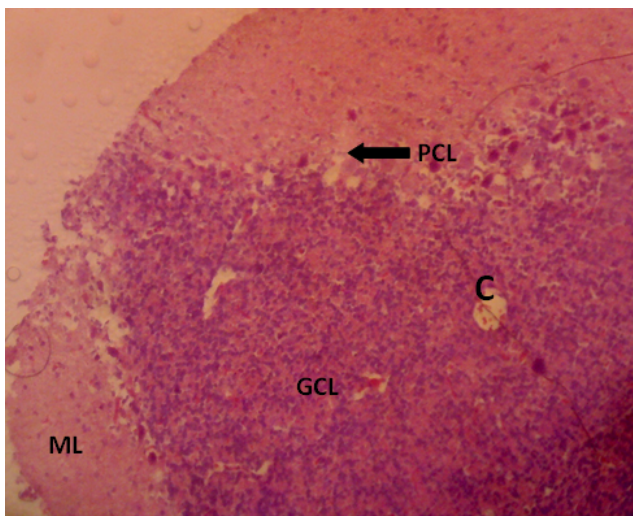
**Fig. 7:** H&E Section of the cerebellum of test group B showing mild congestion of the cerebellar blood vessels (C). Purkinje cell layer (PCL), Granular cell layer (GCL) and Molecular layer (ML). MAGNIFICATION=X100

weight of rats in groups B, C and D respectively when compared with their initial weight; this may be due to dose difference and duration of MTZ administration.

The present study reports the toxicological effects of metronidazole on the cerebellum and pituitary gland of female wistar rats treated with 50mg, 200mg and 400mg therapeutic doses of metronidazole. It was observed that the histology of the cerebellum of the control group showed normal cortex, prominent purkinje cells and granular layer, that is, they were under normal histological limits. The group treated with 50mg/kg/day of MTZ (group B), showed



**Fig. 8:** H&E Section of the cerebellum of the test group C showing mild displacement of the purkinje cells (PCL) with congestion of blood vessels (C). Granular cell layer (GCL) and Molecular layer (ML). MAGNIFICATION=X100.



**Fig. 9:** H&E section of the cerebellum of the test group D showing displacement of the purkinje cell layer (PCL), the granular cell layer (GCL) with congestion (C), and the Molecular layer (ML). MAGNIFICATION=X100.

mild congestion of the cerebellar blood vessel and mild congestion. This agrees with the report of Oda, (2012) whose histologic examination of the cerebellum showed congestion of cerebral and cerebellar blood vessels and hemorrhage as well as a depletion in the granular cell layer. The effects may be due to the action of metabolites present in metronidazole.

Furthermore, the experimental animals treated with 200mg/kg/day of MTZ (group C) showed mild displacement of the purkinje cells with congestion. This may imply

a progressive disruption of the cerebellum with increasing dosage of metronidazole over a prolonged period of time which may lead to cerebellar toxicity. This is in consonance with the report by Agarwal et al.<sup>16</sup> Interestingly, the last experimental animal group treated with 400mg/kg/day of MTZ (group D) showed displacement of the purkinje cells and the granular layer. This agrees with report by Oda, (2012), and may be as a result of destruction of DNA of the cells. The results of this work agree with previous researches that metronidazole has toxicological effect on brain cells.<sup>14,17</sup>

However, the pituitary gland of the experimental animals in group A (control) showed normal chromophobes, acidophils, basophil, pars distalis and pars nervosa. Also, the experimental group treated with 50mg/kg/day of MTZ (group B) showed no significant changes but the animal group treated with 200mg/kg/day of MTZ (group C) showed that the pars nervosa (cells of the posterior pituitary) of the pituitary gland displays the pituicytes with resultant mild necrosis. The last group treated with 400mg/kg/day of MTZ showed reduction in the presence of pituicytes and mild congestion of the blood vessel. This also agrees with report of Azadeh, (2014), because when there is damage or distortion of pituitary cells, it could result in pituitary tumor. Several other studies had earlier documented varying degrees of neurotoxicity arising from metronidazole use.<sup>18</sup> The results of this work agree with previous studies that metronidazole has toxicological effect on brain cells. Therefore, the dosage, pharmacodynamic, pharmacokinetics and bioavailability of this drug should be properly considered especially when requiring high dosage for therapeutic management over a prolonged period of time in order to avoid destruction of cerebellum and pituitary gland.

## 5. Conclusion

The result of this study suggests that long term systematic administration of metronidazole and high dosage in rats, can be toxic to the brain, that is it causes distortion or displacement of the cells of the cerebellum and also causes damage to the cells of the pituitary gland. However, there is an urgent need for more studies in this area with the view of better understanding the mechanism underlying metronidazole induced toxicity.

## 6. Limitations of the work

Due to financial constraints we could not use large sample size for the study. Therefore, we suggest that further studies in this area be conducted using larger sample size whenever possible to further ascertain the present claims.

## 7. Source of funding

None.

## 8. Conflict of interest

None.

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