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## Histopathological Changes of the Testes of Wistar Rats Following Metronidazole Administration

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### ABSTRACT

Damage to the human spermatozoa membrane due to oxidative stress caused by the toxic effects of metronidazole has been implicated as an important pathophysiological mechanism in male fertility. This study was designed to investigate the direct effects of metronidazole on the testicular tissue and that of the introduction of vitamin E to metronidazole treated rats. It also looked at the natural reversal effects on abstinence from medication for 8 weeks. A total of 105 adult male and 50 female wistar rats weighing  $170 \pm 10$ g (70-90 day old) were used for the experiment. The rats were randomly divided into a control and experimental groups. There were four major groups with 5 subgroups consisting of 5 rats each. Varying doses of 15 mg/kg, 30 mg/kg, 200 mg/kg and 400 mg/kg of metronidazole were administered in experiment 1 to 4 respectively. Each experimental has 5 sub-groups: A; control, B; group administered with the experimental dose, C; experimental dose with vitamin E, D; experimental dose with testosterone, E; treated with experimental dose, vitamin E and testosterone. Sub-group F is a reversal group which was left for 8 weeks after cessation of treatment. At the end of the experiment, animals were sacrificed and testicular tissues were dissected out and weighed. Tissues were preserved in 50% Bouin fluid and thereafter prepared for histological studies. Results showed that metronidazole at the therapeutic dose of 15 mg/kg did not have significant negative effect on histology of the testis. At the dose of 30 mg/kg, 200 mg/kg and 400 mg/kg some pathological changes were observed which were dose depended ranging from mild interstitial edema, loss of germinal epithelium, foci of tubular wall thickening and widening of the interstitial spaces. Groups in which metronidazole was concurrently administered with vitamin E and or testosterone showed some levels of protection. The reversal groups showed normal to near normal histological features. The results of this study indicate that metronidazole administration (200 mg/kg or 400 mg/kg), for 8 weeks, caused a harmful effect on the architecture of the testes and these effects were reversible after 8 weeks of abstinence.

**Key words:** Metronidazole, histo-architecture, testis, vitamin E, testosterone

### INTRODUCTION

Metronidazole, an anti-infective drug is used mainly in the treatment of infections caused by anaerobic bacteria and protozoa such as amoeba. Metronidazole is taken up by diffusion and it is selectively absorbed by anaerobic bacteria and sensitive protozoa and once it is taken up by the anaerobes, it is non-enzymatically reduced by reacting with reduced ferredoxin which is generated by pyruvate oxido-reductase.<sup>1</sup> This reduction causes the production of toxic products to anaerobic cells, and allows for selective accumulation in anaerobes.<sup>1</sup> The metronidazole metabolites are taken up into bacterial DNA, and form unstable molecules. This function only occurs when metronidazole is partially reduced, and because this reduction usually happens only in anaerobic cells, it has relatively little effect upon human cells or aerobic bacteria.<sup>2</sup>

The anti-spermatogenic effect of metronidazole has also been shown in some studies. Organisms, such as flagellated protozoa, are more resistant to metronidazole and chemicals that kill these organisms might be toxic to flagellated sperm cell as well.<sup>3,4</sup> Other derivatives of metronidazole as well as ornidazole exert a rapid and reversible anti-fertility effect in male rats.<sup>5,6</sup> In dogs, humans and rats, one of the metabolites of ornidazole is the C3-chloro side – chain of the nitroimidazole ring which can produce 3-chloro-lactaldehyde and  $\alpha$ -chloro-hydrin, the known inhibitors of the glycolytic enzymes such as glyceraldehydes-3-Phosphate dehydrogenase (GAPDH) and triose-phosphate isomerase (TPI) in the spermatozoa.<sup>7,8</sup> Although teratogenic in some animals, metronidazole has not been associated with this effect in humans, but it is best avoided in pregnant and nursing women.<sup>9</sup>

Metronidazole 500mg/kg administration via the nasogastric route for 14 days caused a harmful effect on male fertility in mice after one month of administration.<sup>1</sup> The effect of metronidazole on the male reproductive system cannot be overlooked because there is a growing concern over reduction in male sexual capacity which has been reported to be declining over the past 50 years.<sup>10,11,12</sup>

This study is therefore, aimed at determining the effect of metronidazole on the histology of the male wistar rats testes.

## MATERIALS AND METHODS

The research was given due approval by the Research Ethic Committee of the Niger University College of Health Sciences before commencement of work.

Metronidazole, vitamin E and testosterone were purchased from Cynflac Pharmacy, hospital road, Yenagoa, Bayelsa State.

### Animals and Treatment

A total of 105 adult male and 50 female wistar rats weighing 170±10g (70-90 day old) were obtained from the Animal house of the College of Health Sciences, Niger Delta University, Wilberforce Island. They were maintained in 12 h light and 12h dark conditions at a temperature of 27°C - 30°C in the animal house. The standard laboratory chew and tap water were available ad libitum. After the acclimatization period of two weeks, the rats were randomly divided into a control and experimental groups. There were four major groups with 5 subgroups consisting of 5 rats each.

Experiment 1: adult rats treated with 15 mg/kg of metronidazole

Adult male rats weighing 75 g ± 5, about 70-90 days old at the commencement of the experiment were used. There were divided into a control group (1A), a group treated with metronidazole only (1B), administered with metronidazole and vitamin E (1C), treated with metronidazole and testosterone (1D), treated with metronidazole, vitamin E and testosterone concurrently (1E). A metronidazole reversal group (Group 1F) after 8 weeks of cessation of treatment.

Each sub-group had 5 rats randomly divided into the groups. The vitamin E and testosterone were administered at the dose of 400 mg/kg/day and 0.36 mg/kg/day respectfully.

Experiment 2: adult male rats treated with 30 mg/kg of metronidazole

Adult male and female rats weighing 160 g ± 0.5 about 70-90 day old at the commencement of the experiment were used. There were divided into a control group (2A), a group treated with metronidazole only (2B), administered with metronidazole and vitamin E (2C), treated with metronidazole and testosterone (2D), treated with metronidazole, vitamin E and testosterone concurrently (2E). Metronidazole reversal group (Group 2F) after 8 weeks of cessation of treatment.

Each group had 5 rats randomly divided into the groups. The metronidazole was delivered as a single dose in

0.2ml of distilled water via the nasogastric route.

Experiment 3: Adult rats treated with 200 mg/kg of metronidazole

In experiment 3, adult male rats weighing 170g±0.9, about 70-90 day old at the commencement of the experiment were used. There were divided into a control group (3A), a group treated with metronidazole only (3B), administered with metronidazole and vitamin E (3C), treated with metronidazole and testosterone (3D), treated with metronidazole, vitamin E and testosterone concurrently (3E). A metronidazole reversal group (Group 3F) after 8 weeks of cessation of treatment. The metronidazole was delivered as a single dose in 0.63ml of distilled water via the naso gastric route.

Experiment 4: Adult rats fed with 400mg/kg of metronidazole.

In experiment 4, adult male rats weighing 200g±5, about 70-90 week old at the commencement of the experiment were used. There were divided into a control group (4A), a group treated with metronidazole only (4B), administered with metronidazole and vitamin E (4C), treated with metronidazole and testosterone (4D), treated with metronidazole, vitamin E and testosterone concurrently (4E). A metronidazole reversal group (Group 4F) after 8 weeks of cessation of treatment. The metronidazole was delivered as a single dose in 1.25ml of distilled water via the nasogastric route.

### Dose of Metronidazole

The dose of 400mg/kg and 200mg/kg respectively were selected because the LD50 of metronidazole (p.o) which was earlier determined was 5000mg/kg.<sup>13</sup> The 400mg/kg dose taken in this study is less than 1/8 of the lethal dose and 200mg/kg is less than 1/16 of the lethal dose. While 15mg/kg and 30mg/kg were therapeutic doses.<sup>14</sup>

### Route of administration

The tablet form of metronidazole and vitamin E were administered through the naso-gastric route while the testosterone injection was given intramuscularly.

### Retrieval of tissues

At termination, the rats were anaesthetized with ketamine 1 mg/kg (intramuscularly). To remove the testis, a pair of scissors was inserted into the preputial orifice and cut through the ventral foreskin that covers the tip of the penis. Then penis was pulled caudally and the midventral abdominal muscles removed to expose the pubic symphysis. With the scissors, the pubic symphysis was carefully cut through and the hindlegs pulled apart to expose the pelvic canal. On the surface of the testis is the epididymis a coiled tube identified. The testis removed by cutting the spermatic cord. Testes were then collected in 50% buffered Buin's fluid.

### Organ weights

The testes were separated through dissection after

trimming off the attached tissues and weighed using the volume displacement technique. The relative weight of the organs was expressed per 100g body weight.

#### Tissue Preparation for Histology

The testes dehydrated through ascending grades of alcohol (70%, 80%, 90%, absolute) for about 24 hours. The tissues were then cleared in xylene, infiltrated and embedded in paraffin wax. Sections of 5 micron thickness were cut on Reichert ultra microtome, mounted on slides and stained with Haematoxylin first then rinsed, followed by Eosin. The slides were dehydrated in ascending alcohol solution, cleared. A coverslip is mounted onto a labeled glass slide with Permount. Tissue prepared was examined for differences in comparison to the control group by an anatomical pathologist blinded to the nature of the experiment.

#### Statistical Analysis

Data are expressed as mean  $\pm$  SD and the test of significance analyzed by the student's t-test. The differences were considered significant at  $p < 0.05$ .

### RESULTS

Testicular weight of control and rats treated with metronidazole:

There was no significant reduction in testicular weight of rats treated with 15mg/kg and 30mg/kg of metronidazole ( $p > 0.05$ ) in comparison to the control. Significant reduction was recorded in rats treated with 200 mg/kg and 400 mg/kg ( $p < 0.05$ ). There was no significant reduction in the groups in which metronidazole was concurrently administered with vitamin E and or testosterone when compared to the control ( $p < 0.05$ ) as shown in table 1.

#### Histo-pathological changes

Control: sections of testes from control rats showed normal histological features of spherical seminiferous tubules surrounded by connective tissue interstitium. The interstitium was found to be normal with no Leydig cells abnormalities. Within the seminiferous tubules were spermatogenic cells at different stages of development from spermatogonia to matured spermatozoa located from the basement membrane towards the lumen. The Sertoli cells also showed normal as shown in fig. 1

Rats treated with 15 mg/kg and 30 mg/kg of metronidazole.

Histological sections of testes from all the sub-groups (1B-1F) in this experimental group 1 showed similar histological features as in control above as shown in fig. 1A-D below. Likewise, histological sections in all the sub-groups of experiment 2 showed no pathological features. Sections show normal germinal epithelium, no Sertoli or Leydig cells abnormalities (fig. 1F-H). However, 2B which was treated with only 30 mg/kg of metronidazole showed interstitial edema in comparison to the control (fig. 1E).

#### Rats treated with 200mg/kg of metronidazole

Sub-groups 3B which was treated with 200mg/kg of metronidazole and 3D which was administered with 200mg of metronidazole and 0.36mg/kg/day of testosterone simultaneously showed mild loss of germinal epithelial cells with few spermatozoa present. No Leydig cell or Sertoli cell abnormalities (1E and 1G). However, 3C which was treated with 200mg/kg of metronidazole and 400mg/kg of vitamin E concurrently and 3E which was administered with 200mg/kg of metronidazole, 400mg/kg of vitamin E and 0.36mg/kg of testosterone did not show any observable abnormal features compared with the control as shown fig. 1F and H.

#### Rats treated with 400mg/kg of metronidazole

Histological sections of testes of rats treated with 400mg/kg of metronidazole showed moderate to severe loss of germinal epithelial cells with few spermatozoa present. Interstitial edema was also seen (fig. 2A). However, testes of rats treated concurrently with metronidazole and vitamin E showed mild loss of germinal epithelium with normal spermatogenic series but showed widening of the interstitial spaces (fig. 2F). Foci of thickening of the walls of the seminiferous tubules were seen in the group which was simultaneously administered with 400mg/kg of testosterone, vitamin E and testosterone (fig. 2D). Sub-group 4A showed marked loss of germinal epithelium, thickening of the walls of seminiferous and reduction of Leydig and Sertoli cell number in comparison to control (fig. 2E)

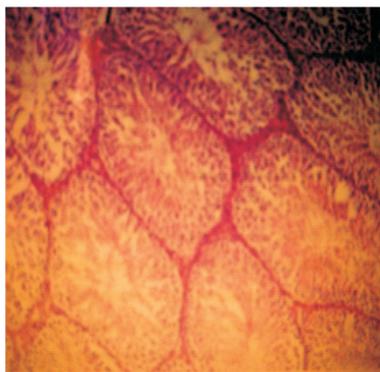
#### Reversal groups

Histological sections of testes of rats treated with 400mg/kg of metronidazole was left for eight weeks after cessation of treatment showed almost normal histology in all the reversal groups in comparison to the control. This suggested evidence of recovery from the effect of metronidazole as illustrated in fig. 2H.

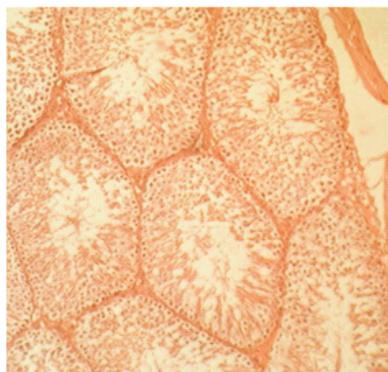
**Table 1.** Approximate testicular weights using volume displacement technique.

GROUPS	DOSE 15 mg/kg	DOSE 30 mg/kg	DOSE 200 mg/kg	DOSE 400 mg/kg
Control group	.....	0.90±0.02	0.90±0.07	0.90±0.03
Met only	0.96±0.08	0.93±0.10	0.76±0.05*	0.74±0.01*
Me + vit E	0.95±0.04	0.93±0.02	0.83±0.09	0.87±0.03
Met + Testosterone	1.03±0.05	0.87±0.01	0.97±0.06	0.90±0.13
Me + vitE + Testosterone	1.03±0.06	1.01±0.05	0.99±0.03	0.86±0.01
Met reversal	1.32±0.70	1.50±0.65	1.30±0.04	1.24±0.41

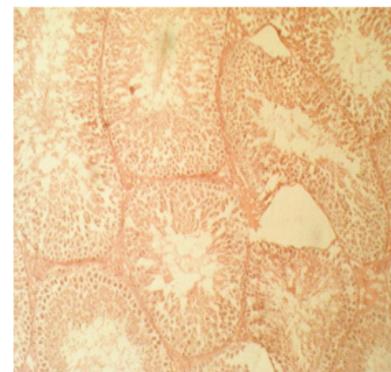
Values are expressed as mean ± standard deviation, n=5, \*=p<0.05 (significant)



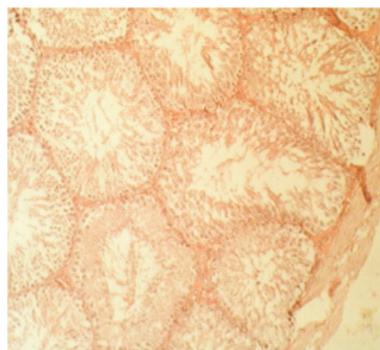
**Figure 1.** Histology of testis from control group H&E stained showing normal histo-architecture. Mag.x200



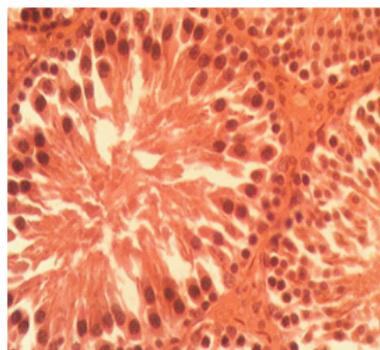
**Figure 1A** Histology of testis from 1A, treated with metronidazole only, showing normal histo-architecture.



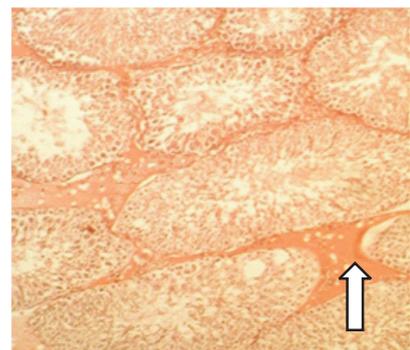
**Figure 1B.** Histology of rats from 1C, treated with metronidazole and vitamin E. Shows normal histo-architecture.



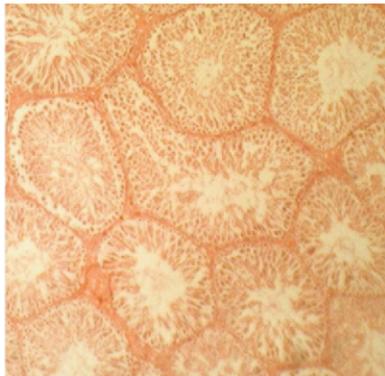
**Figure 1C.**Histology of testis from sub-group 1D, administered with metronidazole and testosterone showing normal features of germinal epithelium, interstitium and Leydig and Sertoli cell. Mag x 200. H&E



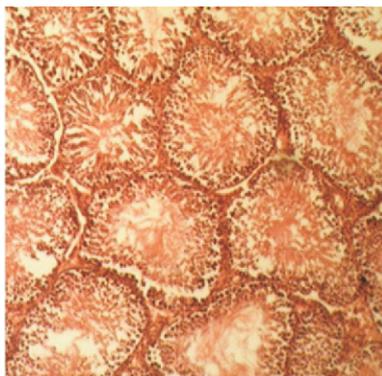
**Figure 1D** Histology of testis from 1E; metronidazole, vitamin E and testosterone treated showing normal histo-architecture. Mag. x 400. H&E



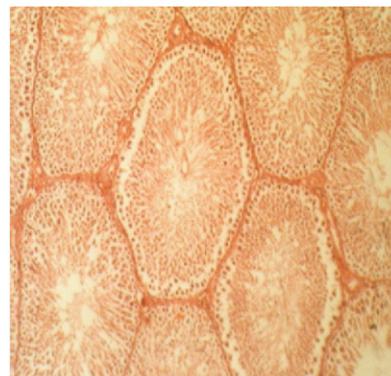
**Figure 1E** Histology of testis of rat from sub-group 2B; treated with metronidazole only showing mild interstitial edema. Mag. x 200. H&E



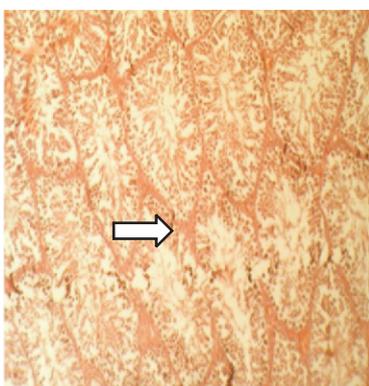
**Figure 1F** Histology of testis from rat in sub-group 2C; metronidazole and vit E treated showing near normal histology. Mag. x 200. H&E



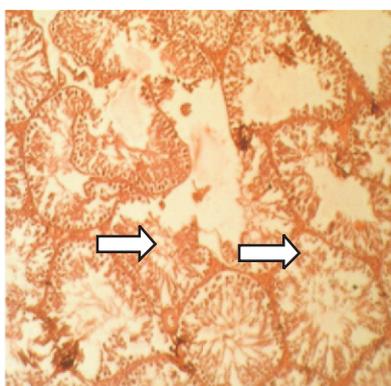
**Figure 1G.** Histology of testis of rat from sub-group 2D, treated with 30mg/kg of metronidazole and 0.36mg/kg of testosterone concurrently. Shows normal histo-architecture. Mag. x 200



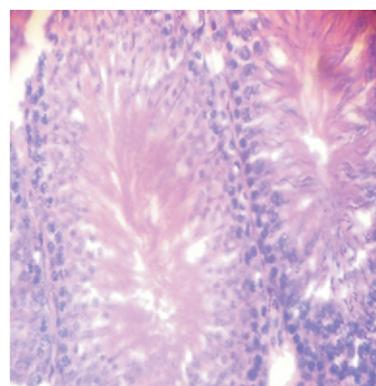
**Figure 1HI.** Histology of testis from 2E, treated with metronidazole and testosterone showing normal features of germinal epithelium, interstitium and Leydig and Sertoli cell. Mag x 200. H&E



**Figure 2A.** Histology of testis from sub-group 3B; treated with metronidazole only, shows loss of germinal epithelium. Mag. x 200



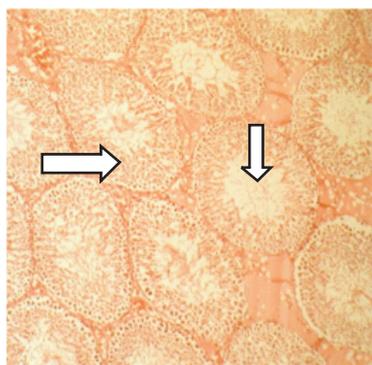
**Figure 2B.** Histology of testis from 3D, metronidazole and testosterone teated, showing disruption of the normal architecture with loss of germinal epithelium. Mag. x 200. H&E



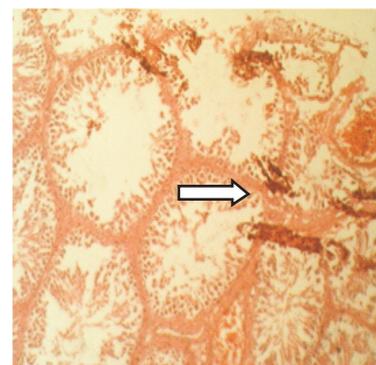
**Figure 2C.** Histology of rats from sub-group 3C, treated with metronidazole and vit E concurrently shows normal histo-architecture. Mag. x 400



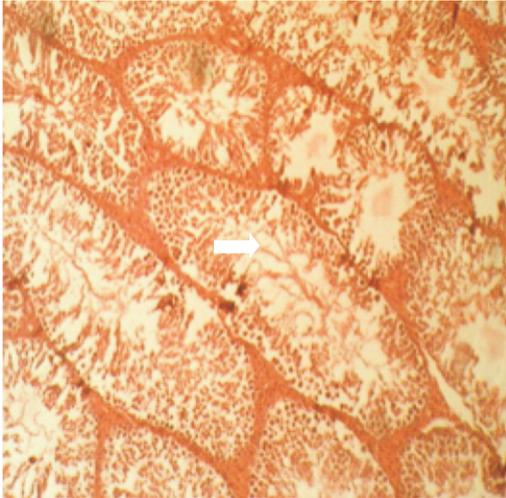
**Figure 2D.** Histology of testis of rat from 3E; administered with metronidazole, vit E and testosterone showing interstitial edema but normal germinal epithelium, Leydig and Sertoli cells. Mag. x 200



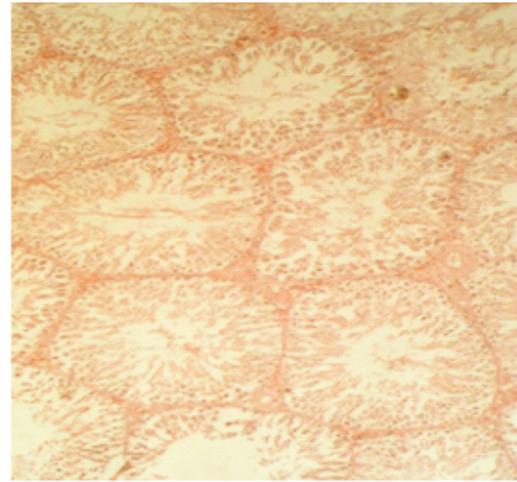
**Figure 2E.** Histology of testis of rat from 4B; treated with metronidazole, only, shows moderate to severe loss of germinal epithelium with thickening of the walls of the seminiferous tubules. Mag. x 200 . H&E



**Figure 2F** Histology of testis from 4C; treated with metronidazole and vitamin E, showing normal germinal epithelium but widened of interstitial space. Mag. x 200. H&E



**Figure 2G.** Histology of testis from sub-group 4E; treated with metronidazole, vit E and testosterone showing loss of germinal epithelium and thickening of the walls of the seminiferous tubules. Mag. x 200. H&E



**Figure 2H** Histology of testis of rat from reversal group, showing normal histo-architecture. Mag. x 200. H&E

## DISCUSSION

The mutagenic and toxic potentials of the drugs or environmental chemicals on the male germ cells have become an important area of serious concern.<sup>15</sup> Metronidazole, a 5-nitroimidazole drug has been reported to decrease testicular weight and to cause abnormal sperm morphology with degeneration of seminiferous tubules within 6 weeks of administration of metronidazole at 400mg/kg dose.<sup>4</sup> The use of metronidazole is increasing, however, its carcinogenicity has not been discarded.<sup>16</sup> Our results demonstrate that the daily treatment of 200 and 400 mg/kg/day metronidazole, for 8 consecutive weeks significantly decreased the weight of the testes. Previous studies have shown that a single oral dose of metronidazole 250 mg/kg drastically reduces the testicular weight and causes infertility in rats after 2–3 weeks, lasting for 3–4 weeks.<sup>16</sup> In our study, the effect of metronidazole administration resulted in a persistent decrease in testicular weight and histo-architectural changes ranging from interstitial oedema, widening of the interstitial spaces and mild to moderate loss of germinal epithelium. The decrease in weight of testes may be attributed to the decreased testosterone levels at all periods of the experiment in this study as earlier explained by Davood et al.<sup>1</sup> Moderate to severe degeneration of germinal epithelium was observed in the testes of rats administered with 200 and 400 mg/kg of metronidazole respectively, the tubules were generally devoid of primary or secondary spermatocytes and spermatids. Rats treated with low and middle doses of metronidazole exhibited normal testicular weights. Minimal histological changes were observed in the testes of mid dose rats including degenerative fragmentation of spermatozoa and spermatid which tallies with work done by McClain et al.<sup>5</sup>

The histological changes were restored in most rats by the end of 8 weeks after the cessation of the treatment

and this also supports earlier work done by McClain et al.<sup>5</sup> hence the effect of metronidazole on fertility and testicular function in male rats is considered reversible. The present study has shown a beneficial effect of the administration of antioxidants on testicular germinal epithelium functions in metronidazole treated rats. Detrimental effects induced by the administration of therapeutic dose of this drug were minimally observed at 30mg/kg dose in all rats during this experimental period. Significantly normal to near normal germinal epithelium, interstitium, Leydig and Sertoli cells were observed in rats concurrently treated with antioxidant. Furthermore, treatment with antioxidants and testosterone improve testicular functions and ameliorated the loss of germ cells, tubular wall thickening and interstitial spacing. However, it should be emphasized that the values of quantitative and qualitative germinal epithelium remained lower than the control values, although the antioxidants improved these testicular parameters in metronidazole treated rats.

## CONCLUSION

The results of this study indicate that metronidazole administration (200 or 400 mg/kg), for 8 weeks, caused a harmful effect on histo-architecture of the testes of rats. It appears that the primary site of metronidazole action may be on testis but further is necessary to ascertain its effect on the hypothalamo-pituitary axis.

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