METRONIDAZOLE INDUCES SIGNIFICANT PATHOLOGICAL ALTERATIONS IN THE MALE REPRODUCTIVE SYSTEM OF MICE

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ABSTRACT

The present study was aimed to investigate the possible effects caused by the antibiotic, metronidazole, on the histology of the testis and the morphology of the sperms of mice. To achieve this, adult male albino Swiss Mice were orally administrated with 0.1 ml (100 mg\ kg) of the commercially used metronidazole (Flagyl) for 60 consecutive days. The results showed significant decreases ($p<0.05$) in the mean weight of body and testis in the treated mice as compared to the distilled water-treated control group. The treatment also caused several histopathological changes in the testis which included necrosis, congestion, hemorrhage, edema, germ cell downfall and rupture in the seminiferous tubules. The tests of the sperm morphology revealed a number of deformations as a result of the treatment, such as the appearance of headless, bend, broken-tailed, hook-tailed, and two-headed sperms, as well as sperms with cytoplasmic droplets. We conclude that from these results that orally administered Metronidazole exerts negative effects on the male reproductive system of mice .

Keywords : flagyl , metronidazole , testis , sperms

التغيرات المرضية في الجهاز التناسلي الذكري للفئران والمتسببة عن الميترونيدازول

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مدرس

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المستخلص

صممت الدراسة الحالية لمعرفة التأثيرات المحتملة التي تسببها المضادات الحياتية، الميترونيدازول، على نسجية الخصى والشكل المظهري للحيوانات المنوية في الفئران. ولتحقيق ذلك، جرعت ذكور الفئران البيض السويسرية البالغة فمويا 0.1 مللتر من الميترونيدازول (الفلاجيل) بتركيز (100ملغم/كغم) المستعمل تجاريا ولمدة 60 يوما متتاليا. أشارت النتائج الى حصول انخفاض معنوي ($p<0.05$) في معدل وزن الجسم والخصى للفئران المعاملة، مقارنة بمجموعة السيطرة والمعاملة بالماء المقطر . كما سببت المعاملة ايضا تغيرات نسجية في الخصى شملت، تنخر، احتقان , نزف , ظهور وذمة , تساقط الخلايا الجرثومية وحصول تمزق في النبيبات المنوية. اظهرت الاختبارات المظهرية للحيوانات المنوية عدد من التشوهات نتيجة المعاملة بالعقار مثل حيوانات منوية بدون رؤوس , حيوانات منوية بأذناب ملتوية , مكسورة وشصية . حيوانات منوية برأسين , فضلا عن وجود القطيرة السايتوبلازمية في البعض منها . يمكن الاستنتاج بان للتجريع الفموي بالميترونيدازول تاثيرا سلبيا في الجهاز التناسلي الذكري للفئران .

الكلمات المفتاحية: فلاجيل، ميترونيدازول، الخصى، الحيوانات المنوية

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INTRODUCTION

Metronidazole is a synthetic drug derived from 1-,8 hydroxyethyl-2- methyl-5-nitro-imidazole. For more than half a century, the drug has been widely used as the preferred medication to treat a variety of infections caused by protozoans and anaerobic bacteria, particularly those having low resistance levels (1). Upon oral administration into the patient, the drug is normally in its inactive 5-nitroimidazole form, while it acquires the metabolic activity when it becomes subjected to partial reduction upon arrival to the targeted tissue (19) , where it acts via its capability to suppress the synthesis of nucleic acids and thereby to disrupt DNA of the cells of the infectious organism. Only cells of protozoans and anaerobic bacteria can reduce nitroimidazole and, thus, it exerts its effects on these cells rather than the cells of human body. Therefore, humans show a high level of tolerance and a low level of side effects to the drug. Recent reports by published by the Randomized Controlled Trial (RCT) recommends the use of metronidazole, whether as a first- or a second-line treatment to eradicate *H pylori*. The high activity of the drug against acute pouchitis was also described, with a remarkable decrease of Pemphigus Disease Area Index (PDAI) scores (18). Nevertheless, the occurrence of peripheral, but not central, neuropathies has been commonly described as side effects (9). Another investigation demonstrated rare neurotoxic effects but other remarkable side effects in subjects treated with metronidazole (4). In Japan, there was no approval for the use of metronidazole as an eradication treatment for infectious agents such as *H pylori* in patients of childhood and adult ages. Antibiotic resistance was also reported against metronidazole, which has been reported to be increased in a wide range of countries, but not in Japan (15). Reports on the effects of metronidazole on the reproductive system were also published and the drug was shown to affect most of the characteristics of sperms in humans and rabbits. A significant decrease in sperm movement long with a significant increase in the numbers of mutated sperms in male rats were demonstrated one month following an initial oral dose of 500 mg \kg bwt given for 14 days. The treatment also significantly reduced the weight of the testis , prostate and seminal vesicles. Another study conducted on male rats reported that metronidazole administration causes tumors in Leydig cells along with reductions in the levels of testosterone, and thyroid hormones (21) . The aim of this study is to demonstrate the pathological alterations of Metronidazole in the reproductive system of male albino mice.

MATERIALS AND METHODS

Mature mice were taken at the age of 16-18 weeks and their weights ranged from 25-30 grams.The study was conducted on 10 male mice obtained from the Animal House of the Biotechnology Research Center / Nahrain University. The animals were divided into two groups, a treated treatment group which was treated orally with 0.1 ml of of metronidazole (Flagyl) (the product is from the State Company for Medicines and Medical Appliances in Samarra - Iraq (S. D. I. IRAQ) with a concentration of 100 mg\ kg as well as a control group which was treated with 0.1 mm of distilled water. The dosage was givien continued for 60 days before and after using standard laboratory balance. The weight of the testis of the animals was measured by a Sartorius analytical sensitive balance. Animals sacrificed by cervical dislocation, a longitudinal incision was made in the abdomen, the surrounding tissue was carefully removed, and then site of incision was washed with a physiologic solution. The entire organs were kept in 10 % formalin fixative until tissue sections were prepared . For the purpose of studying the specifications of the sperms, the epididymis tail was removed and mixed with 2 ml of saline solution (NaCl 0.9) in a clean dish . Then a drop of the solution was taken and mixed on a clean glass slide with a drop of both eosin and necrosin stains using another slide. After the mixture was smeared along the slide, it was dried out and incubated at 37 °C random fields of the sperm preparation were microscopically examined to determine the percentage of sperms with abnormalities , which include changes in the head, tail ,and the position of the cytoplasmic , following the equation below drpolets .

Percentage of abnormal sperms = Number of abnormal sperms \ Total number × 100 %: Histological sections of the testes of the treated and control group were prepared according to the routinely used procedure, i.e., dehydration, clearing, filtration, embedding, sectioning, stained with Haematoxylin-Eosin stains , mounting and microscopic photography .

Statistical analysis

Statistical analysis of the data was performed by using ANOVA we applied factorial within completely randomized design with thirty replications. Least significant differences (LSD) were used to compare between means at 0.05 level.

RESULTS AND DISSCUSIONS

The results in table 1 show significant decrease in the mean values of body weights of the orally treated mice with a concentration of 100 mg/kg metronidazole for 60 days, as compared with the control group .

Table 1. The effect of Flagyl in the rate of body weights after 60 days of dosage

Treatment Groups	Average ± Standard error (g)	
	Primary weight	Final weight
The control	a 25 ± 0.38	a 29.10 ± 0.62
Trented group	a 28.38 ± 1.61	b 24.76 ± 0.31
LSD value	a 3.011 NS	*3.478

Results, in Table 2 reveal a significant decrease in the testis weights of the treated animals , as compared with the control group .

Table 2 . The effect of Flagyl in the rate of testes weights after 60 days of dosage

Treatment Group	Average ± Standard error (mg) (60 days post treatment)
Control	a 316.87 ± 20.6
Trented group	b 189.96 ± 4.95

The results in Table 3 show significant differences in the percentage of the total and abnormal sperms compared to the control group. The percentage of sperms in the treated group of was significantly decreased as compared with the control group. The results showed the appearance of deformed sperms, including headless and bend sperms (Figure 4) as well as cytoplasmic droplets (Figure 8) , a hooked tail (Figure 3), two tail (Figure 2), atrophy head (Figure 5) .

Table 3. The percentage of normal and deformed sperm (Rate ± slandered error) in the tail of epididymis of male mice treated with the Flagyl concentration is 100 mg / kg

Groups	Number of animals	Normal sperms	Abnormal sperm
Control	5	a 93.98±0.73	a 0.73±1.070
Flagyl 100 mg/kg	5	b 33.55±8.22	b 66.45±8.22

This drug targets the genetic material and therefore the cells of the body are exposed to obvious damages , as previously shown by other authors (13) . The safety and maintenance of the sperm is important for men to have good reproductive health, because healthy sperm must be free from deformities and defects , able to easily move for long distances and has the ability to efficiently penetrate the egg during fertilization (20) .

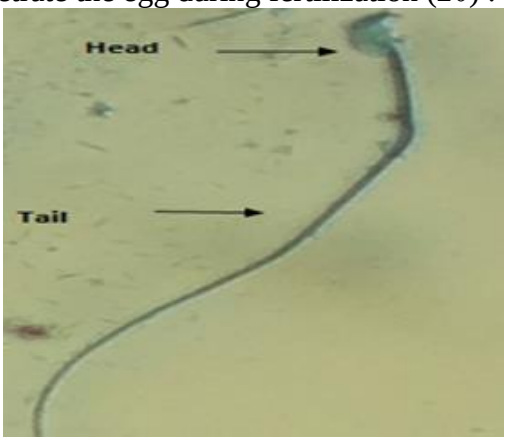

Fig 1. Normal sperm of mice

Fig 2. (H) hooked tail and (T) two tail

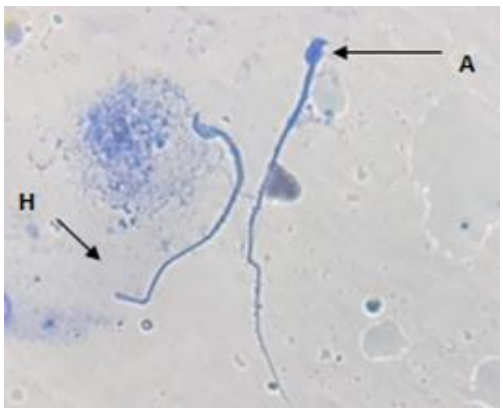

Fig 3. (H) Hooked tail (A) Amorphus

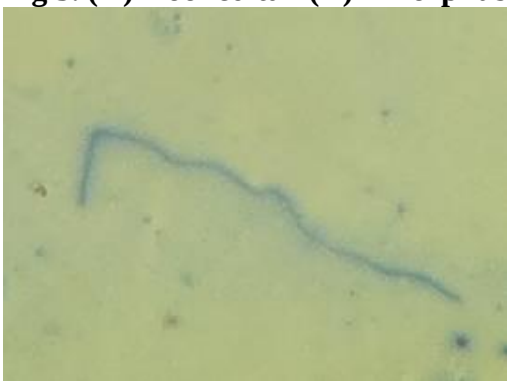

Fig 4. Headless and wavy tail

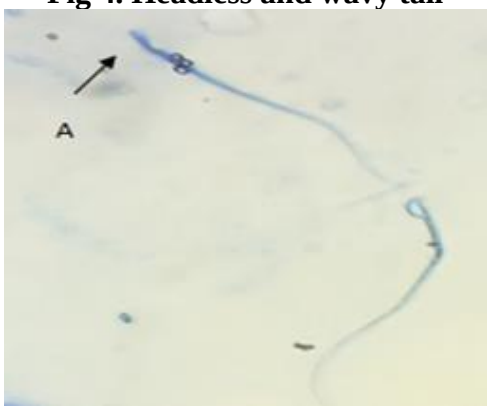

Fig 5. Head atrophy

Fig 6. Folded tail

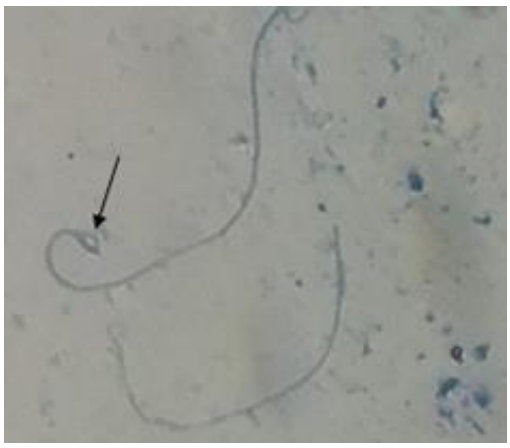

Fig 7. Hook head

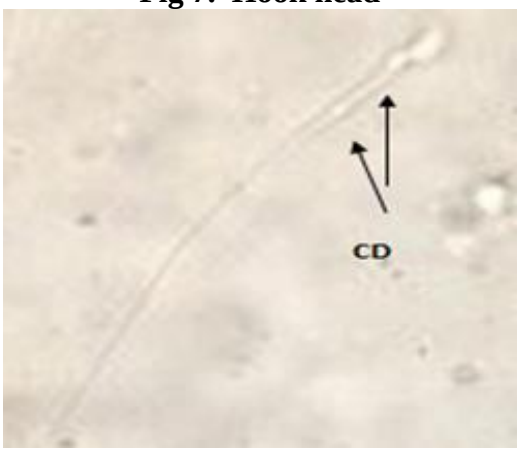

Fig 8. cytoplasmic droplets

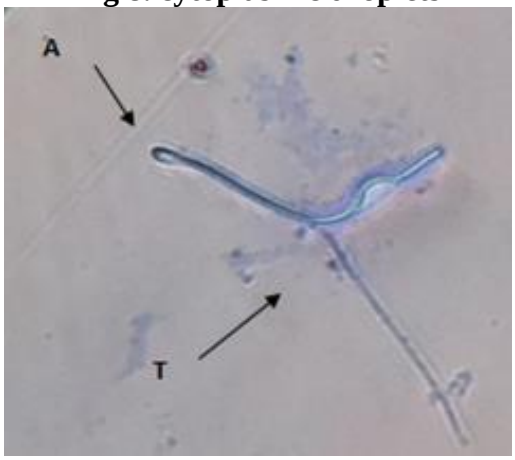

Fig 9. Amorphous head with two tail

Drugs and chemical materials disposed to the environment have been frequently reported as an issue of major concern for the male reproductive system, particularly in relation to the fact that they could cause toxicity and mutagenesis to male's germ cells. Previous investigations revealed dramatic reduction in the body weight of mice (2) , as well as decreased weight of the testis and infertility, in a period of 2 to 3 weeks following a single oral administration with 250 mg/kg of metronidazole. These effects were reported to last for 33 days to 4 weeks (16). Similarly,

another study showed that metronidazole, used in a high dose, rendered male rats infertile (11). Moreover, animals treated with 200 mg/kg metronidazole for 6 weeks showed decreased weight of testis and number of spertmatids in the testis and epididymis. The same treatment also caused the sperm shape to be abnormal and the seminiferous tubules to be degenerated (8). Other authors also demonstrated significantly reduced testicular and epididymal weights in mice orally treated for consecutive 33 days with 100 mg/ kg/day metronidazole (12) . One reason for the observed reduction in the proportion of the motile sperms and the elevation in the numbers of the abnormal sperms is possibly that metronidazole can pass through the blood-testis barrier and approach the germ cells located within the seminiferous tubules. The barrier separating the blood circulation from the testis was suggested to has an essential role in controlling the interactions between the mutagenic drugs and the germ cells (5). Another research demonstrated the effect of metronidazole on decreasing the release of testosterone as being associated with the damaging effects on Leydig cells and the sperms following the success of the drug to pass the barrier (7). Based on the examination of the histological sections of the testes in mice treated with metronidazole, it was possible to observe the various effects caused by this drug on the testicular tissue,. The damage in the seminiferous tubules were caused by the degeneration and necrosis, and they were associated with damages of the surrounding tissues, In contrast, histological sections from the control group showed a normal shape and size of the seminiferous tubules with clarity of the stages of spermatogenesis from spermatogonia untill mature sperm (Figure 10). Histological sections of the treated group showed necrosis in the cells as well as the decline of germ cells counts in the tubule cavity , . In addition, the occurrence of edema was observed, probably due to the contraction of the seminiferous tubules and the accumulation of the fluid in the surrounding tissue (Figure 11). Moreover, the histological examination revealed other types of malformations in the treated mice. These included the occurrence of pyknosis and germ cell counts (Figure 12), congestion (Figure 13), hemorrhage and sloughing of spermatocytes (Figure 14). In addition demonstrates severe rupture in the seminiferous tubules of treated testes (Figure 15). Regressive changes in the seminiferous tubules in the testis of mice after treatment with metronidazole (200 mg/kg BW/day) for 28 days were previously reported (10) . The changes were indicated by shrinkage of the seminiferous tubules, depletion, disorganization, intraepithelial vacuolization, and sloughing of germinal layer, as well as the appearance of multinucleated giant cells. Our results are also consistent with those from other groups (14), particularly as related to the alterations observed in the tubules and the shape of the sperm (6). Other authors proposed that the damages caused to the cells by Metronidazole or other agents can lead the basal membrane to be thickened . Previous research also proved that drugs belonging to the group of metronidazole can necrosis ,these results are in agreement with previous report(3), and kill the cells through causing breaks in DNA strands. Damages to cells were also demonstrated to be caused by mechanisms that directly or indirectly involve oxidative stress as a result of exposure to Metronidazole (17, 22) . We conclude that , orally administered of Metronidazole has negative effects on the male reproductive system of mice

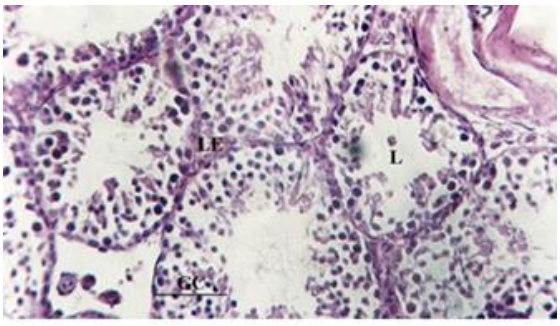

Fig 10 . Cross section of testicular tissue of control group Shows: (GC) germ cell , (L) lumen , (LE) Leydig cell .400X (H&E).

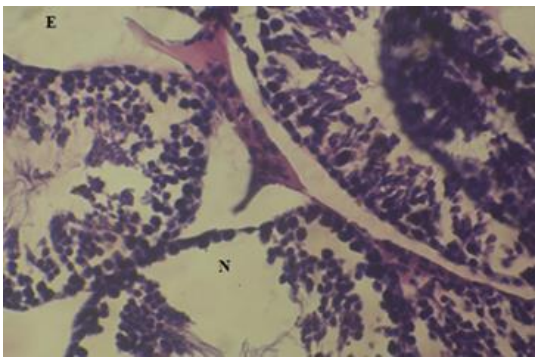

Fig 11 . Cross section of testicular tissue of treated group Shows: (N) Necrosis , (E) Edema . 400X (H&E).

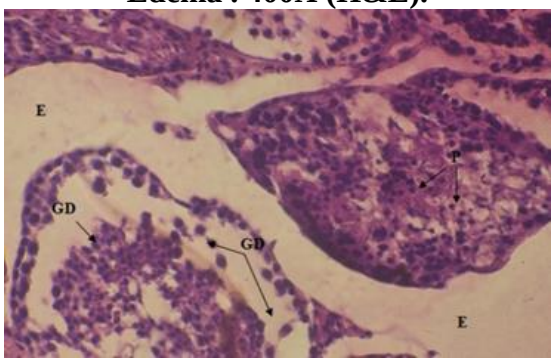

Fig12 . Cross section of testicular tissue of treated group shows: (E)edema (P) pyknosis (GD) germ cell downfall .400X (H&E).

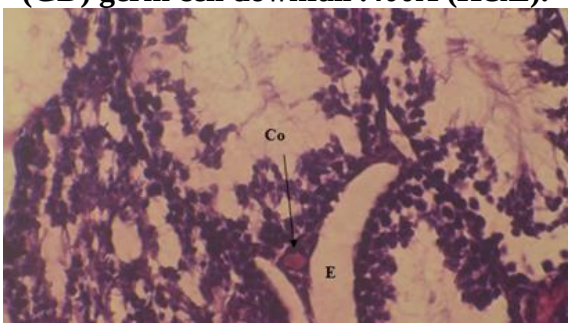

Fig13 . Cross section of testicular tissue of treated group shows: (Co) congestion (E) edema . 400X (H&E).

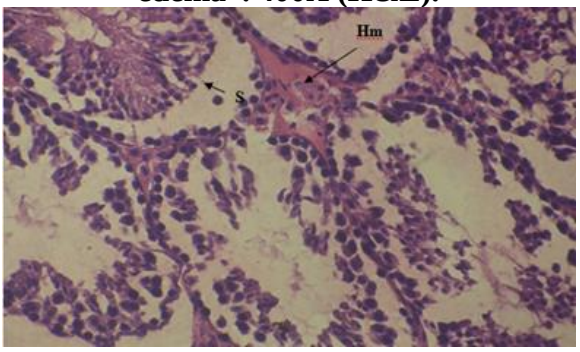

Fig 14 .Cross section of testicular tissue of treated group shows: hemorrhage (S) Sloughing of germinal layer . 400X(H&E).

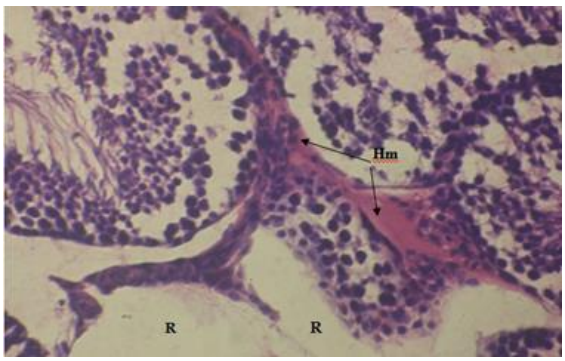

Fig 15 . Cross section of testicular tissue of treated group shows: (Hm) hemorrhage (R) rupture . 400X (H&E).

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