Global Veterinaria 9 (3): 303-310, 2012
ISSN 1992-6197

DOI: 10.5829/idosi.gv.2012.9.3.65175

Histopathological and Biochemical Alterations of Metronidazole-Induced Toxicity in Male Rats

Samah S. Oda

Department of Pathology, Faculty of Veterinary Medicine, Alexandria University, Egypt

Abstract: The present study was carried out to investigate the toxic effects of metronidazole (MTZ) on male albino rats based on biochemical and histopathological alterations as well as reproductive impairment. Rats were divided into three groups (n=8): group A control; group B rats orally administered 135 mg/kg bwt/day MTZ and group C rats orally administered 540 mg/kg bwt/day MTZ for 60 days. No significant changes in the serum biochemical parameters evaluated in the group B rats. Group B rats showed significant reduction in the testes index weight without significant changes in epididymis and accessory sex glands index weight. High dose of MTZ treatment caused a significant elevation of serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST), a significant reduction in the serum albumin level. Testes and epididymis index weight was significantly decreased in group C and no significant alterations were occurred in the accessory sex glands index weight. Moreover, serum testosterone levels were significantly decreased in MTZ treated groups. Histopatholoical alterations were marked in brain, liver, kidney, testes and epididymis of rats treated with high dose of MTZ. The results indicate that therapeutic and high dose of MTZ treatment caused neurotoxicity, impaired male fertility and to lesser extent hepato and nephrotoxicity in rats.

Key words: Metronidazole (MTZ) · Toxicity · Brain · Testes · Histopathology

INTRODUCTION

Metronidazole (MTZ) is a 5-nitroimidazole drug widely used in veterinary and human medicine for the treatment of trichomoniasis, giardiasis, amebiasis and anaerobic bacterial infections [1-3]. In addition to its anti-protozoal and bactericidal properties, MTZ is thought to have some immunomodulatory effects and is commonly used to treat inflammatory bowel disease (IBD) in both dogs and cats [4, 5]. MTZ is rapidly absorbed through gastrointestinal tract with maximum concentrations in both serum and tissues. It is metabolized in the liver and is excreted mainly via the kidneys in urine and to lesser extent through the intestinal wall with feces [6]. MTZ interacts with DNA to create a disruption of the helical DNA structure and strand breakage, resulting in inhibition of protein synthesis and cell death in susceptible organisms. However, MTZ is not a nontoxic drug [7] and has been shown to rapidly cross the blood brain-barrier [4, 8]. Central nervous system (CNS) side effects associated with MTZ toxicosis have been reported in humans [7] and in veterinary species, including rats [9], dogs [10] and cats [11]. In dogs and cats, central vestibular and cerebellar dysfunctions resulting in ataxia, nystagmus, head tilt, tremors and seizures are commonly recorded in cases of MTZ toxicosis [4]. Sohrabi *et al.* [12] reported the direct hazardous effects of MTZ on the germ and Leydig cells after penetration into the blood-testis barrier. Moreover, MTZ administration (200 or 400 mg/kg), for 8 weeks, caused a harmful effect on the testes of male rats [13]. Few reports are available concerning the hepatotoxic [14-16] and nephrotoxic [16] effects of nitromidazole derivatives. The genotoxic effect of MTZ was studied by Mudry *et al*. [17] and EL-Nahas and EL-Ashmawy [18]. Although MTZ is considered safe and is widely used in the human and veterinarian populations, it is necessary to clarify the potential biological risks in the use of MTZ. Moreover, histopathological studies concerning MTZ toxicosis are scarce. Therefore, the aim of the present study was to assess the toxic

Corresponding Author: Samah Shehata Oda, Department of Pathology, Faculty of Veterinary Medicine, Alexandria University, P.O. Box 22758, Edfina-Rashid-Behera, Egypt.
Tel: +20-1006503624, Fax: +20-452960450.

effects of MTZ on male albino rats on the bases of biochemical alterations, reproductive impairments and histopathological changes.

MATERIALS AND METHODS

Animals and Experimental Design: Twenty four adult male albino rats (weighing 180-200g) were used. Rats were housed in cages and were maintained on commercial basal food and water *ad libitum*. Rats were received humane care incompliance with the guidelines of the National Institutes of Health (NIH) of Animal Care and the local committee approved this study. After 2 weeks of adaptation, all animals were randomly divided into three groups of eight rats each: group A served as control and orally received 1ml physiological saline once daily. Group B rats orally administered 135 mg/kg bwt MTZ (Flagyl®) tablet 250 mg (a product of Rhone-Poulenc, U.K., manufactured by Alexandria Pharmaceutical Co. Egypt). Group C rats orally administered 540 mg/kg bwt MTZ. The doses of MTZ represent the therapeutic dose and 4 times the therapeutic dose of rat according to Paget and Barnes [19]. MTZ was suspended in physiological saline and was administered by gavage daily for 60 days.

Evaluated Parameters

Serum Biochemical Analysis: Rats were anesthetized with ether then blood was collected from the inner canthus of the eye by heparinized capillary tube into clean dry test tube. The blood was centrifuged with 3000 rpm for 10 min. to separate the serum. The activities of the serum alanine aminotransferase (ALT) and aspartate aminotransferase (AST) were estimated according to Reitman and Frankel [20], total protein [21], albumin [22], urea [23] and creatinine [24]. Total serum testosterone was estimated according to Demetrious [25].

Reproductive Organs Weight: Immediately after the collection of blood samples, rats were euthanized. After dissection, testes, epididymis and accessory sex glands (prostates and seminal vesicles) were collected, examined grossly then weighed. The index weight (I.W.) of the organ was calculated by Matousek [26] I.W. = organ weight (g)/body weight (g) x 100.

Histopathological Evaluation: After necropsy, tissue specimens of the brain, spinal cord, sciatic nerve, liver, kidneys, testes, epididymis and accessory sex glands (prostates and seminal vesicles) were collected and then rapidly fixed in 10% neutral-buffered formalin for at least 24 h. The fixed specimens were processed through the conventional paraffin-embedding technique [27], sectioned at 5 µm and stained with Mayer's haematoxylin and eosin (HE).

Statistical Analysis: Results were analyzed statistically by one-way analysis of variance followed by Duncan's multiple range tests [28]. Data are presented as means plus or minus the standard error. The minimum level of significance was set at $P \leq 0.05$.

RESULTS

Clinical Signs: No clinical signs were observed in rats of groups A and B. On the other hand, rats of group C exhibited weight loss, depression, ataxia and dizziness.

Serum Biochemical Analysis: Table 1 shows that group B rats exhibited non significant alterations in serum AST, ALT, total protein, albumin, globulin, urea and creatinine levels compared to the control rats. Conversely, there were significant increase ($P \leq 0.05$) in serum ALT and AST levels and a significant reduction in serum albumin level. Moreover, no significant changes in the serum total protein, globulin, urea and creatinine levels of group C rats.

Reproductive Organs Weight and Serum Testosterone Level: The index weight of the testes was decreased significantly ($P \leq 0.05$) in MTZ treated groups compared to the control group (Table 2). This reduction was marked in the group C. The index weight of the epididymis was decreased significantly in group C rats; however, no significant change was demonstrated in group B rats. Besides, no significant changes were observed in the index weight of the accessory sex glands in MTZ treated groups. Regarding serum testosterone levels, there was a significant reduction ($P \leq 0.05$) in the groups B and C compared to the control group. The greatest reduction was seen in the group C (Table 2).

Histopathological Findings

Brain: Brain specimens of the control rats were within normal histological limits. The cerebral cortex of group B rats showed satellite perineuronal oligodendroglias surrounding small shrunken neurons with contracted dense nuclei and condensed cytoplasm (Fig. 1A).

Table 1: Effect of metronidazole on serum biochemical parameters of male albino rats

Group	ALT (u/l)	AST (u/l)	Total protein (g/dl)	Albumin (g/dl)	Globulin (g/dl)	Urea (mg/dl)	Creatinine (mg/dl)
A	27.90±0.06b	166.50±5.48b	7.08±0.27a	4.55±0.14a	2.53±0.12a	37.00±0.06a	0.40±0.06a
B	30.30±1.56ab	180.40±3.12ab	6.79±0.10a	4.40±0.06a	2.39±0.16a	38.45±0.89a	0.50±0.06a
C	34.60±0.46a	202.33±8.21a	6.76±0.31a	3.85±0.03b	2.91±0.28a	39.40±1.27a	0.53±0.07a

Values are expressed as mean ± standard errors. Values with different letters at the same column are significantly different at P=0.05 (anova) with Duncan's multiple range test. A = control, B = 135 mg / kg bwt metronidazole-treated, C = 540 mg / kg bwt metronidazole-treated.

Table 2: Effect of metronidazole on reproductive organs weight and serum testosterone levels of male albino rats

Group	Testes I.W.	Epididymis I.W.	Accessory sex glands I.W.	Testosterone (ng /ml)
A	1.39±0.03a	0.51±0.01a	1.01±0.03a	2.91±0.14a
B	1.13±0.02b	0.49±0.01a	0.92±0.01a	2.39±0.04b
C	0.58±0.08c	0.31±0.03b	0.91±0.04a	1.55±0.11c

Values are expressed as mean ± standard errors. Values with different letters at the same column are significantly different at P=0.05 (anova) with Duncan's multiple range test. I.W. = organ weight (g) / body weight (g) x 100. A = control, B = 135 mg / kg bwt metronidazole-treated, C = 540 mg / kg bwt metronidazole-treated.

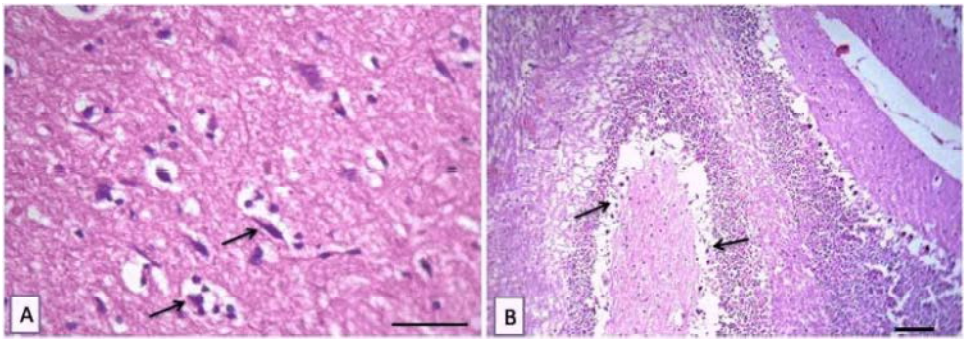

Fig. 1: Photomicrograph of rat brain stained with HE. (A) Cerebral cortex of a rat treated with 135 mg/kg bwt metronidazole: Satellite perineuronal oligodendroglias surrounding small shrunken neurons with contracted dense nuclei and condensed cytoplasm (arrows). (Bar = 50 µm). (B) Cerebellum of a rat treated with 540 mg/kg bwt: Selective necrosis of Purkinje cells and depletion in granule cells layer of a cerebellar folium (arrows). (Bar= 100 µm).

Neuronophagia as well as cerebrocortical hemorrhage were evident. The microscopic changes of group C rats were consisted of congestion of cerebral and cerebellar blood vessels and hemorrhage. Satellitosis and neuronophagia were also noticed. 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 (Fig. 1B). The necrotic Purkinje cells were shrunken and hyperchromatic, sometimes disappear, leaving empty spaces and a fenestrated ground layer.

Liver: Liver of the control rats showed normal histoarchitecture of intact portal areas and normal hepatocytes. Liver of group B rats exhibited minute foci of hepatic cell necrosis accompanied by mononuclear cell infiltrates. Liver tissues of group C rats revealed remarkable degenerative changes represented by diffuse disorganization of the hepatic cords and cytoplasmic vacuolations. Dilatation and congestion of hepatic sinusoids were noticed. In addition, minute foci of hepatic cell necrosis and associated mononuclear cell infiltrates were evident almost adjacent to the portal areas (Fig. 2).

Kidneys: Kidneys of the control rats had normal morphology of the renal parenchyma with well-defined glomeruli and tubules. Conversely, kidneys of groups B and C rats showed variable degenerative and necrotic changes that were clear in the latter group. Lesions were consisted of vacuolation of the renal lining epithelium and presence of eosinophilic hyaline casts in the lumina of renal tubules. Moreover, focal areas of tubulonecrosis accompanied by mononuclear cell infiltrates were noticed, particularly at the corticomedullary junction (Fig. 3).

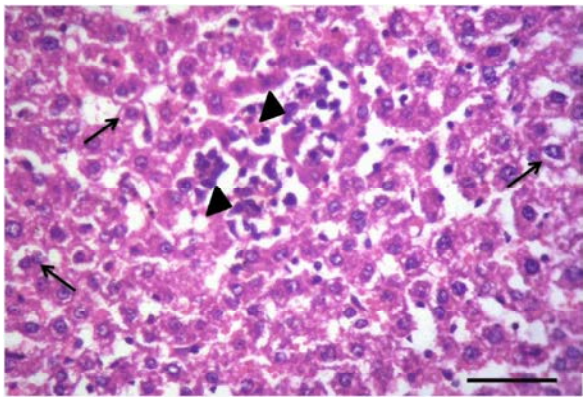

Fig. 2: Photomicrograph of a liver from rat treated with 540 mg/kg bwt metronidazole: Minute foci of hepatic cell necrosis and associated mononuclear cell infiltrates (arrow heads) and mild hepatocytic vacuolation (arrows). (HE, bar= 50 µm).

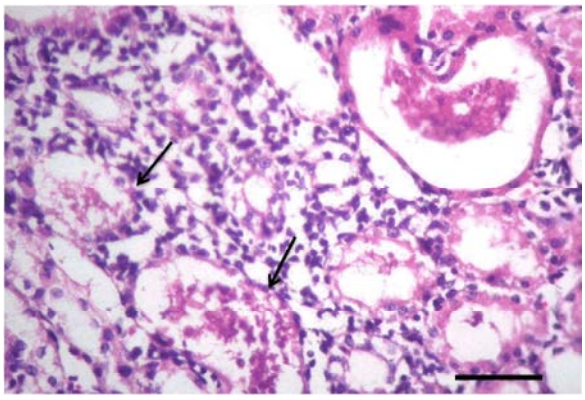

Fig. 3: Photomicrograph of a kidney from rat treated with 540 mg/kg bwt metronidazole: Tubulonecrosis accompanied by mononuclear cell infiltrates at corticomedullary junction with presence of eosinophilic hyaline casts in the lumina of renal tubules (arrows). (HE, bar= 50 µm).

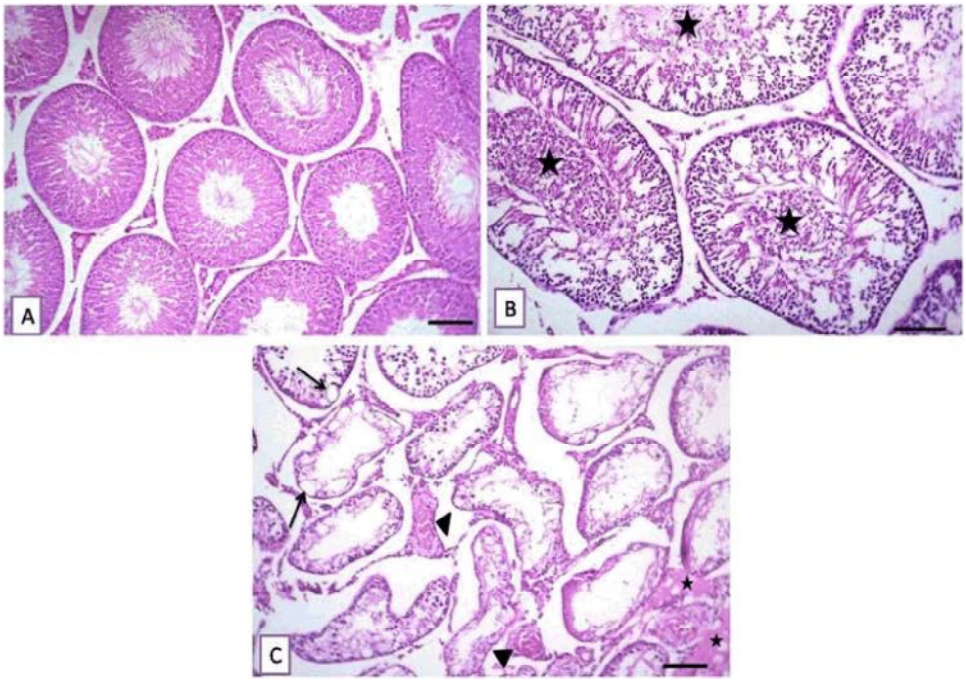

Fig. 4: Photomicrograph of rat testis stained with HE. (Bar= 50 µm). (A) Normal testis histo-architecture of a control rat. (B) Degenerated testis of rat treated with 135 mg/kg bwt metronidazole: Lumina of the seminiferous tubules contained sloughed germinal epithelial cells (stars). (C) Degenerated testis of rat treated with 540 mg/kg bwt metronidazole: The seminiferous tubules had single or double cell layers, vacuolated germinal epithelium (arrows), interstitial congestion (arrow heads) and edema (stars).

Testes: Testes of the control rats had normal histoarchitecture and were composed of uniform, well-organized seminiferous tubules with complete spermatogenesis and interstitial connective tissue (Fig. 4A). Testes of group B rats had small, disorganized seminiferous tubules with reduced spermatogenesis. Lumina of some seminiferous tubules contained sloughed germinal epithelial cells (Fig. 4B) and few multinucleated giant cell formations. However, marked degenerative changes were detected in group C rats (Fig. 4C).

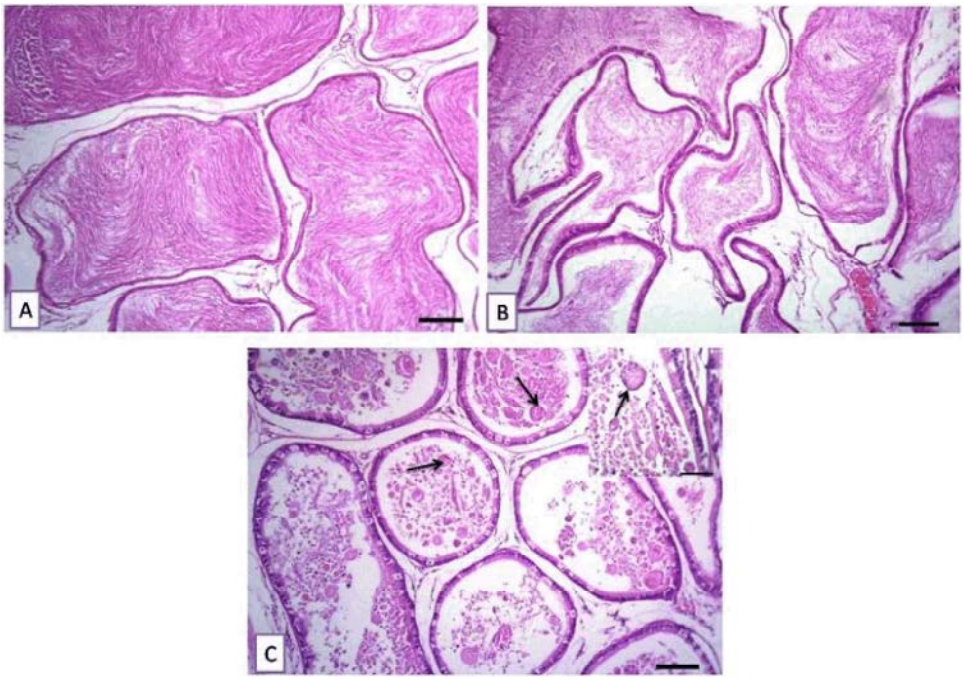

Fig. 5: Photomicrograph of rat epididymis stained with HE. (Bar= 100 µm). (A) Cauda epididymis of a control rat with normal histological structure and sperm density. (B) cauda epididymis of rat treated with 135 mg/kg bwt metronidazole with relatively low sperm density. (C) Cauda epididymis of rat treated with 540 mg/kg bwt metronidazole: The epididymal ductules were free from mature spermatozoa and contained numerous sloughed germ cells with multinucleated giant cell formations (arrows) (inset, bar= 50 µm).

Wherein, lesions were consisted of small, disorganized seminiferous tubules with buckled basement membrane. The majority of seminiferous tubules had single or double cell layers indicative of cessation of spermatogenesis. Vacuolation of germ and Sertoli cells was also seen. Some tubules had sloughed germinal epithelial cells within their lumina with numerous multinucleated giant cell formations. Regarding to the interstitial tissue, there were congestion of the intertubular blood vessels and edema.

Epididymis: Epididymis of the control rats showed normal histological structure with normal sperm density (Fig. 5A). Epididymal ductules of group B rats had a relatively low density of spermatozoa when compared to control group (Fig. 5B). The majorities of epididymal ductules of group C rats were free from mature spermatozoa and contained numerous number of sloughed germinal epithelium with numerous multinucleated giant cell formations (Fig. 5C).

DISCUSSION

This study was conducted to assess the adverse effect of MTZ toxicosis on male albino rats based on biochemical and histopathologic alterations as well as reproductive dysfunction. The results of this study revealed the hepatotoxic effect of the high dose of MTZ treatment as there was significant elevation of the hepatic enzymes (ALT and AST) and a significant reduction in serum albumin level. These findings were parallel to the histopathological changes in the liver, particularly in the rats of group C. It has been suggested that metabolites of MTZ may bind to RNA instead of DNA, possibly inhibiting RNA protein synthesis [11]. Yamamoto *et al*. [29] recorded that MTZ-induced encephalopathy developed in patients with liver cirrhosis. They have been reported that severe liver dysfunction due to cirrhosis would facilitate the occurrence of the side effects because MTZ is mainly metabolized by the liver [30]. No significant changes were detected in the serum urea and creatinine levels in MTZ-treated groups. However, microscopic examination of kidney tissues of MTZ-treated groups revealed various degrees of degenerative and necrotic changes. Ahmad *et al.* [16] reported that a single therapy of ofloxacin, ornidazole and MTZ drugs increases hepatotoxicity and renal toxicity. Clinically, there were signs of central nervous system dysfunction represented by ataxia and dizziness. Besides, brain lesions were

remarkable, particularly in rats treated with high dose of MTZ. The cerebellar lesions were similar to those found by Schärer *et al.* [31] who found clinical signs associated with CNS dysfunction in dogs given 4-6 doses of MTZ at 250 mg/kg/d. Histologic examination of brain specimens showed degeneration (swelling, vacuolation and clumping of protoplasm) and selective loss of Purkinje cells. These dogs all had clinical neurologic abnormalities within 4-6 days and prior to the development of histologic lesions in the brain; most of the dogs died within 1 week after the onset of clinical signs. The pathogenesis of MTZ neurotoxicity is currently unknown and there are relatively few publications addressing the mechanism of MTZ neurotoxicity. It has been suggested that metabolites of MTZ may bind to RNA instead of DNA, possibly inhibiting RNA protein synthesis, which could potentially lead to axonal degeneration [11]. Another proposed mechanism involves the modulation of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) receptor within the cerebellar and vestibular systems [32]. In support of this hypothesis, it has recently been demonstrated that diazepam, a benzodiazepine with major effects on GABA neurotransmitters in the brain, dramatically improved the recovery times for dogs with MTZ toxicosis [32]. The mutagenic and toxic potentials of drugs or environmental chemicals on male germ cells have become an important area of environmental concern [33]. MTZ, a 5-nitroimidazole drug has been reported to decrease testicular weight, testicular and epididymal spermatid counts and to cause abnormal sperm morphology with degeneration of seminiferous tubules within 6 weeks of administration of MTZ at 400 mg/kg dose [34]. The present study showed marked reproductive dysfunction; particularly in the high dose MTZ-treated group. The index weight of testes and epididymis were greatly reduced in group C rats [12, 18, 35]. These findings were compatible with the histopathological results: small, disorganized seminiferous tubules with buckled basement membrane and the majority of seminiferous tubules had single or double cell layers indicative of sudden cessation of spermatogenesis. The significant reduction in the testes and the epididymis weight as well as histopathological alterations in the testes and epididymis may be attributed by the significant reduction in serum testosterone levels. The reduction in testosterone may be due to MTZ which reaches the blood testis barrier and gains access to the germ cells in the seminiferous tubules. Since, the blood testis barrier was possibly an important aspect when considering reproductive and mutagenic effects of drugs and environmental chemicals [36]. The permeability characteristics for the blood testis barrier are generally similar to those limit penetration of membranes of the central nervous system [37]. MTZ is distributed to all tissues including the blood brain barrier and seminal fluid [5]. Similarly, MTZ immobilized rat spermatozoa *in vitro* [38]. The percentage of motile rabbit and human sperm incubated with MTZ was affected [33]. These could explain the direct hazard effects of MTZ on sperm and Leydig cells through decreased testosterone secretion after penetration of the blood testis barrier. Likewise, MTZ administration (15mg/kg, 200mg/kg or 400 mg/kg), for 8 weeks, caused an oxidative stress on the testes of male rats that evidenced by the high level of malondialdehyde (an index of lipid peroxidation) in the MTZ treated groups [13].

In conclusion, therapeutic and high dose of MTZ treatment for 60 days caused neurotoxicity, harmful effect on male fertility and to lesser extent MTZ induced hepato and nephrotoxicity in rats.

ACKNOWLEDGMENT

The author gratefully thanks Dr. Mahmoud M. A. Elmaghraby professor of Animal Breeding and Production, Department of Animal Husbandry and Animal Wealth Development, Faculty of Veterinary Medicine, Alexandria University, Egypt for performing the statistical analysis.

REFERENCES

1. Reynolds, A.V., J.M.T. Hamilton-Miller and W. Brumfill, 1975. A comparison of the *in vitro* activity of metronidazole, tinidazole and nimorazole against Gram-negative anaerobic bacilli. Journal of Clinical Pathology, 28: 775-778.
2. Bergan, T., 1985. Antibacterial activity and pharmacokinetics of nitroimidazole. A review. Scandinavian Journal of Infectious Diseases, 46: 64-71.
3. Frey, H.H. and W. Löscher, 1996. Lehrbuch der Pharmakologie und Toxikologie für die Veterinar-medizin. Enke, Stuttgart., pp: 502-503.
4. Groman, R., 2000. Metronidazole. Compend Cont Educ., 22: 1104-1107, 1130.
5. Plumb, D.C., 2002. Veterinary Drug Handbook, 4th ed., Iowa State University Press, Ames, IA., pp: 549-552.

6. Finch, R.G. and I.S. Snyder, 1986. Antiprotozoan drugs. In: Modern pharmacology. Eds.: C.R. Craige and R.E. Stitzel. Little, Brown Co. Boston, pp: 729-740.
7. Gupta, A.K., M.P. Agarwal, R. Avasthi, D.P. Bhadoria and N. Rohatgi, 2003. Metronidazole-induced neurotoxicity. Journal of the Association of Physicians of India, 51: 617-618.
8. Finegold, S.M., 1980. Metronidazole. Annals of Internal Medicine, 93: 585-587.
9. Rogulja, P.V., W. Kovac and H. Schmid, 1973. Metronidazol-Encephalopathie der Ratte. Acta Neuropathologica (Berlin), 25: 35-45.
10. Wright, K.H. and J.W. Tyler, 2003. Recognizing metronidazole toxicosis in dogs. Veterinary Medicine, 98: 410-418.
11. Caylor, K.B. and M.K. Cassimatis, 2001. Metronidazole neurotoxicosis in two cats. Journal of the American Animal Hospital Association, 37: 258-262.
12. Sohrabi, D., M. Alipour and A. Mellati, 2007. Effect of metronidazole on spermatogenesis, plasma gonadotrophins and testosterone in rats. Iranian Journal of Reproductive Medicine, 5: 69-72.
13. Ligha, A.E. and C.W. Paul, 2011. Oxidative Effect of metronidazole on The Testes of Wistar Rats. Australian Journal of Basic and Applied Sciences, 5: 1339-1344.
14. Tabak, F., R. Ozaras, Y. Erzin, A.F. Celik, G. Ozbay and H. Senturk, 2003. Ornidazole-induced liver damage: Report of three cases and review of the literature. Liver International, 23: 351-418.
15. Harputluoglu, M.M., U. Demirel, N. Karadag, D. Karahan, M. Aladag, M. Karincaoglu and F. Hilmioglu, 2007. Severe hepatitis with prolonged cholestasis and bile duct injury due to the long-term use of ornidazole. Acta Gastroenterolgica Belgica, 70: 293-295.
16. Ahmad, A., M. Chaudhary, A. Soni, A. Payasi and V.K. Dwivedi, 2010. Comparative toxicity profile study of mebatic vs. ofloxacin, ornidazole and metronidazole drugs in rat model. Asian Journal of Biochemistry, pp: 1-11.
17. Mudry, M.D., M.A. Carballo, M.D. Labal de Vinuesa, M. Gonz´alez Cid and I. Larripa, 1994. Mutagenic bioassay of certain pharmacological drugs: III. Metronidazole (MTZ). Mutation Research, 86: 243-77.
18. EL-Nahas, A.F. and I.M. EL-Ashmawy, 2004. Reproductive and cytogenetic toxicity of metronidazole in male mice. Basic and Clinical Pharmacology and Toxicology, 94: 226-231.
19. Paget, G.E. and J.M. Barnes, 1964. Toxicity tests. In: Evaluation of drug activities and pharmacometrics. Eds.: D.R. Lautrance and A.L. Bacharach. Academic Press, London and New York, pp: 135-166.
20. Reitman, S. and S. Frankel, 1957. A colorimetric method for determination of serum glutamate oxaloacetic acid and pyruvic acid transaminases. American Journal of Clinical Pathology, 29: 56-63.
21. Doumas, B.T., D.D. Bayse, R.J. Carter, T. Peters and R. Schaffer, 1981. Acandidate reference method for determination of total protein in serum. I. Development and validation. Clinical Chemistry, 27: 1642-1650.
22. Reinhold, R.P., 1953. Determination of serum albumin. Clinical Chemistry, 21: 1370-1372.
23. Patton, C.J. and S.R. Crouch, 1977. Enzymatic determination of urea. Analytical Chemistry, 49: 464-469.
24. Henry, R.J., 1974. Principles and techniques. In: Clinical chemistry. Ed.: Harper and Row, pp: 525.
25. Demetrious, J.A., 1987. Testosterone in methods. In: Kapalan LA, editor. Clinical chemistry tech AG. 2nd ed. CVMOS Co., pp: 268.
26. Matousek, J., 1969. Effect on spermatogenesis in guinea pigs, rabbits and sheep after their immunization with sexual fluid of bulls. Journal of Reporoductive Fertility, 19: 63-72.
27. Culling, C.F., 1983. Hand book of histological and histochemical techniques. 3rd ed. London, Boston: Butterworth.
28. SAS, 2001. Statistical analysis system. Users guide: statistics. Cary, NC: SAS Institute.
29. Yamamoto, T., K. Abe, H. Anjiki, T. Ishii and Y. Kuyama, 2012. Metronidazole-Induced Neurotoxicity Developed in Liver Cirrhosis. Journal of Clin. Medical Research, 4: 295-298.
30. Cheong, H.C., T.G. Jeong, Y.B. Cho, B.J. Yang, T.H. Kim, H.C. Kim and E.Y. Cho, 2011. Metronidazole-induced encephalopathy in a patient with liver cirrhosis. Korean Journal of Hepatology, 17: 157-160.

31. Schärer, K., 1972. Selektive Purkinje-Zellschadigungen nach oraler Verabreichung großer Dosen von Nitroimidazol-Derivaten am Hund. Verhandlungen der Deutschen Gesellschaft für Pathologie Deutsche Gesellschaft für Pathologie, 56: 407-410.
32. Evans, J., D. Levesque, K. Knowles, R. Longshore and S. Plummer, 2003. Diazepam as a treatment for metronidazole toxicosis in dogs: a retrospective study of 21 cases. Journal of Veterinary Internal Medicine, 17: 304-310.
33. Foote, R.H., 2002. Effect of metronidazole, Ipronidazole and dibromochloropropane on rabbit and human sperm motility and fertility. Reproductive Toxicology, 16: 749-755.
34. Grover, J.K., V. Vats, M. Srinavas, S.N. Das, P. Jha, D.K. Gupta and D.K. Mitra, 2001. Effect of metronidazole on spermatogenesis and FSH, LH and testosterone levels of pre-Pubertal rats. Indian Journal of Experimental Biology, 39: 1160-1166.
35. Noorafshan, A., S. Karbalay-Doust, A. Valizadeh, E. Aliabadi and H. Mirkhani, 2010. Ameliorative Effects of Curcumin on the Seminiferous Epithelium in metronidazole-Treated Mice: A Stereological Study. Toxicologic Pathology, 38: 366-371.
36. Dixon, R.L. and I.P. Lee, 1977. Possible role of the blood-testis barrier in dominant lethal testing. Environmental Health Perspectives, 6: 59-63.
37. Okumura, K., I.O. Lee and R.L. Dixon, 1975. Permeability of selected drugs and chemicals across the blood testis barrier of the rat. Journal of Pharmacology and Experimental Therapeutics, 191: 89-95.
38. El-Ashmawy, I.M., 1988. Effect of some drugs on male fertility in the rat. M.V.Sc. Thesis Presented to Pharmacology Department, Faculty of Veterinary Medicine, Alexandria University, Egypt.