Evaluation of Histopathological and Biochemical Effects of Nephrotoxicity Induced by Metronidazole in Male Albino Mice

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ABSTRACT:

The aim of the current study was to assess the adverse effects of daily single injections of metronidazole (MTZ) at different doses on renal functions and investigate histopathological changes in the kidneys of male albino mice. The mice were divided into four equal groups, each containing 6 animals. The first group received saline intraperitoneally (i.p) and served as a control. The second group was administered MTZ at a dose of 125 mg/kg i.p. once a day, the third group received MTZ at a dose of 250 mg/kg i.p. once a day, and the fourth group received 500 mg/kg/day i.p. MTZ for three consecutive weeks. The results revealed that the high doses of MTZ in groups 3 and 4 led to a significant increase in urea and creatinine values. Additionally, histopathological alterations were observed kidneys of these groups. However, MTZ at a dose of 125 mg/kg did not exhibit marked changes in the aforementioned parameters. This study highlights a substantial degree of renal damage resulting from MTZ treatment at high doses, specifically at levels 2 to 3 times the therapeutic dose in mice.

Key words: *Metronidazole (MTZ), nephrotoxicity, histopathology, mice*.

الملخص:

تم إجراء الدراسة الحالية لتقييم التأثيرات الضارة لحقنة يومية واحدة من الميترونيدازول (MTZ) بجرعات مختلفة على وظائف الكلى. كما تم فحص التغيرات النسجية في الكلى الناتجة عن MTZ في فئران الذكور البيضاء. تم تقسيم الفئران إلى أربع مجموعات متساوية، كل منها تحتوي على 6 حيوانات، حيث تم إعطاء المجموعة الأولى محلول ملحي عن طريق الحقن الداخلي (i.p) واعتبرت المجموعة الطبيعية. تلقت المجموعة الثانية MTZ بجرعة 125 ملغ/كغ i.p يوميا، بينما تم إعطاء المجموعة الثالثة جرعة 250

ملغ/كغ i.p يوميا، وتلقت المجموعة الرابعة جرعة 500 ملغ/كغ i.p يوميا لمدة ثلاثة أسابيع متتالية. أظهرت النتائج المتحصل عليها أن جرعة عالية من MTZ في المجموعتين 3 و 4 أدت إلى ارتفاع كبير في قيم اليوريا والكرياتينين. وبالإضافة إلى ذلك، تم العثور على تغيرات نسيجية في الكلى. ومع ذلك، لم تظهر MTZ بجرعة 125 ملغ/كغ أي تغييرات ملحوظة في المعايير السابقة. أظهرت الدراسة الحالية أن هناك درجة كبيرة من الضرر الكلوي ناتج عن علاج MTZ بهذا المستوى العالي من الجرعة (ضعفين إلى ثلاث مرات من الجرعة العلاجية في الفئران .

INTRODUCTION

Metronidazole (MTZ) is a 5-nitroimidazole drug widely used in both veterinary and human medicine to treat conditions such as trichomoniasis, giardiasis, amebiasis, and anaerobic bacterial infections (1). In addition to its anti-protozoal and bactericidal properties, MTZ is believed to have immunomodulatory effects and is commonly employed in the treatment of inflammatory bowel disease (IBD) in dogs and cats (2,3). MTZ is quickly absorbed through the gastrointestinal tract, reaching peak concentrations in both serum and tissues. It undergoes hepatic metabolism and is primarily excreted through the kidneys in urine, with a smaller amount eliminated through the intestinal wall in the animals' feces (4). However, it's essential to note that MTZ is not without toxicity and has been shown to rapidly cross the blood-brain barrier [5]. In dermatology, Metronidazole is used to treat conditions such as Rosacea, and it is marketed by Galderna under the trade names Rozex and Metrogel (6). It is available over the counter (OTC) in pharmacies and sometimes in the open market in Nigeria. Pfizer markets it under the trade name Flagyl in the US, while Sanofi-Aventis uses the same tradename Flagyl internationally (7). Metronidazole, when taken orally, is selectively absorbed by anaerobic bacteria and sensitive protozoa. Once absorbed by anaerobes, it undergoes nonenzymatic reduction by reacting with reduced ferredoxin, which is generated by pyruvate oxidoreductase (8).Additionally the metronidazole has activity against a wide range of microorganisms, including anaerobic bacteria, protozoa, and microaerophilic bacteria. Additionally, it notes that Clostridium spp. species are typically susceptible to metronidazole (9&10) .metronidazole has the ability to effectively cross the blood-brain barrier. As a result, it has the potential to cause

various neurological adverse events, particularly when used at dosages exceeding 2 grams per day for extended periods. The mentioned neurological adverse events include peripheral neuropathy (damage to peripheral nerves), encephalopathy (brain disease, damage, or malfunction), cerebellar dysfunction (problems with the cerebellum, a part of the brain), and seizures.(13&14). Metronidazole (MTZ), a synthetic drug, is the preferred choice for treating infections caused by various protozoa diseases such as amoebiasis, giardiasis, and trichomoniasis. It is particularly indicated for the treatment of intestinal and hepatic amoebiasis, as well as forms of urethritis and vaginitis caused by Trichomonas vaginalis, and anaerobic bacterial infections (13&15). Importantly, MTZ does not affect facultative aerobic bacteria, preserving the normal population of these organisms in the human body. MTZ is rapidly and nearly completely absorbed from the gastrointestinal tract, with peak concentrations in the blood achieved approximately hours after administration. Metabolized in the liver, it is primarily excreted via the kidneys in urine and, to a lesser extent, through the intestinal wall with feces (15). The drug crosses the placenta and is present in breast milk at concentrations corresponding to those in the mother's serum. It reaches therapeutic concentrations in bones, abscesses, and the central nervous system (CNS), readily crossing the placenta and entering the fetal circulation (17). Adverse effects of MTZ have been documented in both humans (12&16) and veterinary species, including rats (16) . In veterinary medicine, both treated cases and experimental studies report common side effects of oral administration of MTZ, including lethargy, anorexia, vomiting, and diarrhea. Limited reports are available regarding the hepatotoxic (17&18) and nephrotoxic (18) effects of nitroimidazole derivatives. Despite its widespread use and general safety in human and veterinary populations, it is essential to clarify potential biological risks associated with the use of MTZ. Additionally, histopathological studies on MTZ toxicosis are scarce. Therefore, the present study aimed to investigate the side effects and histopathological changes associated with MTZ administration in male albino mice.

Materials and methods

Drugs and Chemicals
Metronidazole, procured from ROTEXMEDICA in a 500mg/100ml formulation from Germany,
was employed for the study. A stock solution was prepared using distilled water, and subsequent dilutions were adjusted according to the recorded body weights of the animals. renal function tests
were carried out at Al-Saleem Laboratory in Benghazi.

Animals

The study involved 24 adult male albino mice with a weight range of 20-25 grams. These mice were accommodated in the controlled laboratory environment at the Pharmacology Department, University of Benghazi. The mice were housed in plastic cages, maintained at a controlled
temperature of 24±2°C, with a 12:12-hour light/dark cycle. They had free access to a standard rat diet and tap water. The mice were acclimatized to these conditions for a period of 2 weeks before the initiation of the study. All experiments were conducted between 9:00 a.m. and 2:00 p.m.

Experimental design

The animals were randomly divided into four groups, each comprising six mice. They received daily intraperitoneal (i.p) treatments for 21 consecutive days as follows:
Groups: Four groups of male albino mice, each with 6 animals.
Control Group (Group 1): Received saline intraperitoneally (i.p) as a control.
Group 2: Received MTZ at a dose of 125 mg/kg i.p. once/day.
Group 3: Received MTZ at a dose of 250 mg/kg i.p. once/day.
Group 4: Received a higher dose of MTZ at 500 mg/kg i.p. once/day for three consecutive weeks.

Determination of biochemical parameters

At the end of the experiment, animals that had undergone overnight fasting (both in the control and experimental groups) were euthanized through cervical dislocation, followed by decapitation. Blood samples were acquired, placed into regular sample tubes, and centrifuged at 10,000 revolutions per minute for 2 minutes to separate the serum. The serum was then preserved in Eppendorf tubes and dispatched to

Al-Saleem Medical Laboratory for evaluating serum urea and creatinine levels using Roche diagnostic kits from the USA. The absorbance of the reaction was measured at 690 nm using the COBAS INTEGRA 400 PLUS instrument.

Histopathological studies

Following the sacrifice of the animals, a postmortem examination was conducted, and the kidneys of the mice were identified and meticulously dissected as a whole for subsequent histopathological examination. After rinsing the dissected kidneys with normal saline, sections were extracted from each organ. The tissue was then fixed in 10% neutral-buffered formalin, underwent gradual dehydration in ethanol (50-100%), thorough washing in running tap water, additional dehydration, cleansing in xylene, and finally, embedding in paraffin. Kidney sections were sliced to a thickness of 4-5µm and subsequently stained with hematoxylin and eosin dye for microscopic observations.

Statistical analysis

The data derived from the results of the aforementioned experiments have been presented as mean ± standard error (mean ± SEM). Statistical analysis was conducted using GraphPad Prism (version 6.01) for Windows, employing one-way analysis of variance (ANOVA) followed by Tukey's multiple comparisons test. This analysis aimed to determine whether the scores of different groups exhibited significant differences. The level of significance was categorized as highly significant at $P < 0.001$, moderately significant at $P < 0.01$, and significant at $P < 0.05$.

Results and Discussion

Serum biochemical analysis

(Table 1) Showed the effect MTZ on the urea and creatinine levels. There are an increase in the level of urea and creatinine in group 3 treated mice ($P < 0.05$) and ($P < 0.05$) respectively, and group 4 treated mice ($P < 0.001$) and ($P < 0.001$) respectively. Moreover, no significant changes in the serum urea and creatinine levels of group 2 compared to the control group.

Table 1: Effects of intraperitoneal administration of different doses of metronidazol on serum renal functions.

Parameter	Control	Metronidazol 125mg/kg	Metronidazol 250mg/kg	Metronidazol 500mg/kg
Urea(mg/dl)	39±1.9	42.83±1.66	48.33±1.11*	58.66±2.46***
Creatinine(mg/dl)	0.41±0.010	0.44±0.02	0.51±0.01*	0.98±0.04***

Values are expressed as mean±SEM.
*,*** represent significant decrease at P<0.05, P<0.001, respectively, when compared to control group.

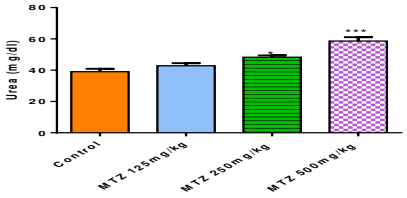

Figure 1. Showed the effect of metronidazole on the urea level. Values are expressed as mean±SEM. *** (P<0.001) and * (P<0.05) denotes significant difference vs. control values.

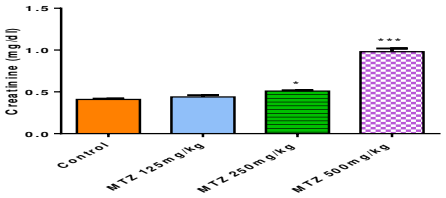

Figure 2: Showed the effect of metronidazole on the creatinine level. Values are expressed as mean±SEM. *** (P<0.001) and * (P<0.05) denotes significant difference vs. control values.

Histopathological evaluation of Effects of metronidazole on Kidney :

kidney of the control mouse showed normal morphology of renal unit composed of glomeruli and tubules (figure. 3). Conversely, kidnyes of group 2 mice revealed cloudy swelling (reversible hydropic degeneration) and tubular casts (figure. 4). Whereas, group 3 exhibited interstital aggregates of inflammatory cells (figure. 5). Kidneys of group 4 mice on renal lobule showing necrotic tubules. (figure. 6).

Figure 3: Photomicrograph of healthy mousc cortico-medullary junction of normal glomeruli and tubules.

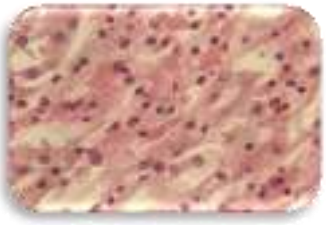

Figure 4: Photomicrgraph of mice kidney treated with 125 mg/kg metronidazole: cloudy swelling and tubular casts.).

Figure 5: Photomicrgraph of mice kidney treated with 250 mg/kg metronidazole: interstital aggregates of inflammatory cells (nephritis).

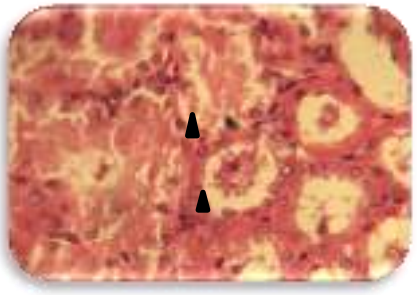

Figure 6: Photomicrgraph of mice/rat kidney treated with 500 mg/kg metronidazole: showing necrotic tubules (head arrows).

Metronidazole as a chemotherapeutic happens in numerous structures and is generally utilized in dentistry, for example in periodontal treatment, and endodontic treatment

The present study evaluated the nephrotoxicity activity of MTZ after prolonged use on laboratory albino mice based on biochemical as well as histopathological alterations for period of 21 days as a measure of chronic toxicity The current study showed marked Nephrotoxicity effect of the high dose of MTZ (250 mg/kg and 500mg/kg) treatment as there was a significant elevation of urea and creatinine .These findings were compatible with the histopathological results especially with group 4. High doses of MTZ (groups 3 and 4) led to a significant increase in urea and creatinine values. This suggests impaired renal function, as elevated levels of these markers are indicative of kidney damage. The study revealed histopathological alterations in kidneys, indicating that MTZ administration at higher doses resulted in structural changes in these organs. The lowest dose of MTZ (125 mg/kg) did not cause significant changes in urea and creatinine levels, suggesting that at this dose, the drug did not have marked adverse effects on renal function. Such observation is in accordance with the data reported by several studies when the albino rats were treated with MTZ.(11,12,13)

Conclusion:

The study concludes that a considerable degree of renal damage resulted from high doses of MTZ (groups 3 and 4), which were

administered at levels 2 to 3 times the therapeutic dose in mice.

References:

1. BerganT (1985) Antibacterial activity and pharmacokinetics of nitro imidazole. A review. Scand J Infect Dis Suppl 46: 64-71.
2. Groman R (2000) Metronidazole. Compend Cont Educ 22: 1104-1107.
3. PlumbDC(2002) Veterinary drug handbook. (4thedtn), Iowa State University Press, United States.
4. Finch RG, Snyder IS (1986) Antiprotozoan drugs. In: Modern pharmacology. (2nd edtn), Little, Brown Co, United States.
5. Gupta AK, Agarwal MP, Avasthi R, Bhadoria DP, Rohatgi N (2003) Metronidazoleinduced neurotoxicity. J Assoc Physicians India 51: 617-618.
6. Oda S (2012) Histopathological and biochemical alterations of metronidazole-induced toxicity in male rats. Glob Vet 9: 303-310.
7. Ahmad A, Chaudhary M, Soni A, Payasi A, Dwivedi VK (2010) Comparative toxicity profile study of mebatic vs. ofloxacin, ornidazole and metronidazole drugs in rat model. Asian J Biochem 5:78-88.
8. Chukwu VO, Akudike CJ, Ezejindu DN, Augustine IC (2015) The protective effects of turmeric on liver enzymes of metronidazole treated adult male Wistar rats. IJASR 1: 255-258
9.Snavely SR, Hodges GR: The neurotoxicity of antibacterial agents. Ann Intern Med 101:92-104, 1984

10.Ahmed A, Loes DJ, Bressler EL: Reversible magnetic resonance imaging findings in metronidazole-induced encephalopathy. Neurology 45:588-589, 1995.
11.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.
12.Alvarez, R.S., Richardson, D.A., Bent, D.A. and Ostergard. D.R.(1983). Central nervous system toxicity related to prolonged metronidazole therapy. Am. J. ObstetGynecol. 145:640-641.
13.Bergan, T., 1985. Antibacterial activity and pharmacokinetics of

nitroimidazole. A review. Scandinavian Journal of Infectious Diseases, 46:64-71.
14.Frey, H.H. and W. Loscher, 1996. Lehrbuch der Pharmakologie und Toxikologie fur die Veterinar-medizin. Enke, Stuttgart., pp:502-503.
15.Gupta, A.K., M.P. Agrwal, R. Avasthi, D.P. Bhadoria and N. Rohatgi, 2003. Meteronidazole-induced neurotoxicity. Journal of the Association of Physicians of India, 51: 617-618.
16.Rogulja, P.V., W. Kovac and H. Schmid, 1973. Meteronidazole-Encephalopathie der Ratte. Acta Neuropathologica (Berlin), 25:35-45.
17.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.
18.Tan, C.H., Chen,Y.F., Chen, C.C.,Chao, C.C., Liou, H.H. and Hsieh, S.T. (2011). Paniful neuropathy due to skin denervation after meteronidazole- induced neurotoxicity. J. Neural NeurosurgPsychiatry. 82:462-466.