Histological Evaluation of the Effect of Metronidazolemedicine on the Brain Tissue in Adult Female White Rats

Yaseen Khalaf Mohammed[1], Ayad Hameed Ibrahim[2], Aziz Khalid Hameed[3]

[1]Researcher: Department of Biology-College of Science, Tikrit University/Iraq, [2]Prof. Dr: Department of Anatomy and Histology-College of Veterinary Medicine- Tikrit University/Iraq, [3]Prof. Dr: Department of Biology-College of Scince- Tikrit University/Iraq

Abstract

The present study was undertaken to assess the neurotoxic effect of metronidazole in pregnant female rats . Twenty adult pregnant female rats were randomly divided into four groups treatment with oral metronidazole at the therapeutic dose 250 mg/kg, 500 mg/kg and 1000 mg/kg from the eight day of pregnant to the twenty day of pregnant (every eight hour), whereas the last group serve as a control. Routine Histological Techniques were done and stained by Hematoxylin and Eosin (H & E). The histopathological examination of brain show the lesion was characterized by a wide spongy cavitation and cavitation was observed around the nerve and glial cells along with atrophy of some of its nuclei and around the axonal and vascular axons with the presence of nuclear thickening of some glia cells, as well as hyperplasia of the glial cells supporting, disintegration and degeneration of nerve fibers, with damage to the meninges. In addition to the presence of bloody congestion. This study revealed that metronidazole have a neurotoxic effect in adult female rats with a severity depended on its dose and duration of administration.

Keywords: *Metronidazole, Brain, Pregnant, Neurotoxic, Hyperplasia, Vacuolation, pyknosis.*

Introduction

Metronidazole is an injectable and oral synthetic[(1).] Metronidazole is an antimicrobial agent commonly used in the treatment of several protozoal and anaerobic infections[(2).]. Its main indications are trichomonal infection, amoebiasis, Helicobacter Pylori infection and Clostridium difficile associated diarrhea. Additionally, it is often used in Crohin disease,Rosacea and hepatic encephalopathy. Though it is well tolerated in common setting, patient may experience serious neurologic side effects in both long term and short term use. This includes peripheral neuropathy, cerebellar dysfunction, visual impairment, vestibulotoxicity, cochleotoxicity, ataxic gait, dysarthria, seizures and encephalopathy[(3;4;5;6;7)].

Corresponding Author:
Yaseen Kalaf Mohammed
Researcher: Department of Biology-College of Science, Tikrit University/Iraq
e-mail: sy19941991@gmail.com

Despite of the passage of sixty years of research, metronidazole metabolism and its cellular toxicity are not clearly known. Metronidazole is considered a primary drug, Prodrug, not in the active formula. It is activated by reducing (reducing the nitro group) when there is a lack of oxygen concentration, which leads to breaking down amidazole and causing cellular toxicity. This is a currency basis for treating anaerobic infection. It has not yet been established whether the reduced (active) metronidazole is responsible for cellular toxicity or some of its metabolic derivatives that are responsible for the events of cellular toxicity[(8; 9)].

Metronidazole crosses the blood-brain barrier, and its induction of cerebral cytotoxicity does not depend on how it is administered orally or intravenously[(10)]. Several studies have been conducted on the neurotoxic mechanism of metronidazole, but until now the mechanism has not been clearly defined by the hypotheses developed by researchers[(11)]. In many experiments were conducted on animals (rats), which showed the occurrence of neuronal modulation after treatment with metronidazole

with damage to the cerebellum, metronidazole and its metabolic derivatives are associated with RNA in the nucleus of the neuron and inhibit the production of proteins and the occurrence of bulging and breakdown of the axon[12].

Since then several cases have been reported and awareness of this entity among clinicians have substantially increased especially in the last decade. But still many questions remain unanswered and hence this entity needs further research and clarifications. Therefore, the present study was carried to investigate brain histopathological alteration associated with metronidazole administration in female rats.

Material and Method

Twenty Sprague-Dawley females rats were used for the purpose of this study. It was purchased from Animal House, Faculty of Veterinary Medicine, Tikrit University. The average weights were (167 grams), and were in a good health. The rats were kept in the animal house, as they were randomly distributed, at the rate of five animals for each group inside the cages intended for breeding with a floor furnished with sawdust. (27 ° C) and a 12-hour light cycle: 12-hour darkness, and give the diet feed (plate) and water in sufficient quantities to feed it throughout the breeding and livestock treatment period. Adult females were placed with adult males rats and by the rate of every two females with one male in each breeding cage for the purpose of mating, and animals were monitored until fertilization occurred by observing the vaginal plug on the next day and the day on which fertilization occurred was considered zero of pregnancy and the day after that was considered The first day of pregnancy. Used metronidazole as a tablet, and one tablet contains 250 or 500 mg/kg of metronidazole. The pharmaceutical industry used in the UAE was Julphar. Doses used for rats were calculated using the following method [13]:

The pregnant animals group divided into four groups and each consist of five pregnant female.

Pregnant Group:

1. **Group 1 (Control group):** Given normal feeding and water.
2. **Group 2:** Given the concentration 250 mg/kg from the metronidazole medicine form the eight day to the twenty day from pregnant (every eight hour).
3. **Group 3:** Given the concentration 500 mg/kg from the metronidazole medicine form the eight day to the twenty day from pregnant (every eight hour).
4. **Group 4:** Given the concentration 1000 mg/kg from the metronidazole medicine form the eight day to the twenty day from pregnant (every eight hour).

After the end of the specified period of experiments, the histological technique of the samples was performed according to the method [14], and then samples were performed on the techniques of preparing the microscopic tissue sections, which were fixation and hardening, washing, and dehydration, clearing, infiltration, embedding, and trimming the mold. Cutting the paraffin sections by using a rotary microtome with thickness (6μm), Mounting and Staining. Hematoxylin and Eosin stain (H & E) used to study the histological changes caused by metronidazole. After completing the preparation of the microscopic tissue sections, they were examined by optical microscopy.

Result

Group 1 (Control group): The meningeal pia mater around the cerebral cortex contained a loose connective tissue with the presented of fibroblasts with blood vessels and appeared beneath it a pale colored Molecular layer that appeared to be spongy with a number of glial cells extending downward with the Granular layer as larger cells continuously connected with the pyramidal neuron layer (Fig.1).

Group 2 (Pregnancy): The cerebral cortex tissue is surrounded by the inner meningeal tissue (pia mater) with another outer membrane, Erachnoid membrane, where the presence of the Subarachnoid space in which the Cerebro-Spinal fluid occurs, the Molecular layer under the membranes of the meninges appeared pale due to the vaculation of many places Glial and neuron cells, especially with large numbers of glial cells in the lower layers of the cerebral cortex (Fig.2).

Group 3 (Pregnancy): The meningeal layer of the brain has damage and loss of continuity of the membrane to the pia mater as well as an increase in the penetration of the molecular and granular layer beneath it, as well as in the area of pyramidal neurons. Glial cells and nerve fibers swell around the glia and nerve cells, which degenerate and disintegrate, which showed frequent Vacuolation and Cavitation in brain tissue (Fig.3).

Group 4 (Pregnancy): The cerebral cortex has an extensive necrosis and necrosis that has extended to the deepest layers of the brain. This enlargement has surrounded all the supporting and nerve cells, extending from under the meninges that have lost the continuity of the coated surface of the brain to the deepest large nervous layers (Fig. 4). The middile of the brain contained nerve fibers in the form of degenerative bundles, missing continuity, i.e., disintegrated surrounding large numbers of inflammatory cells and debris of glial and neuronal cells in their midst and the lesion and cavitation regions of the cerebral cortex were surrounded by degenerated and degraded nerve fibers and there were large numbers of glial and neuronal cells that lost the cytoplasm as their nuclei appeared inside the cavitation sinuses (Fig. 5).

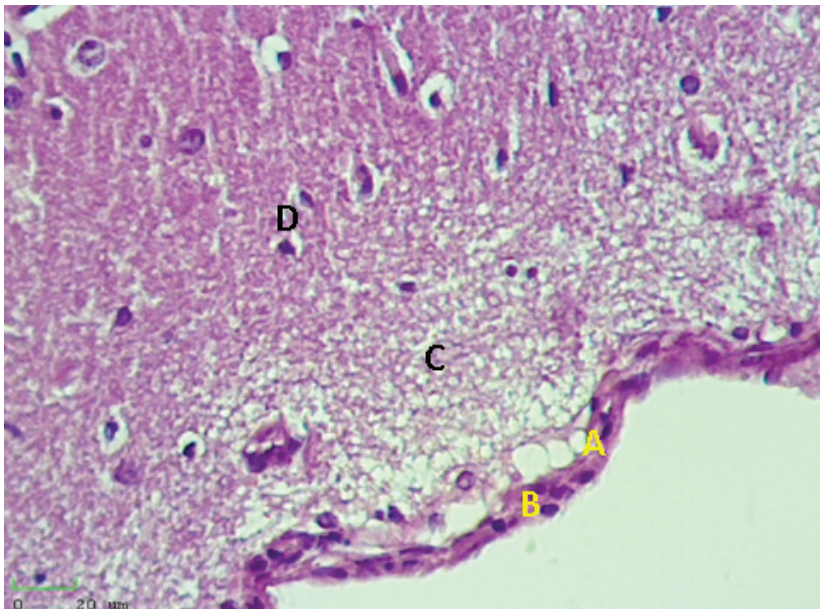

Figure (1): Brain of the female white rat (control group): A - the meningeal membrane of the pia mater. B- Fibroblast. C-Molecular layer D-glial cells. H & E 40X.

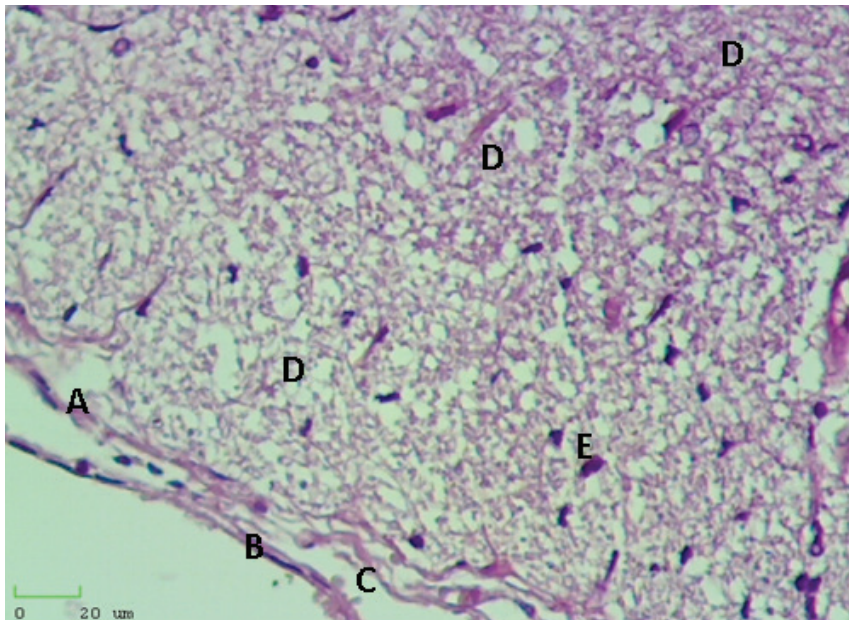

Figure (2): Brain section for female white rat in pregnant stage 20 day treatment the metronidazole medicine (250 mg/kg) as shown: A- inner Meningesmembrane. B- Arachnoid membrane. C-Bleeding in the subarachnoid space. D- molecular layer and thegranular layer and nerve layer in which vaculation. E-glial cells. H & E 40X.

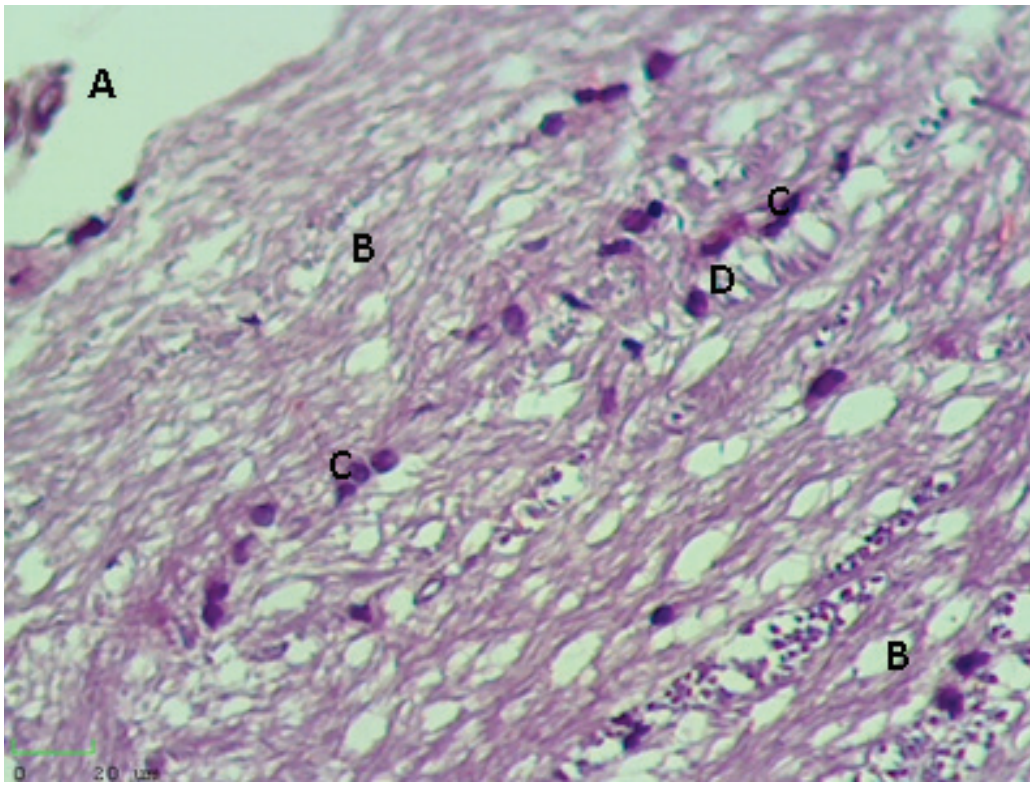

Figure (3): Brain section for female white rat in pregnant stage 20 day treatment the metronidazole medicine (500 mg/kg) as shown: A- A rupture of the meninges membrane. B- vacuolation the molecular and granular layers. C- Glial cells hypertrophy. D- Degeneration of nerve fibers. H & E 40X.

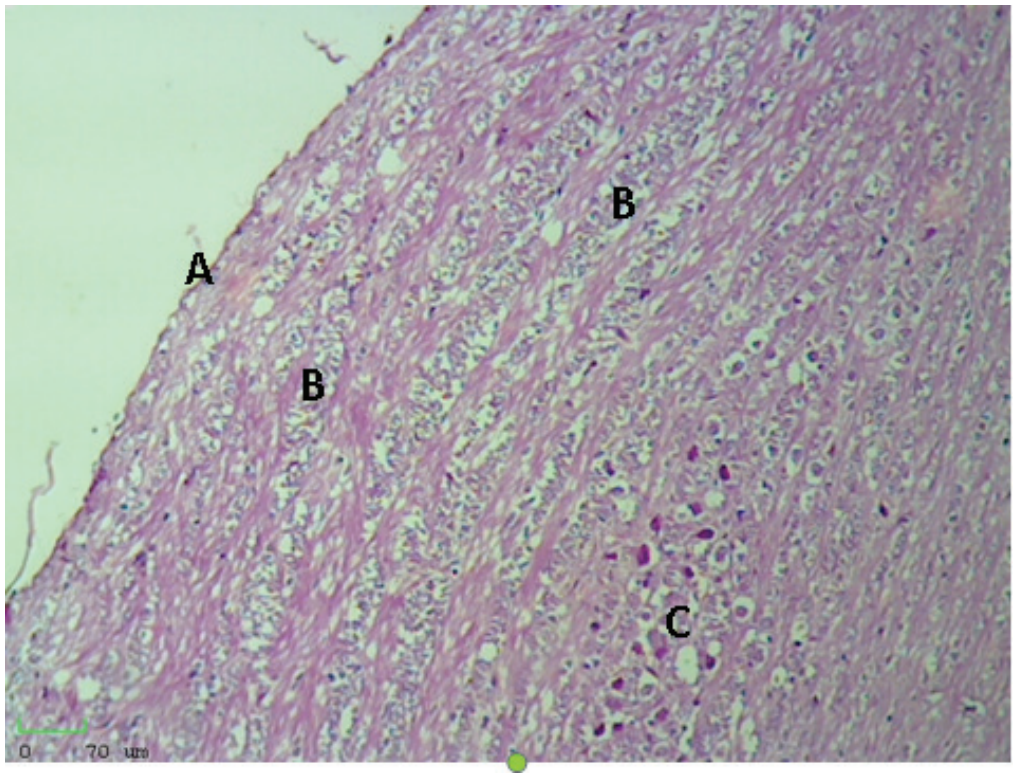

Figure (4): Brain section for female white rat in pregnant stage 20 day treatment the metronidazole medicine (1000 mg/kg) as shown: A- Pia matre damaged. B- Dgeneration of nerve fibers C- vacuolation around nerve cells and supporting glial cells. H & E 40X.

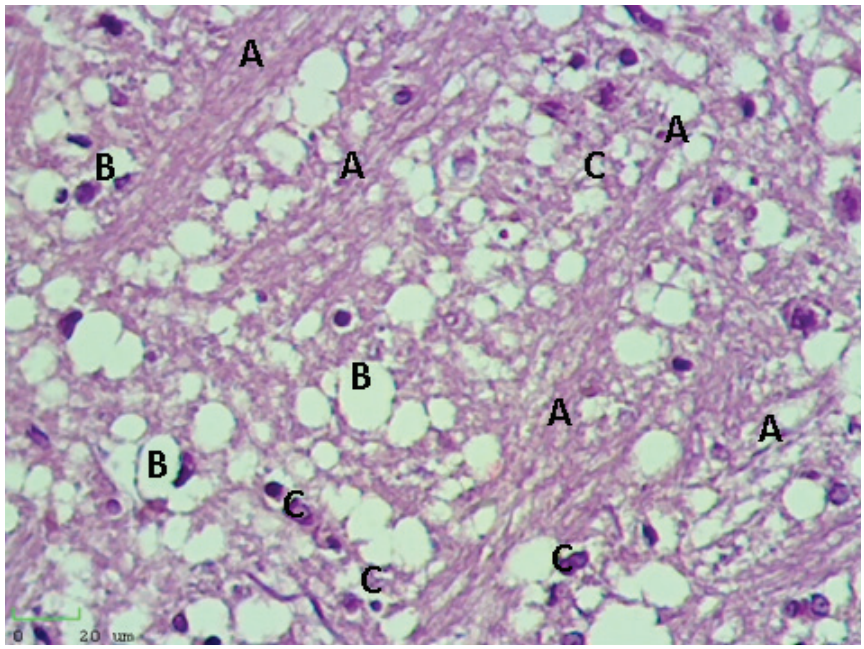

Figure (5): Brain section for female white rat in pregnant stage 20 day treatment the metronidazole medicine (1000 mg/kg) as shown: A-Degenerated and dislocated nervous fibers. B-Cavitation and vacuolation of the cerebral cortex tissue. C- Nuclei of glial and neuronal cells that lost their cytoplasm. H & E 40X.

Discussion

Metronidazole crosses the blood-brain barrier, and its induction of cerebral cytotoxicity does not depend on how it is administered orally or intravenously [10]. Several studies have been conducted on the neurotoxic mechanism of metronidazole, but until now the mechanism has not been clearly defined by the hypotheses developed by researchers[11]. If many experiments were conducted on animals (rats), which showed the occurrence of neuronal modulation after treatment with metronidazole with damage to the cerebellum, metronidazole and its metabolic derivatives are associated with RNA in the nucleus of the neuron and inhibit the production of proteins and the occurrence of bulging and breakdown of the axon [12].

The results of the study showed the occurrence of cavitation and widespread cavitation in the brain tissue with the occurrence of a breakdown of the axons and the occurrence of hemorrhage, and this increased with the increase in the dose given. The concentration of metronidazole is high in the extracellular space in the brain that is likely to cause toxicity to the brain [15]. This is consistent with what the researcher [16] found through histological microscopy, and it was found that the treatment of cats with metronidazole At a dose of 73.5-147 mg/kg for forty days, leaders lead to a loss of the medulla of some cranial nerve axons with necrosis and degeneration of some neurons, an increase in the number of glial cells and swelling of the axons with the presence of large macrophages in the Brainstem . Also, the treatment of rabbits with metronidazole in a dose of 20 and 40 mg/kg, and through microscopic histological examination of the brain and sciatic nerve, led to cavitations in the brain, spongy changes and degeneration of nerve bundles in the sciatic nerve with cell degeneration and the loss of Burkinji cells with severe congestion in the brain with loss of the covering Myeloma of the sciatic nerve axons [17]. [18] also indicated that the treatment of male rats with metronidazole in doses of 135 mg/kg and 540 mg/kg for sixty days, and through histological examination found in brain tissue the occurrence of blood congestion within the blood vessels, hemorrhage, Burkinji cells necrosis and hemorrhage in the granular layer and epidermis around Supper neuron and glial cells Metronidazole metabolic products may be associated with DNA or RNA in neurons [19].

The association of metronidazole or its metabolic derivatives with RNA inhibits the construction of proteins and consequently breaks the axons in nerve

fibers [20; 21; 6]. Metronidazole also induces the oxidation of norepinephrine, dopamine, and all catecholamine derivatives to form nitro-ion roots that reduce tissue oxygen, the superoxide roots, which increase the water content causing swelling of the axons [22]. It may also induce vascular spasm, which may result in a lack of localized oxygen, in which case it leads to any tissue breakdown[23].

Reducing metronidazole as a result of metabolic processes produces a synthetic anti-inflammatory, thiamine, as metronidazole turns into a vitamin B1 analog, and therefore it can be a base substance for the enzyme thiamine, which leads to competition vitamin B1, so metronidazole is converted into a counterpart of thiamine. It leads to harmful feeding of the nerves, which causes neurological diseases[24]. Vitamin B1 is a key coenzyme in the mitochondria of the alpha-ketoclutarite and pyrophite metabolites which are part of the biochemical pathway to produce ATP which is the most basic energy in the cell [25]. In the lobule cells, the metabolic metabolites of metronidazole are associated with RNA instead of the deoxyribonucleic acid (DNA). Therefore, the construction of cellular proteins will be inhibited due to the metronidazole binding to the RNA and thus lead to the breakdown of neuronal modulators[26]. Whereas [11] stated that the neurotoxicity of metronidazole occurs through its inhibitory effect of the-aminobutyric acid neurotransmitter.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of both MOH and MOHSER in Iraq

Conflict of Interest: Non

Funding: Self-funding

References

1. Senhorinho GN, Nakano V, Liu C, Song Y, Finegold SM, Avila-Campos MJ.Occurrence and antimicrobial susceptibility of Porphyromonas spp. and Fusobacteriumspp. in dogs with and without periodontitis. Anaerobe. 2012; 18: 381–385.
2. Lessa FC, Bamberg WM, Beldavs ZG. Burden of Clostridium difficile infection in the United States. N Engl J Med. 2015; 372: 825-834.
3. Roy U, Panwar A, Pandit A, Das SK, Josh B. Clinical and Neuroradiological Spectrum of Metronidazole Induced Encephalopathy: Our Experience and the Review of Literature. J Clin Diagn Res. 2016;10(6): OE01–OE09.
4. Rainer BM, Kan S,Chienb AL. Rosacea: epidemiology, pathogenesis, and treatment. J Am Acad Dermatol. 2018; 9: 1-10.
5. DivakaruniAK,MahabirB, Orrett FA, Adidam Sneha R, Venkata SA, AdidamCR,Divakaruni S. Compliance and Treatment Outcomes of Various Regimens for Trichomoniasis in Trinidad and Tobago. J Med Sci.2019; 7 (10): 97.
6. Ceruelos AH, Romero-Quezada LC, Ledezma J, Ruvalcaba C, Contreras LL. Therapeutic uses of metronidazole and its sid effects: an update. Europ Rev Med pharm sci.2019; 23:397-401.
7. Leffler DA, Lamont JT. Clostridium difficile infection. N Engl J Med. 2015; 372: 1539-1548.
8. Johnson S, Louie TJ, Gerding DN, Cornely OA, Chasan-Taber S, Fitts D, Gelone SP, Broom C, Davidson DM. Vancomycin, metronidazole, or tolevamer for Clostridium difficile infection: results from two multinational, randomized, controlled trials. Clin Infect Dis. 2014;59(3):355-7.
9. Chacho J, Pramod K, Sinha S, Mahadevan M,BharathRD, Bindu PS,Yasha TC,Taly SB. Affiliations expand. Clinical, neuroimaging and pathological features of 5-nitromidazole-induced encephalo-neuropathy in two patients: Insights into possible pathologenesis. Neurol Ind.2011; 59: 743-747.
10. Agarwal A, Kanekar S, Sabat S, Thamburaj K. Metronidazole-induced cerebellar toxicity. Neurol Inter.2016;8: 6365.
11. Evans J, Levesque D, Knowles K, Longshore R,Plummer S. Diazepam as a treatment for metronidazole toxicosis in dogs: a retrospective study of 21 cases. J Vet Intern Med. 2003; 17:304–310.
12. Bradley WG, Karlsson IJ, Rassol CG. Metronidazole neuropathy. Br Med J.1977; 2:610–611.
13. Nair AB, Jacob S.Asimple practice guide for dose conversion between animals and human. J B Clini pharm.2016; Vol 7: 27-31.
14. Suvarna K, Layton C, Bancroft J.Bancroft's theory and practice of histological techniques.8th ed . Elsevier Health Sci. 2018; pp79- 186 .
15. Frasca D, Dahyot-Fizelier C, Adier C, Mimo O, Debaene B, Couet W, Marchand S. Metronidazole and hydroxymetronidazole central nervous system

distribution: 1.microdialysis assessment of brain extracellular fluid concentrations in patiants with acute brain injury. Antimicrob Agents Chem.2014; 58(2): 1019-1023.

16. Olson EJ, Morales SC, Mcvey AS,Hayden DW. Putative metronidazole neurotoxicosis in a Cat. Vet Pathol.2005; 42: 665-669.
17. Al shibani WH, Ghelab KG, Naji HB. An evaluation of neurotoxic effect of metronidazole in rabbits. Kufa J Vet Med Sci. 2011; 2(2).
18. Oda SS. Histopathological and Biochemical Alterations of Metronidazole-Induced Toxicity in Male Rats. Glob Vet.2012;9 (3): 303-310.
19. Wright KH, Tyler JW. Recognizing metronidazole toxicosis in dogs. Vet Med.2003; 98:410–418.
20. Dingsdag SA, Hunter N. Metronidazole: an update on metabolism, structure-cytotoxicity and resistance mechanisms. J Antimicrob Chemother. 2018; 73: 265-279.
21. Goolsby TA, Jakeman B, Gaynes RP. Clinical relevance of metronidazole and peripheral neuropathy: a systematic review of the literature. Int J Antimicrob Agents.2017; 51: 319-325.
22. Ra DN, Mason RP. Generation of nitro radical anions of some 5-nitrofurans, 2-and 5-nitroimidazole by norepinephrine, dopamine, and serotonin. A possible mechanism for neurotoxicity caused by nitroheterocyclicdruge. J Biol Chem,1987; 262:11731-11736.
23. Ahmed A, Lose DJ, Bressler EL. Reversible magnetic resonance imaging findings in metronidazole induced encephalopathty. Neuro,1995; 45; 588-9.
24. Alston TA, Abeles RH. Enzymatic conversion of the antibiotic metronidazole to analog of thiamine. Arch. Biochem. Biophys. 1987; 257(2):357-362.
25. Butterworth RF. Pathophysiologic mechanisms responsible for the reversible (thiamine-responsive) and irreversible (thiamine non-responsive) neurologicale symptoms of Wernicke's encephalopathy. Drug Alcohol Rev. 1993; 12(3):315-322.
26. Aronson JK. Metronidazole side effects of drug 16th ed : the international encyclopedia of adverse drug reaction and interactions. Elsevier, Amst. Indian J Pharmacol. 2016; 48(2): 224.