An evaluation of neurotoxic effect of metronidazole in rabbits

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Abstract:

The present study was undertaken to assess the neurotoxic effect of metronidazole in rabbits .Eighteen rabbits were randomly divided into three equal groups ,first group was injected with metronidazole at the therapeutic dose 20 mg/kg.B.W. , second group was injected with metronidazole at double therapeutic dose where as the last group serve as a control .Each animal in all groups was administered intraperitoneally twice daily for (20) days .

The evaluations markers have used in this study were monitering of clinical symptoms and determination of histopathological changes in brain and sciatic nerve. The careful observation of clinical signs ,reflexes (patellar ,cross extensor ,and papillary light) and certain responses (pupil size ,nystagmus and a menance response) in first and second group showed a remarkable nervous system dysfunction has improved by histopathological examination of brain and sciatic nerve .there were sever vacuolation in brain ,where the lesion was characterized by spongiform changes ,and degeneration of the nerve bundles in sciatic nerve were detected in rabbits treated with metronidazole at therapeutic dose ,where as there is degeneration and selective loss of parkinjii cells with sever congestion in brain as well as demylination and degeneration in sciatic nerve in rabbits treated with metronidazole at double therapeutic dose .This study revealed that metronidazole has a neurotoxic effect in rabbits (both centrally and peripherally) with a severity depended on its dose and duration of administration.

تقييم السمية العصبية للمترونيدازول في الأرانب

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الخلاصة:

أُجريت هذه الدراسة لتقيم ألتأثير السمي العصبي للمترونيدازول في الأرنب. تم استخدام ثمانية عشر أرنب قسمت عشوائياً إلى ثلاث مجاميع متساوية ،حقنت المجموعة الأولى بالجرعة العلاجية للمترونيدازول (٢٠) ملغم/كغم من وزن الجسم ،وحقنت المجموعة الثانية بضعف الجرعة العلاجية ، في حين اعتبرت المجموعة الأخيرة كمجموعة سيطرة .جميع الحيوانات في كل المجاميع تم حقنها عن طريق البريتون بواقع مرتين باليوم ولمدة (٢٠) يوم .أن مؤشرات التقييم التي تم استخدامها في هذه الدراسة تضمنت مراقبة العلامات السريرية وتحديد التغيرات النسيجية المرضية في الدماغ والعب ألوركي.

أن المشاهدة الدقيقة للعلامات السريرية ،بعض المنعكسات (المنعكس الرضفي ،منعكس الانبساط المستعرض، ومنعكس البؤبؤ للضوء) ،وبعض الاستجابات المحددة (حجم البؤبؤ ،الرأرأة ،والاستجابة لخطر التهديد)في المجموعتين الأولى والثانية أظهرت اعتلالاً في الجهاز العصبي تم تأكيدهُ من خلال الفحص النسيجي المرضي للدماغ والعصب ألوركي .حيث تم رصد تفاج في الدماغ ،أذ تميزت الآفة بتغيرات أسفنجية الهيئة ، وتنكس الحزم العصبية في العصب ألوركي للأرانب المعالجة بالمترونيدازول وبالجرعة العلاجية ،بينما لوحظ تنكس الخلايا والفقدان النوعي لخلايا بيركنجي مع احتقان شديد في الدماغ بالإضافة إلى التجرد من النخاعين وتنكس الخلايا في العصب ألوركي للأرانب المعالجة بالمترونيدازول بضعف الجرعة العلاجية.

لقد بينت هذه الدراسة بـأن للمترونيدازول تـأثيرات سمية عصبية (محيطية ومركزية)في الأرانب تعتمد في شدتها على الجرعة ومدة الإعطاء.

Introduction:

Metronidazole is a unique antimicrobial agent that has very little effect on most aerobic Gram-positive and Gram-negative bacteria, but is highly effective against anaerobic bacteria (1). It has antiprotozoal properties where it used routinely in treatment of Giardiasis, Trichomoniasis, and Amebiasis (2).

Metronidazole is lipophilic and distributes widely where peritoneal fluid and milk concentrations approach plasma concentrations .It reaches therapeutic concentrations in bones , abscesses ,and the CNS. It readily cross the placenta and enters the fetal circulation (1).It is rapidly take up by obligate anaerobic microorganism and converted into active form by reduction of its nitro group then binding to DNA and prevent nuclic acid formation (2).Metronidazole is metabolized primarily in the liver ,both unchanged drug and metabolites are eliminated in urine and feces .

Metronidazole in rabbits has been cited as a treatment of choice for enterotoxaemia caused by *Clostridium spiroforme* ,which is considered one of the most common and dangerous disease in rabbits (3),also it has been found to be effective in preventing abscess formation after experimental septic peritonitis in rabbits(4).

The adverse effects of metronidazole were documented in both human (5&6) and animals (7). In veterinary medicine ,in both treated cases and experimental studies , the most commonly

reported side effects of oral administration of metronidazole include lethargy ,anroxia ,vomiting ,and diarrhae in dogs (8&9),in addition to salivation /ptyalism in cats (10&7).Another study using 14c- labeled metronidazole detected accumulation of the unchanged drug in the cerebellum and hippocampal areas of mice after intravenous administration(11).

The studies concerning the neurotoxicity of metronidazole in rabbits ,a popular pet, are still rare where veterinary practitioners should be a ware of susceptibility of rabbits and the form of metronidazole neurotoxicity in these species where they still questionable .

Therefore, the present study was carried out to investigate the side effect and neuronal histopathological alteration associated with metronidazole administration in rabbits.

Materials and Methods:

The experiment of this study was conducted in the animal house at veterinary medicine college of Al-Qadisiya university .Eighteen healthy adult of local breed rabbits of either sex with a mean of body weight 2.58±0.44 Kg .All rabbits were clinically examined before the beginning of the experiment, then they were randomly assigned into three groups (six animals per group).Rabbits of all groups were injected intraperitoneally twice daily for (20) days with equal volume as follow:-

Group (A): injected with metronidazole (flagel) at recommended therapeutic dose in rabbits (20) mg /Kg.B.W. (12).

Group (B): injected with metronidazole at (40) mg /Kg.B.W.

Group (C): injected with normal saline and serve as control group.

─ Parameters: the following parameters were assayed:

1. Careful observation of general clinical signs showed by animals along the period of experiment.
2. Assessment of some reflexes and responses that have beneficial role in determination of the severity and location of nerve lesion including:

─Patellar reflex.
─Cross extensor reflex.
─Pupillary light reflex .
─Eye pupil size .
─ A menace response .

3. At the end of the experiment rabbits in all groups were then sacrificed, and the brain and sciatic nerve were taken in order to examine histopathological change.

Results:

Clinical observations of the animals along the duration of the experiment explained that metronidazole treated animals showed general signs reflected nervous dysfunction as weakness, lethargy, ataxic, tremor, and marked alteration in response to certain reflexes and external stimuli (table, 1).

The onset of these signs and the response to reflexes and external

stimuli were obviously increased proportional to the dose used and the duration of treatment with mitronidazole (table, 1).

Rabbits treatment with mitronidazole at dose of (20 mg/Kg B.W.I.P.) showed clear nervous signs compared with animal in control group ,but it is less prominent as compared with rabbits received (40 mg/Kg B.W.I.P.) dose of metronidazole, where they showed more prominent clinical nervous signs especially in the second ten day period of the experimt as weakness of movement ,knuckling , incomplete flexion ,extension cause wobble ,ataxia ,falls down easily ,difficulty in rising ,nystigmus ,abnormal pupil size and tetraparesis.

Table (1): Reflexes and response to external stimuli in rabbits treated with metronidazole for (20) days.

Time Period	Reflexes and Responses	Group A	Group B	Group C
(1-10) Day	Patellar reflex	Present *	Present *	Reduced in (2) treated animals
	Cross - Extensor Reflex	Present *	Present *	Mild Reduction in (2) animals
	Pupillary Light Reflex	Present *	Present *	Slow Response *
	Pupil Size	Bilateral Normal size *	Bilateral Normal size *	2 Animals showed bilateral miotic pupil size
	A menance Response	Present *	Present *	Present *
	Nystagmus	Absent *	Absent *	Absent *
(10-20) Day	Patellar Reflex	Present *	Mild Reduction *	greatly reduced*
	Cross - Extensor Reflex	Present *	Mild Reduction *	greatly reduced*
	Pupillary Light Reflex	Present *	Slow *	slow and consensual response*
	Pupil Size	Bilateral Normal size *	Bilateral Mild miotic pupil size *	clear bilateral miotic pupil size*
	A menance Response	Present *	Reduced *	reduced *
	Nystagmus	Absent *	Present in (2) animals	Present *

Group A: rabbits treated with metronidazole at dose of (20)mg/Kg.B.W.I.P. Twice daily.
Group B: rabbits treated with metronidazole at dose of (40)mg/Kg.B.W.I.P .Twice daily.
Group C: rabbits injected I.P. with normal saline twice daily.
* In all animals in the group.

Microscopic examination of brain sections from animals of both treated groups was much different from the histological section of control group (fig.1). In sections taken from brain of rabbits treated with metronidazole at dose of (20) mg/kg. B.W.I.P at multiple sites there were several fairly well demarcated areas of necrosis and increased cellularity ,affecting different regions of brain within the necrotic foci ,there was no apparent preservation of neurons, a strocytes or oligodendrocytes (fig.2&3).

Brain lesions also were characterized by spongiform changes due to cytoplasmic vacuolation within the neurons (fig.4&5).

Histopathological examination of brain speciemens from animals treated with 40 mg/kg B.W.of metronidazole showed more prominent degeneration (swelling and vacuolation) compared with animals received metronidazole at dose of (20) mg/kg B.W. and also there was selective loss of purkinji cells with sever brain congestion (fig.6&7).

The histopathological changes in sciatic nerves sections from rabbits treated with metronidazole at therapeutic dose for (20)days were swollen axons with degeneration in bundles of nerve fibers (fig.9&10), where as the nerves in sciatic nerves of animals received double therapeutic dose for (20) days undergo demyelination and sever vacuolation and degeneration of axons (fig. 11&12).Compared with microscopic alterations in sciatic nerve sections from both treated groups there were no evidence of detected histopathological alteration in sciatic nerve sections from rabbits in control group (fig.8).

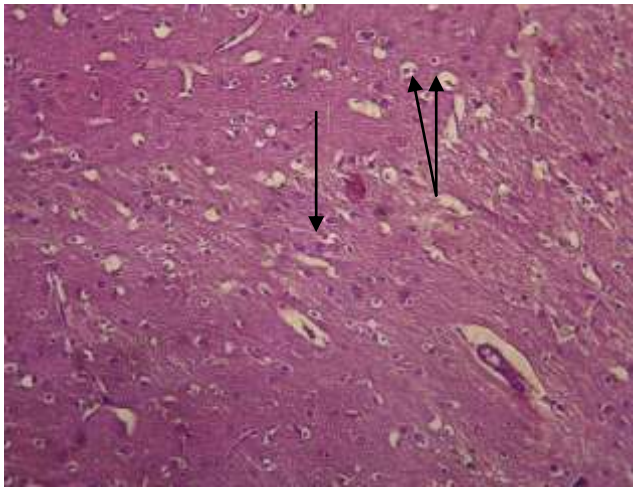

Figure (1): Brain. Control group. There is normal brain tissue and normal neurons (double arrows) and presence of normal pyramidal cells (arrows). 50X H&E.

Figure (2): Brain. There were several fairly well demarcated areas of necrosis (arrows). 50X H & E.

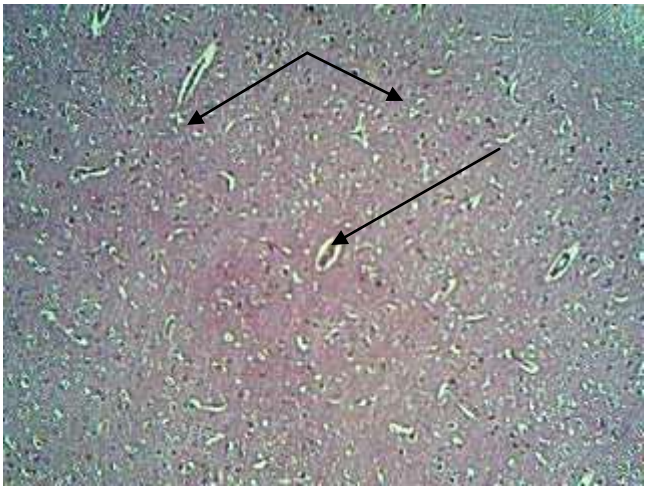

Figure (3): Brain. There were several fairly well demarcated areas of necrosis (arrow) and increased cellularity, affecting different regions of brain (double arrows). 50X H & E.

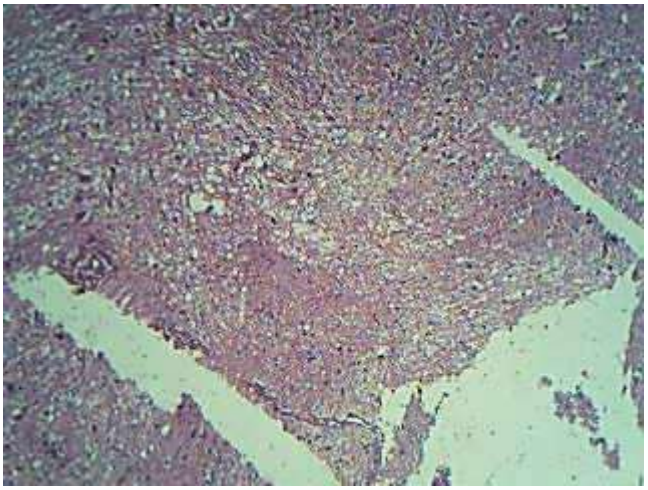

Figure (4): Brain. There are sever vacuolation in the brain, the lesion was characterized by spongiform changes due to cytoplasmic vacuolation within the neurons (arrows). 50X H & E.

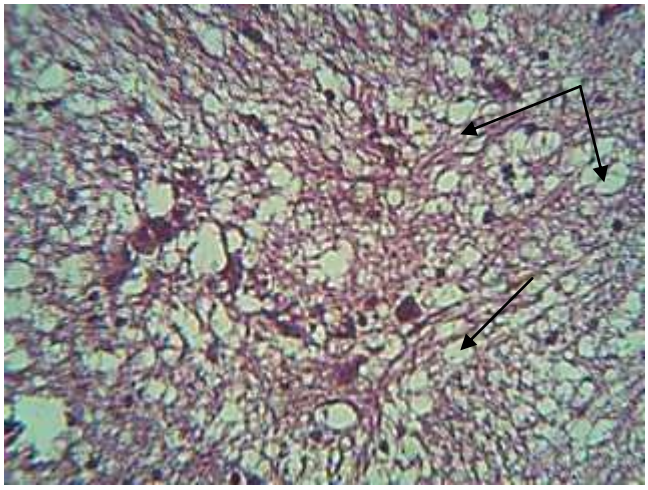

Figure (5): Brain. There are sever vacuolation in the brain, the lesion was characterized by spongiform changes due to cytoplasmic vacuolation within the neurons (arrows). 200X H & E.

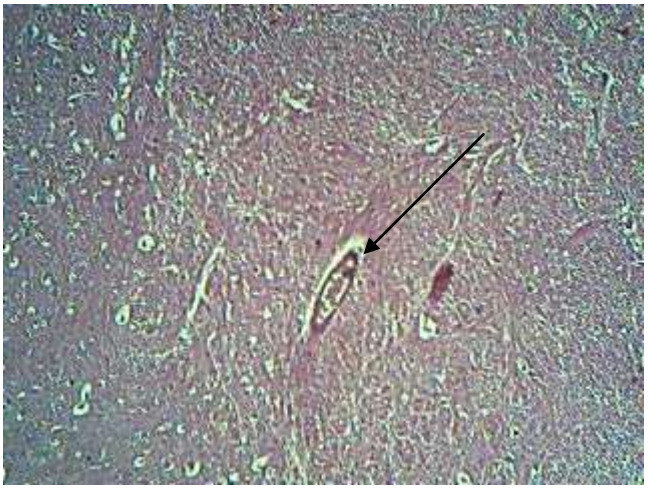

Figure (6): Brain. There is degeneration (swelling and vacuolation) and selective loss of purkinji cells with severs brain congestion (arrow). 50X H & E.

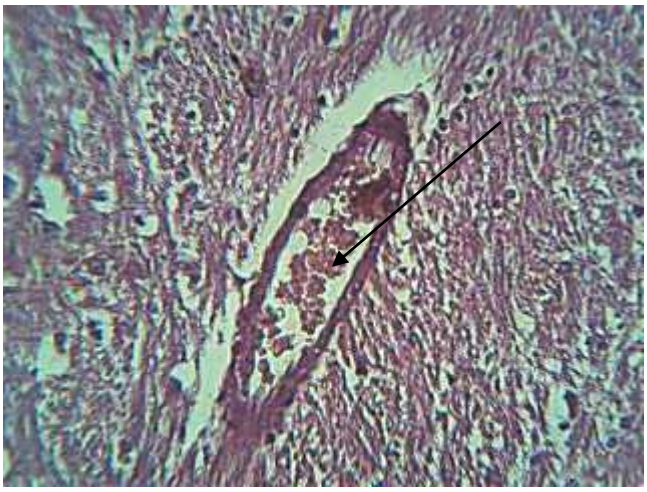

Figure (7): Brain. There is degeneration (swelling and vacuolation) and selective loss of purkinji cells with sever brain congestion (arrow). 200X H & E.

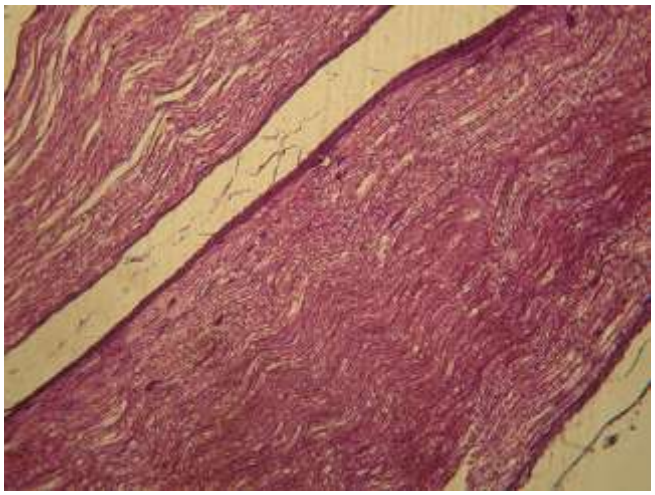

Figure (8): Sciatic nerves. Control group. There are normal axons with normal bundles of nerve fibers (arrow). 50X H & E.

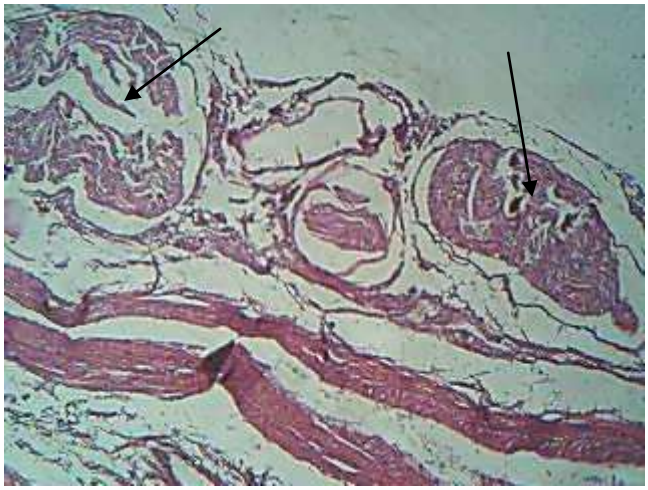

Figure (9): Sciatic nerves. There are swollen axons (arrows) with degeneration in the bundles of nerve fibers. 200X H & E.

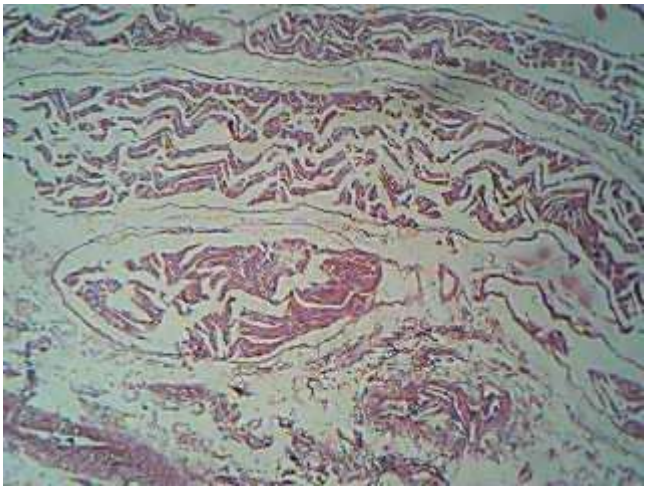

Figure (10): Sciatic nerves. There are swollen axons (arrows) with degeneration in the bundles of nerve fibers. 50X H & E.

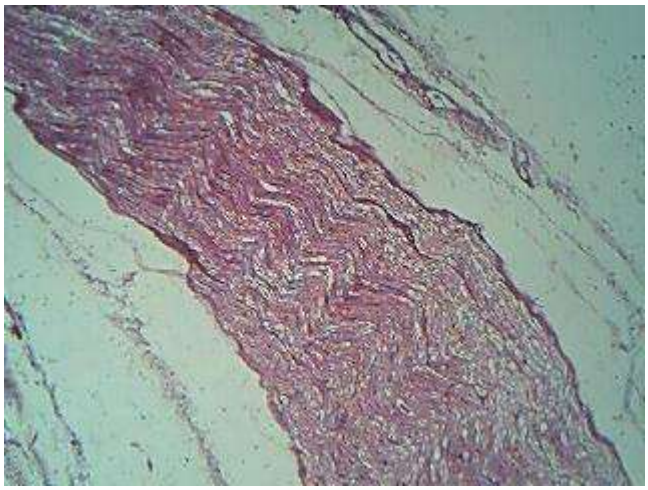

Figure (11): Sciatic nerves: there is demyeliation with vacuolaion and sever degeneration of axons. 50X H & E.

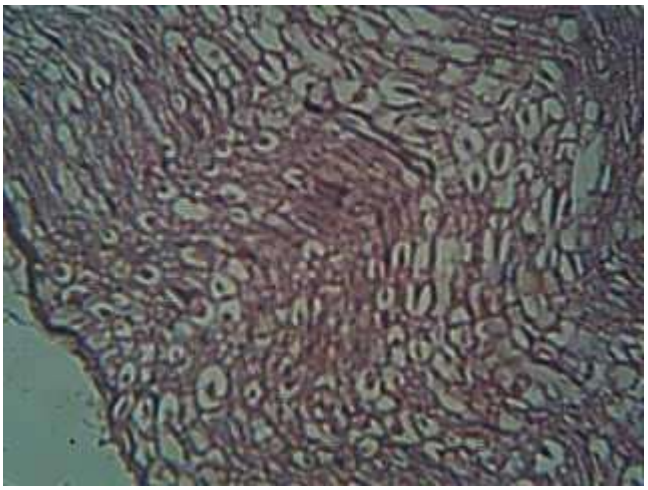

Figure (12): Sciatic nerves: there is demyeliation with vacuolaion and sever degeneration of axons. 200X H & E.

Discussion:

The obvious reduction in sensation and motor activity in metronidazole treated rabbit was manifisted by weakness in limbs movement, decrease in peripheral reflexes (patellar reflex and cross-extensor reflex) could be altributed to the degenerative lesion in peripheral nerves (particularly axonopathies and /or peripheral myelinopathies) as it showed by the present study .Furthermore, as the axons degenerate and /or the disruption of myelin is diffusely occurred generate a global neurological deficit, when they are limited to peripheral nerves produces the symptoms of peripheral neurpathy (13&14), in which impairment in sensation and motor strength are first impaired in the most distal extent of the axonal process particularly in the fore and hind limbs (13).The axanopathy recorded in this study come in agreement with that referred by (15) where metronidazole causes sensory and peripheral neuropathy.

The results of the present study showed that treated animals suffered from weakness of movement ,ataxia ,falling down easily ,difficulty in rising (tetraparesis) ,nystagmus and abnormal of the pupil size and reflexes raised the possibility of a central function lesion as it was confirmed by histopathological examination in this study .It was documented by (16) that nystagmus that is usually occurred with impaired consciousness, abnormal pupils and opithotonus originated from cerebrallar –potine area to midbrain area injury and increased intraocular pressure.

The result of CNS dysfunction that recorded in this study was consistent with previous studies in dog (8& 9), cat (7) and human (5& 6).

The mechanism of toxic effects of metronidazole has not been identified (17) .The neurotoxic effect of metronidazole may be due to one or more of the following speculations:

The first proposed that the mechanism of toxicity is well linked to the fact that metronidazole and it's reduced metabolites ,bear close structural resemblance to the antineuritic nutrient ,thiamine ,where metronidazole toxicity may be due ,wholly or in part ,to its conversion to thiamine analogue and consequent vitamin B1 antagonism (18)

Consistent with this hypthesis , the drug is accepted as substrate for the thiaminase (18) so conversion of metronidazole to an analog of thiamine may mimic nutrition deficiency neuropathy (18&19) .

Vitamin B1 is an essential co enzyme in the mitochondrial metabolism of α-ketoglutarate and pyruvate ,where they are part of biochemical pathways that resulted in generation of ATP, a major form of energy for the cell(20) .Also pyruvate dehydrogenase is involved in the production of acetylcholine, and for myelin synthesis (21) ,and

this may explain the demylination that observed in histopathological sections in this study .In consistency with this speculation we will start a study focusing on using of vitamin B1 to amiolerate the neurotoxicity of metronidazole .

Second speculation, the intermediate metabolities of metronidazole are thought to be capable of binding to and diruspting of cellular DNA and induce cell death in anaerobic microganism (22&23) .In mammalian cell it has been proposed that metronidazole and /or its metabolites may bind to RNA instead of DNA (22), so the neuronal protein synthesis may be inhibited by metronidazole – mediated RNA binding ,which causes axonal degeneration (24&25)

Another speculation, postulated that nitroradical anions and semiquinone generated during reactions between catecholamines and metronidazole contribute to metronidazole neurotoxicity (26).

Further speculation for metronidazole neurotoxicity based on metronidazole affinity for the GABA receptor site was based on the similarity of both the chemical structure and clinical signs of toxicity of metronidazole and benzodiazepine antagonist flumazenil ,which also known to attach to the GABA receptor (27), where stimulation of these receptors results in CNS depression ,somnolence, fatigue , lethargy ,ataxia and muscular incoordination (28&29) which also may explain the clinical signs observed in this study .

A dose –dependent and duration – dependent relationship between central and peripheral neurotoxicity induced and metronidazole administered was demonstrated in rabbits.

Further studies are required to overcome or amiolerat the neurotoxic effect of metronidazole as our future study that raise a question about using of thiamine in alleviation of neurotoxicity induced by metronidazole.

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