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Full Length Research Paper

Pharmacodynamic and pharmacokinetics of metronidazole in protein malnourished rats

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The aim of this study is to evaluate the relationships between metronidazole toxicity and pharmacokinetics in protein malnourished rats. The study was carried out on two sets of rats, normally-fed set and protein malnourished set. Each set was divided into a control group and three-treated groups that received metronidazole daily in one dose of 200, 400 or 800 mg/kg for 30 days. Liver enzymes as well as testosterone and gonadotropin hormones levels were estimated. The pharmacokinetic profiles were carried out over a period of 48 h. Metronidazole plasma levels were determined by a validated high-performance liquid chromatography (HPLC) method. Metronidazole has dose dependent effects on hematological profile, liver enzymes, testosterone and gonadotropins which potentiated in protein malnourished rats. Protein malnutrition elevated metronidazole C_{max}, AUC_{0-48}, and $AUC0_{-inf}$, prolonged $t_{1/2}$ and MRT, and decreased elimination rate constant. The clinical significance of this work should be stressed in case of protein malnourished individuals due to the risk of accumulation of the drug after repeated doses.

Keywords: Protein malnutrition, rats, Metronidazole, pharmacokinetics, pharmacodynamic, Carbamazepine.

INTRODUCTION

Metronidazole is an antimicrobial drug used in treatment of protozoal and anaerobic bacterial infection. It is rapidly and completely absorbed from the gastrointestinal tract (Ralf, 1983; Fredricsson et al., 1987; Tracy and Webster, 2001) and widely distributed in most tissues and body fluids (Schwartz et al., 1979; Sattar et al., 1982; Nagar et al., 1989). Metronidazole administration in high doses caused harmful effects on some organs and affects males' fertility in rats (Schwartz et al., 1979; McClain and Downing, 1988; Bone et al., 1998; Mudry et al., 2007). Metronidazole toxicity may induce several neurologic side effects, including peripheral neuropathy, ataxic gait, dysarthria, convulsive seizures, and encephalopathy (Hobson et al., 2006; McGrath et al., 2007).

The frequency of protein malnutrition (PM) is rapidly increasing in developing countries (Merino-Sanjuán et al., 2011). Dietary protein deprivation during early life is known to have adverse effects on brain anatomy, physiology, and biochemistry (Torún and Chew, 1993). PM was found to alter the safety and efficacy of some drugs by affecting their pharmacokinetics (González-Hernández et al., 2008), while it has no effect on pharma-cokinetics of other drugs (del Carmen et al., 2008). PM produces adverse functional effects such as loss of muscle (Lee et al., 2004; Araujoi et al., 2005), alteration of the immune system (Lehmann, 1991) that enhances the susceptibility to infections, and leads to mucosal atrophy (Reynolds et

Abbreviations: HPLC, High-performance liquid chromatography; **AUC_{0-48},** total area under the plasma concentration-time curve from time zero to 48 h; **AUC_{0-inf},** total area under the plasma concentration-time curve from time zero to time infinite; **C_{max},** peak plasma concentration; **t_{max},** time to reach a C_{max}; **k_{el},** elimination rate; **NF,** normally fed; **PM,** protein malnourished; **MTZ,** metronidazole.

al., 1996). The use of metronidazole has increased markedly, particularly in developing countries, where the association of malnutrition and parasitosis is very common (Lares-Asseff et al., 1993). This study was conducted to examine the effect of different doses of metronidazole in association with protein malnutrition on testosterone, Luteinizing hormone (LH) and follicle-stimulating hormone (FSH), hematological profile, liver function, and histopathology. In addition, metronidazole pharmacokinetics was evaluated by sensitive, simple, and accurate validated high - performance liquid chromatography (HPLC) method that was developed in our laboratory. This method was characterized by the utilization of a small volume of plasma and was simple for metronidazole extraction.

MATERIALS AND METHODS

Chemicals and reagents

Metronidazole was provided by Alex Co. for pharmaceutical industries (Egypt). Working standard of metronidazole (purity, 99.3%), and the internal standard carbamazepine (purity 99.7%) were obtained from internal references standard unit, National Organization for Drug Control and Research (NODCAR), Egypt. Sodium acetate and HPLC grade acetonitrile were purchased from E- Merck, Darmstadt, Germany. Analytical grade acetonitrile, acetic acid, perchloric acid, and methanol were supplied by Labscan, Ireland. De-ionised water was prepared on site (MILLIPORE, France).

Study design

Adult male Sprague Dawley rats weighing 180 ± 10 g, obtained from the animal house of NODCAR (Egypt), were maintained in a 12 h light and 12 h dark regime at a temperature of 25 ± 1°C. Synthetic standard diets and tap water were available *ad libitum*. This study was carried out in two sets of rats: a normally fed set and a protein malnourished set. Each set was divided into four groups, one control and three treated groups. Each group comprised 10 individuals. The animals were fed in standard synthetic diet containing either 20% casein for normally fed animals (Bamji and Sharada, 1972) or 5% casein for protein malnourished rats (Edozien, 1968). Metronidazole was administered orally for 30 days with doses of 200, 400 or 800 mg/kg. At the end of treatment (day 30), blood samples were collected under light ether anesthesia from the retro-orbital vein at 0.0, 0.5, 1.0, 1.5, 2.0, 4.0, 6.0, 8.0, 10.0, 24.0, and 48.0 h post-dose. Liver was reserved in 10% formalin-saline solution for subsequent histopathological investigations. The protocol was approved by the council and the ethical committee of the general division for basic medical science, NODCAR, Egypt.

Biochemical analysis

Plasma alanine (ALT) and aspartate transaminase (AST) activities were determined according to Reitman and Frankel (1957). Alkaline phosphatase was assessed using the alkaline phosphatase colorimetric assay kit of Abcam (UK), while creatinine evaluation was done according to the quantitative kinetic colorimetric method using kits obtained from Roch Diagnostics (Mannheim, Germany). Urea was determined using urea kits of Diamond Diagnostic (Hanover, Germany). Albumin was assessed by Brilliant Blue G (BBG) (Bromocresol Green Complex) method using albumin kit of Clinical system Co. (India). Shimadzu, UV 160 spectrophotometer was used to measure the values of the earlier mentioned parameters.

Blood analysis

Erythrocytes, leukocytes count, haematocrit, and hemoglobin percentage were evaluated using autohematology analyzer (Maxom, shenzhen Marcom Electronic Co., China).

Hormonal investigation

Plasma FSH, LH, and testosterone levels were measured by the radioimmunoassay method using enzyme-linked immunosorbent assay (ELISA) reader (BioTEK. Instruments Inc., ELx 808, USA), as described in the instructions provided with the Monobind Inc, USA kit. Testosterone was assayed in accordance to the manufacturer's recommendations (kit by IBL immune biological laboratories, Japan).

Histopathological examination

Liver specimens of the different groups were fixed in 10% formalin saline for 24 h. Paraffin tissue blocks were sectioned at 4 microns thickness and the tissue slide sections were then deparaffinised, stained by haematoxylin and eosin, and examined under the light electric microscope (Banchroft et al., 1996).

Liver biopsy specimens obtained from animals of different groups were prospectively studied. Histopathology changes were observed and grouped based on two main criteria: vascular changes include vessel congestion, fatty changes, and collagen fiber; and necrotic changes include necrosis, fibrosis, nuclear changes, abscesses, and cell regeneration. The morphological changes were assessed semi-quantitatively, blind by two independent investigators assessors. The histological diagnosis based on a 7-feature (portal ductal proliferation, bile plugs in portal ductules, porto-portal bridging, lymphocytic infiltration in portal region, multinucleated hepatocytes, neutrophilic infiltration, and hepatocellular swelling) and 15-point (0 to 15) scoring system. The points of specimen for the same group collected together and the average point per group was evaluated (Lee et al., 2008).

Determination of metronidazole levels

Validation of HPLC method was carried out according to bioanalytical method validation guideline of Food and Drug Administration concerning partial validation of bioanalytical methods (FDA Guidance for Bioanalytical Method Validation, 2001). The plasma calibrations curves were constructed in the range of 0.5 to 30 µg/ml. The extraction procedure involved protein precipitation by methanol following the addition of 25 µl of carbamazepine solution (200 µg/ml) as internal standard to 500 µl plasma. After brief mixing for 10 s and centrifugation for 10 min at 3500 rpm and 4°C, 200 µl of the supernatant was injected into HPLC system which composed of Waters pump and automated injection system (Waters, Milford, USA). The separation was achieved using an analytical Zobrax Eclipse XDB-phenyl (250 × 4.6 mm), 5 µm particle size column (Agilent), and the mobile phase consists of 0.05 M sodium acetate:acetonitrile:glacial acetic acid (75:25:1, v/v/v) and the pH was adjusted to 4.0 by phosphoric acid. The column effluent was monitored by ultraviolet (UV) detector (Waters, Milford, USA) at a value of 315 nm. The system was controlled and monitored by a single computer operated with Millennium software (Waters, Milford, USA).

Pharmacokinetic and statistical analysis

Kinetica® version 5.0 (Thermo fisher Scientific, USA) software was used to calculate metronidazole pharmacokinetics using non-compartmental pharmacokinetic analysis of C_{max}, t_{max}, AUC_{0-48}, AUC_{0-inf}, $t_{½}$, and MRT, and also, it was used to perform the statistical analyses of these pharmacokinetic using two way analysis of variance (ANOVA) after transformation of the data to their logarithmic (ln) values. Using the error variance (S^2) obtained from the ANOVA, the 90% confidence intervals (CI) were calculated from the following equation.

$$90\%\ CI = (X_t - X_r) \pm t\ (V)\ \sqrt{(s^2 \times 2/n)}$$

$X_t - X_r$: the means of the ln transformed values for the test (protein malnourished) and the reference (normally fed); S^2: the error variance obtained from the ANOVA; n: the number of animals; t: t tabulated value for 90% CI; v: the degree of freedom of the error variance from the ANOVA.

No drug-nutrition interaction effect was assumed if the 90% CI was between 0.8 and 1.25 for the log-transformed FDA according to bioequivalence guidelines, as drug nutrition interaction is one of bioequivalence aspect (Steinijans et al., 1991; FDA, 2001). Biochemical parameters statistical analysis was performed by one-way analysis of variance (ANOVA using SPSS statistical software (SPSS Inc Chicago, USA)). For histopathological evaluation, as nonparametric analysis, statistical analysis was performed using Mann-Whitney test for pair-wise comparison between the metronidazole in normally fed and in protein malnourished groups. The differences were considered significant if $P < 0.05$.

RESULTS

Metronidazole caused a decrease in red blood cells (RBCs) and lymphocytes count, and also reduced the hematocrit and hemoglobin percentage. The decrease was dose dependent and potentiated with protein malnutrition (Table 1). In addition, it was found that protein malnutrition decreases testosterone, FSH, and increases LH levels. These effects were of statistically significant. Administ-ration of metronidazole in dose levels of 200, 400, and 800 mg/kg resulted in a decrease in the plasma levels of testosterone, FSH, and LH in both normally fed and protein malnourished rats. These decreases were dose dependent and much more decrease was observed in protein malnourished animals (Table 2).

In control groups protein malnutrition caused a significant decrease in plasma albumin level while it had no effect on alkaline phosphatase or in plasma AST and ALT levels. The administration of metronidazole in doses of 200, 400, and 800 mg/kg, increased in a dose dependent way in all the aforementioned parameters both in NF and PM rats. The greatest effect in AST, ALT, and alkaline phosphatase plasma levels was observed at the highest dose of metronidazole and it started to be statistically significant at 200 and 400 mg/kg in protein malnourished and normal fed rats, respectively (Table 3).

Histopathological findings

The histopathology assessment in liver was performed for all groups. Liver samples of normally fed and protein malnourished groups showed normal well defined histo-logical structures without any signs of vascular or inflammatory changes (Figure 1). Liver sample of protein malnourished group showed mild fatty changes in the hepatocytes without any signs of vascular or inflam-matory changes (Figure 2); however, the signs of toxicity were revealed after administration of metronidazole in both groups. The changes in liver treated with 200 mg/kg metronidazole were not statistically significant in comparison to corresponding control group (Figure 3), while changes (as expressed in score previously mentioned in statistical section) in protein malnourished rats were statistically significant ($P < 0.05$) in comparison to normally-fed control one. These changes included dilatation and congestion in the central vein and inflammatory cells infiltration (Figure 4). Administration of the higher dose of metronidazole (400 mg/kg) resulted in more congestion in the central vein and more cells infiltration in both normally fed as well as in protein malnourished rats. These changes varied from mild in normally fed (Figure 5) to moderate in protein malnourished rats (Figure 6). In the highest dose used (800 mg/kg), the changes reside in vascular vessels congestion and inflammatory cell infiltration in normally fed rats (Figure 7), and these changes became more severe in protein malnourished rats (Figure 8).

HPLC assay

All chromatograms were free from any interference at the retention times of metronidazole or internal standard and both compounds were eluted as completely and it appeared as two separate resolved peaks without peak tailing. The retention times for metronidazole and carbamazepine (internal standard) were 4.063 and 11.526 min, respectively (Figure 9). Linear relationships ranging from 0.05 to 30 µg/ml ($r^2 = 0.9985$) were found between metronidazole plasma concentration and the peak area ratios (metronidazole/internal standard) Figure 10. The accuracy of metronidazole varied from 96.11 to 102.03% and from 92.00 to 101.00% for intra and inter-day, respectively (Table 4). The reproducibility of the method was defined by examining both intra- and inter-day variance. Coefficient of variance values ranged from 0.72 to 8.73% and from 0.48 to 9.68% for intra-day and inter-days assay, respectively (Table 4).

Pharmacokinetic parameters

Protein malnutrition significantly enhanced the rate and extent of metronidazole in all protein malnourished groups in comparison to the corresponding normally fed group. Metronidazole median t_{max} values varied from 1.25 h in groups treated with 200 mg/kg to 5.0 h following dose of

Table 1. Hematological parameters following administration of metronidazole in normally fed and protein malnourished rats.

Dose (mg/kg)	RBCs ($\times 10^6$ mm^{-2})		Leukocytes ($\times 10^3$ mm^{-2})		Haemoglobin (%)		Haematocrite (%)	
	NF	PM	NF	PM	NF	PM	NF	PM
Control	7.80 ± 0.19	5.47 ± 0.021*	7.94 ± 0.21	6.01 ± 0.02$^+$	69.5 ± 13.51	56.15 ± 0.02*	41.02 ± 0.19	34.12 ± 0.13*
200	7.11 ±1.25	4.75 ± 0.02*$^+$	8.01 ± 0.41	5.48 ± 0.02*$^+$	70.01 ± 0.15	53.20 ± 0.04*$^+$	43.02 ± 0.21$^+$	32.10 ± 0.02*$^+$
400	7.01 ± 0.41$^+$	4.65 ± 0.03*$^+$	7.51 ± 0.36$^+$	5.88 ± 0.04*$^+$	70.5 ± 0.46$^+$	52.1 ± 0.02*$^+$	40.0 ± 0.31$^+$	34.2 ± 0.03*$^+$
800	6.01 ± 0.06$^+$	5.01 ± 0.09*$^+$	7.10 ± 0.56$^+$	4.08 ± 0.10*$^+$	67.3 ± 0.51$^+$	51.02 ± 0.10*$^+$	38.01 ± 0.31$^+$	33.09 ± 0.19*$^+$

$^+$Significant in comparison to corresponding control group, $P < 0.05$. *Significantly difference in comparison to corresponding normally fed subgroup, $P < 0.05$.

Table 2. Effect of metronidazole on FSH, LH, and testosterone hormones in normally fed and protein malnourished rats.

Dose (mg/kg)	FSH (mIU/ml)		LH (mIU/ml)		Testosterone (ng/ml)	
	NF	PM	NF	PM	NF	PM
Control	14.6 ± 0.02	11.1 ± 0.03*	11.9 ± 0.01	12.1 ± 0.06*	7.4 ± 0.11	5.6 ± 0.08*
200	13.3 ± 0.15$^+$	9.3 ± 0.04*$^+$	10.9 ± 0.08$^+$	7.6 ± 0.09*$^+$	6.7 ± 0.13$^+$	4.7 ± 0.07*$^+$
400	8.6 ± 0.09$^+$	6.0 ± 0.05*$^+$	7.6 ± 0.11$^+$	5.3 ± 0.07*$^+$	3.9 ± 0.9$^+$	2.7 ± 0.011*$^+$
800	4.7 ± 0.08$^+$	4.9 ± 0.12*$^+$	6.0 ± 0.07$^+$	4.2 ± 0.09*$^+$	2.9 ± 0.13$^+$	2.0 ± 0.09*$^+$

$^+$Significant in comparison to corresponding control group, $P < 0.05$. *Significantly difference in comparison to corresponding normally fed subgroup, $P < 0.05$.

Table 3. Effect of metronidazole on liver aspartase aminotransferase (AST), analine transferase (ALT) activities, albumin, and alkaline phosphatase in rats.

Dose (mg/kg)	Alkaline phosphatase (IU/L)		Albumin (g/dl)		ALT (g/dl)		AST (g/dl)	
	NF	PM	NF	PM	NF	PM	NF	PM
Control	8.13 ± 1.02	7.98 ± 1.05	4.51 ± 1.2	3.12 ± 1.4*	19.01 ± 1.4	20.01 ± 1.2	7.46 ± 1.4	7.10 ± 1.6
200	9.11 ± 1.4	10.12 ± 1.8$^+$	4.42 ± 1.2	3.24 ± 0.9*	20.10 ± 1.2	23.01 ± 1.2*$^+$	7.96 ± 1.7	9.25 ± 0.95*$^+$
400	12.13 ± 2.1$^+$	13.97 ± 1.9*$^+$	4.19 ± 0.9	3.11 ± 1.0*	23.15 ± 2.1$^+$	25.12 ± 1.1*$^+$	9.01 ± 0.95$^+$	10.45 ± 1.4*$^+$
800	14.10 ± 2.0$^+$	17.01 ± 3.01*$^+$	4.21 ± 0.9	3.43 ± 0.8*	26.14 ± 1.1$^+$	28.10 ± 0.9*$^+$	9.15 ± 1.5$^+$	11.62 ±1.2*$^+$

$^+$Significant in comparison to corresponding control group, $P < 0.05$. *Significantly difference in comparison to corresponding normally fed subgroup, $P < 0.05$.

800 mg/kg in NF group (Table 5). The mean metronidazole C_{max} when administered in dose levels of 200, 400, and 800 mg/kg to protein malnourished rats was higher than the corresponding normally-fed groups. These increases are represented by 115.53, 14.63, and 6.62%, respectively. The 90% CI for the protein malnourished/normally fed (test/references) ratio for the log-transformed C_{max} was (0.567 to 1.558), (0.721 to 1.351), and (0.598 to 1.541) and not entirely contained within the 0.80 to 1.25 range, indicating that protein malnutrition significantly increased the rate of absorption of metronidazole (Table 5). The mean metronidazole $AUC_{0\text{-}inf}$ when administered in the aforementioned dose levels to protein

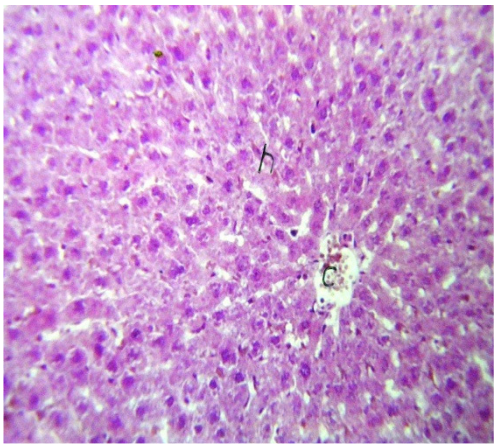

Figure 1. Light micrograph of the liver of normally fed rats showing normal liver structure (H&E stain, X64).

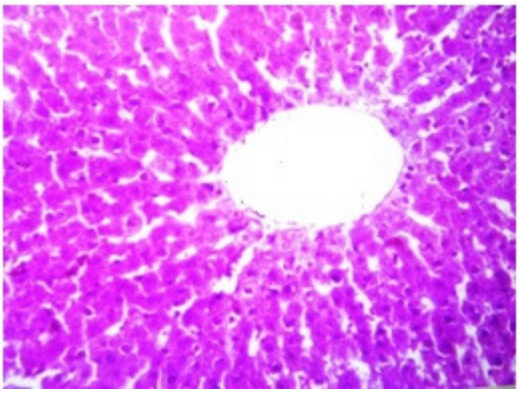

Figure 2. Light micrograph of liver of protein malnourished rats showing fatty change in the hepatocytes.

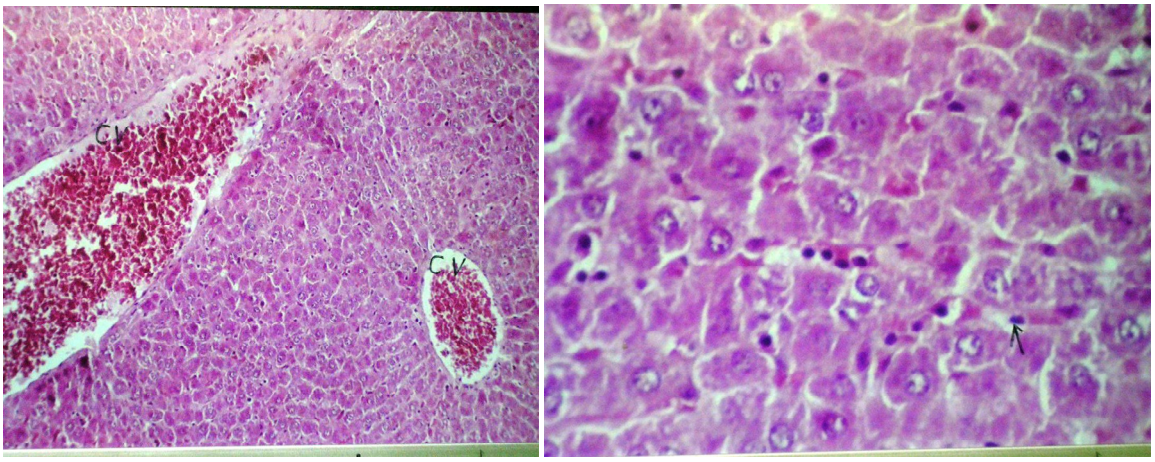

Figure 3. Light micrograph of the liver of normally fed rats treated with 200 mg/kg metronidazole for four weeks showing: (A) dilatation and congestion in the central vein (cv) (H&E stain, X40) and (B) diffuse Kupffur cells proliferation (arrow) in between the hepatocytes (H&E stain, X64).

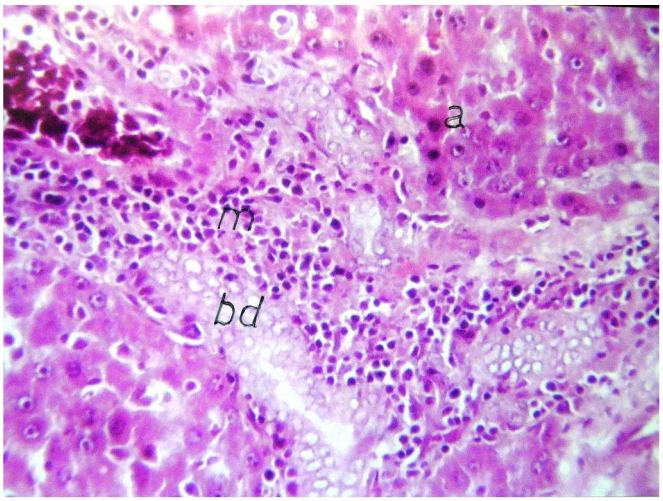

Figure 4. Light micrograph of the liver of protein malnourished rats treated with 200 mg/kg showing inflammatory cells infiltration (m) in between the newly formed bile ducts (bd) at the portal vein (H&E stain X80).

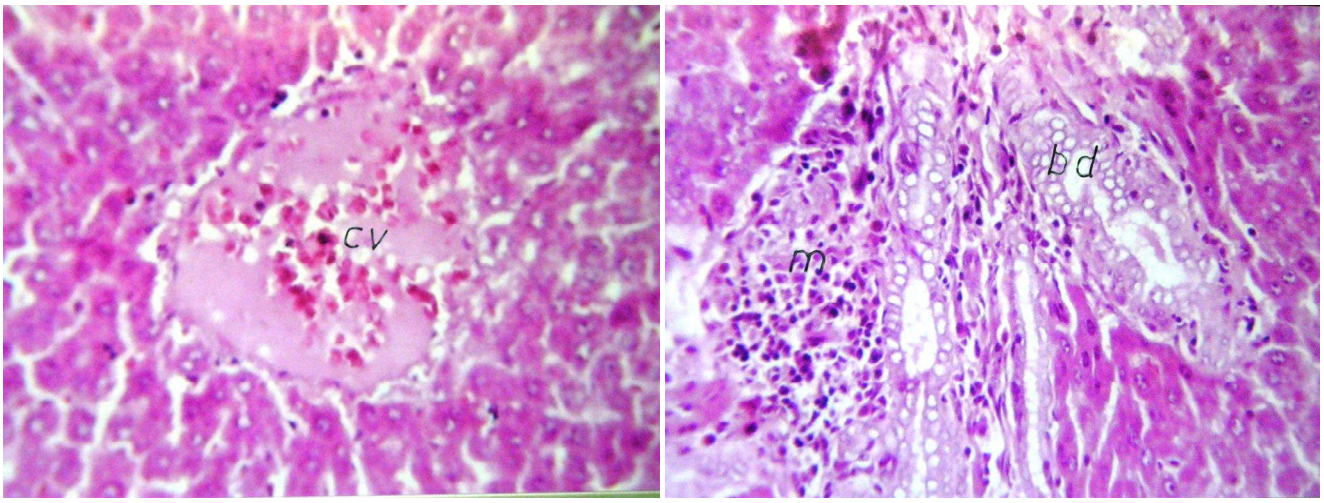

Figure 5. Light micrograph of the liver of normally fed rats treated with 400 mg/kg metronidazole for four weeks showing: (A) dilatation and congestion of the central vein (cv). (H&E stain X80) and (B) diffuse Kupffur cells proliferation in lining epithelium of bile duct (bd) and inflammatory cells infiltration in portal vein (m) (H&E stain, X64).

malnourished rats were approximately 69.48, 30.88, and 84.06%, respectively and they are higher than the mean $AUC_{0\text{-}inf}$ of the corresponding normally fed rats (Table 5). The 90% CI for the protein malnourished/normally fed ratio for the log-transformed $AUC_{0\text{-}inf}$ was (0.614 to 0.825), (0.721 to 1.315), and (0.654 to 1.450), respectively and not entirely contained within the 0.80 to 1.25 range, indicating that protein malnutrition significantly increased not only the rate of absorption, but also the extent of metronidazole absorption. In addition, protein malnutrition significantly prolonged the elimination half-life time and mean residence time of metronidazole as it was slowly eliminated in protein malnourished rats than normally fed rats (Table 5).

DISCUSSION

The main aim of this work was formulated to study toxico-kinetics of metronidazole in different doses in protein malnutrition rats. Induction of PM in this work resulted in several alterations in the investigated biochemical,

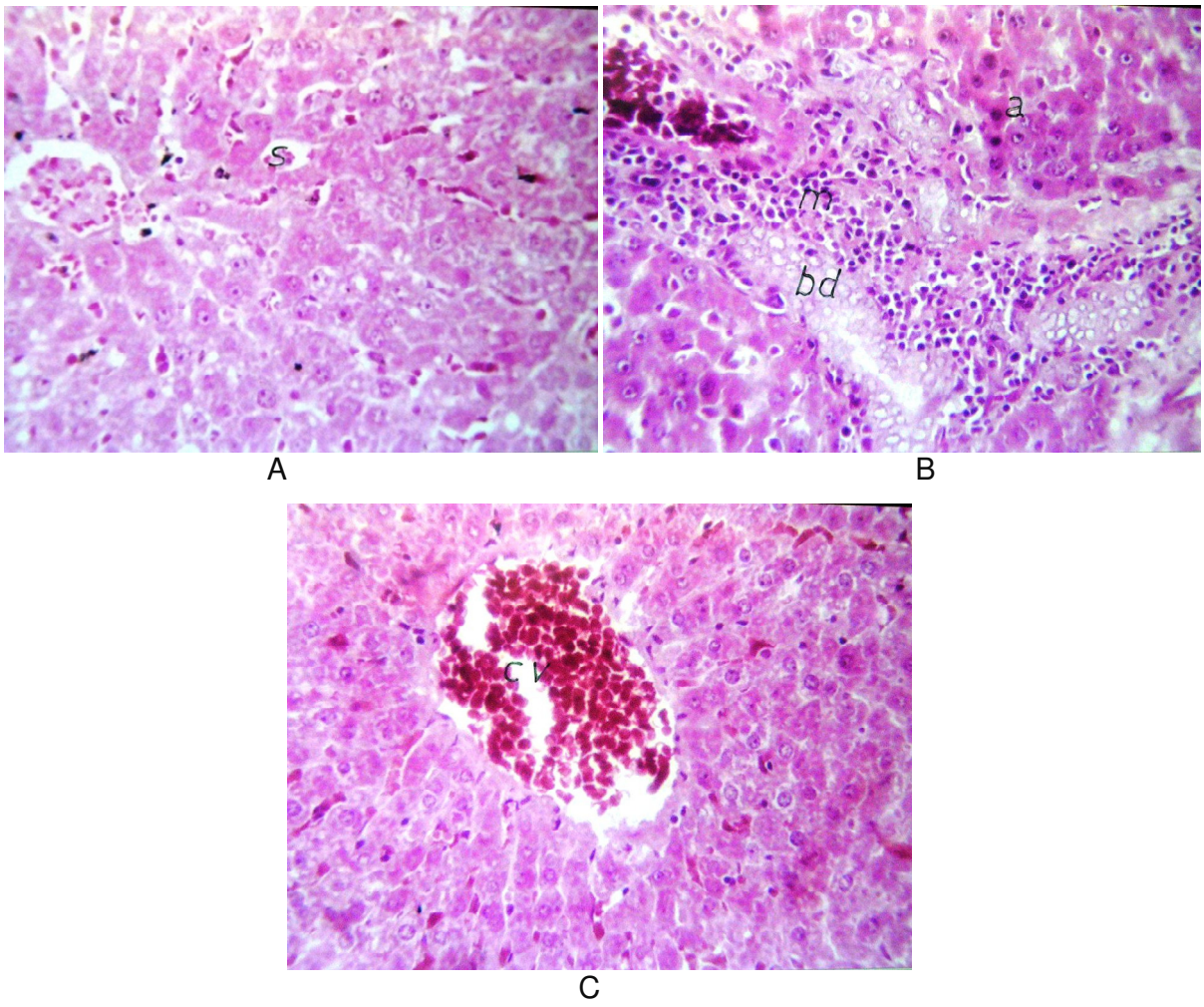

Figure 6. Light micrograph of the liver of protein malnourished rats treated 400 mg/kg metronidazole for four weeks showing: (A) congestion in hepatic sinusoids (H&E X64), (B) cells infiltration (m) in between the newly formed bile ducts (bd) at the portal area (H&E X80), and (C) congestion in central vein (H&E X64).

hormonal, and hematological parameters as well as liver histological examination. In addition, it showed significant changes in metronidazole pharmacokinetic parameters.

Concerning the hematological changes, reduction of RBCs, lymphocytes, haematocrit, and hemoglobin observed in this study is in agreement with the results obtained from previous study (El-Nahas and Ashmawy, 2004). This reduction in hematocrit and hemoglobin may be attributed to inhibition of hematopoiesis, while the decrease in RBCs and lymphocytes counts may be due to osteoclast induced by metronidazole that indicated work by increase in the number of chromosomal aberration in bone marrow cells (El-Nahas and Ashmawy, 2004).

Regarding the hormonal changes, the reduction in plasma levels of testosterone, FSH, and LH hormones following administration of metronidazole may be due to impairment of hypothalamo-hypophyseal-gonadal axis in protein malnourished animals (Herbert, 1980; Lado-Abeal et al., 1999), while the reduction in testosterone levels can be attributed to the direct effect of metronidazole on germ and Leydig cells (Noorafshan et al., 2011) as its penetrate testes blood barrier. The elevation of liver enzymes is compatible with the histopathological findings which indicate that metronidazole induced liver injury and this injury may be due to DNA break down in rat hepatocytes which are relative to the dose level (Martelli et al., 1990).

Generally, the dose dependent effects of metronidazole in normally fed and in protein malnourished animals can be explained from the pharmacokinetics point of view. The results obtained in this study showed that protein malnutrition elevated C_{max}, AUC, prolonged half life time and MRT and decreased elimination rate in all protein malnourished animals in comparison to the corresponding animals of normally fed group.

Following oral administration of metronidazole to normally-fed rat in dose levels 200, 400, and 800 mg/kg,

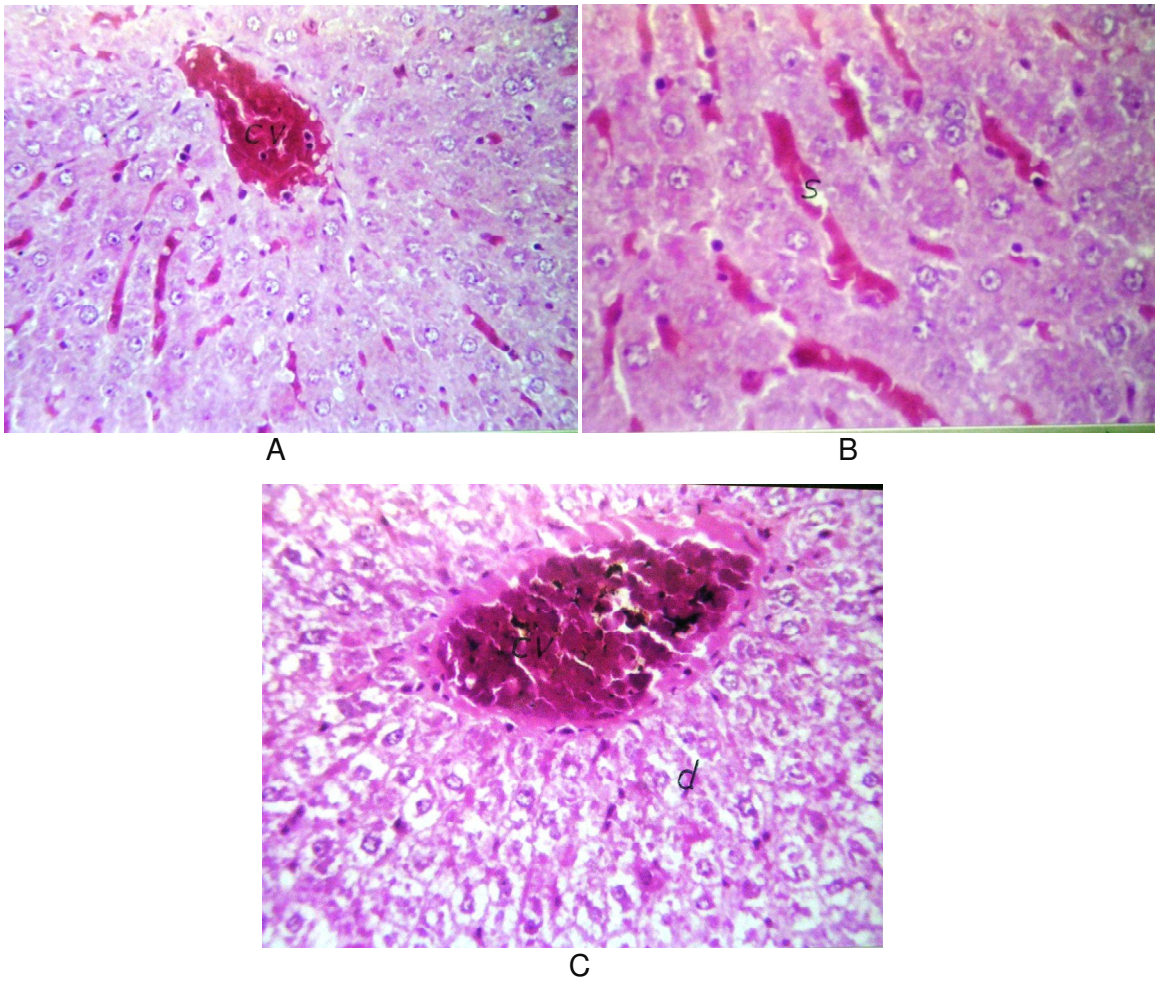

Figure 7. Normally fed rats treated with 800 mg/kg metronidazole for four weeks showing: (A) congestion in central vein and sinusoid, (B) congestion in hepatic sinusoids (H&E stain, X84), and (C) severe congestion in central vein (cv) with degeneration in hepatocytes (H&E stain X160).

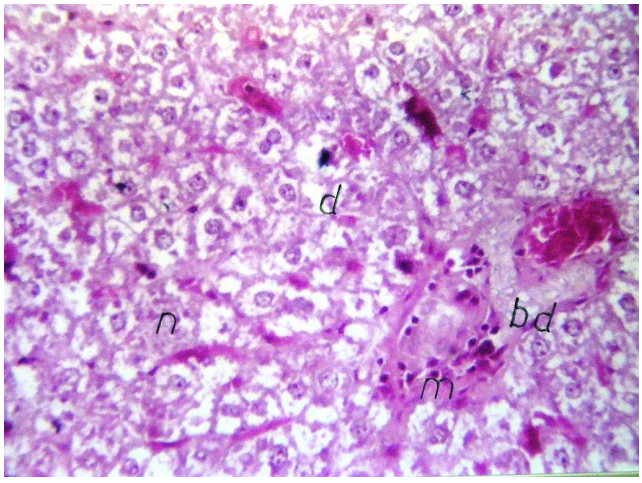

Figure 8. Light micrograph of liver of protein malnourished rats treated with 800 mg/kg metronidazole for four weeks showing few (pv) with inflammatory cell infiltration in between the newly formed bile ductules (bd) in portal vein (H&E stain X80).

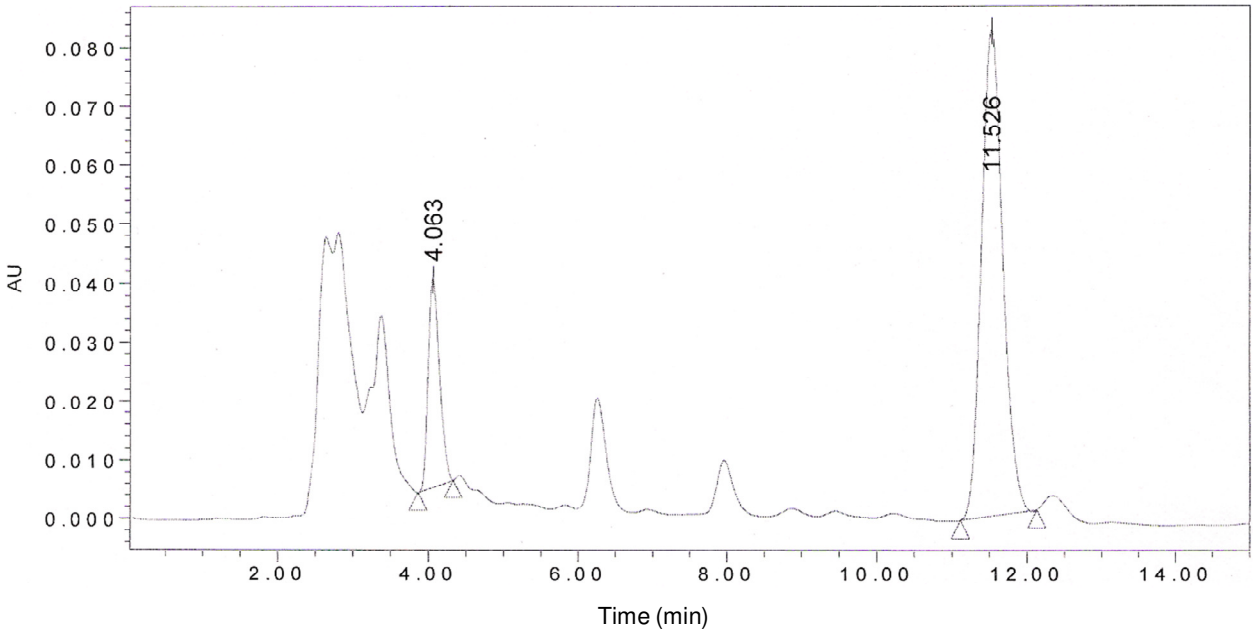

Figure 9. Typical chromatogram of chromatographic separation Metronidazole (4.063 min) and Carbamazepine (internal standard) (11.526 min).

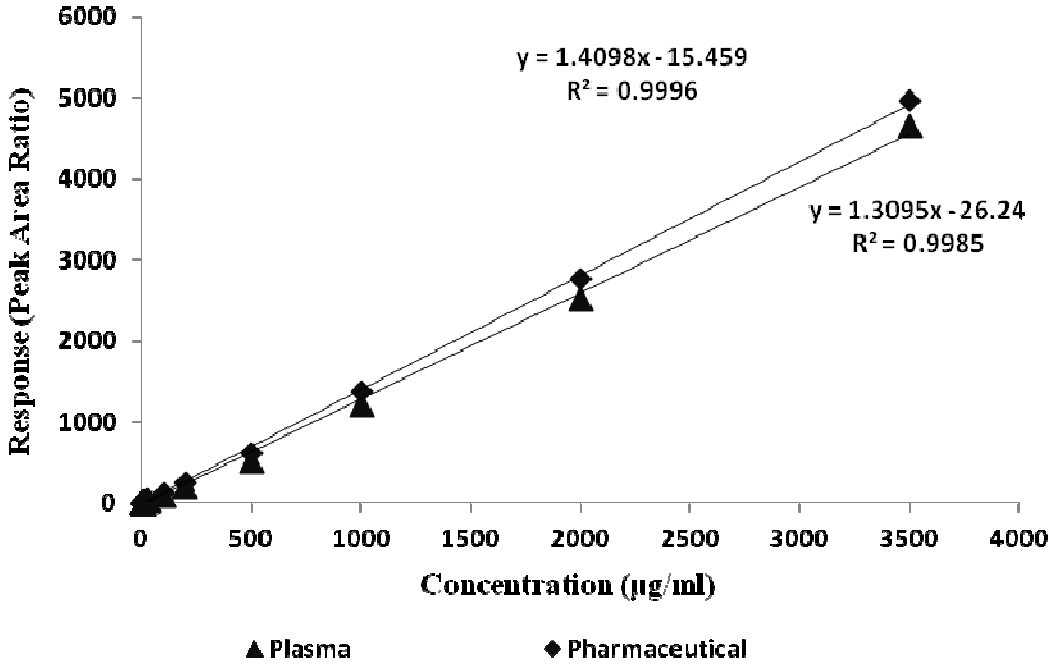

Figure 10. Standard calibration curve of metronidazole dissolved in acetonitrile or after extracted from plasma.

the mean observed peak plasma metronidazole concentration (C_{max}) was dose dependent and much more increase was observed in all malnourished groups in comparison to the corresponding normally fed group. Protein malnutrition leads to significant reduction in total plasma proteins and albumin (Jung and Shah, 1996; González-Hernández et al., 2008) that may be attributed to the decrease in food intake by malnourished animal as they consumed about half of the amount of food intake by normally fed animals in addition to the low protein amount

Table 4. Inter-day and intra-day repeatability of HPLC method for determination of metronidazole in rat plasma.

Theoretical conc. (µg/ml)	Concentrations found					
	Intra-day			Inter-day		
	Mean ± SD	Accuracy (%)	CV (%)	Mean ± SD	Accuracy (%)	CV (%)
0.05	0.049 ± 0.001	98.00	2.04	0.046 ± 0.004	92.00	8.16
0.10	0.101 ± 0.003	101.00	2.97	0.101 ± 0.003	101.00	2.97
0.50	0.510 ± 0.012	102.00	2.35	0.501 ± 0.009	100.20	1.80
1.00	1.0203 ± 0.029	102.03	2.84	0.992 ± 0.08	99.20	8.06
2.00	1.985 ± 0.054	99.25	2.72	2.001 ± 0.061	100.05	3.05
5.00	4.821 ± 0.421	96.42	8.73	4.797 ± 0.3001	95.94	6.03
10.00	9.611 ± 0.312	96.11	3.25	9.821 ± 0.951	98.21	9.68
20.00	20.01 ± 0.145	100.05	0.72	19.884 ± 0.198	99.42	1.00
30.00	29.621 ± 0.195	98.74	7.44	29.084 ± 0.139	96.95	0.48

Table 5. Mean of pharmacokinetic parameters and CI of metronidazole in normally-fed and in protein malnourished rats.

Group (mg/kg)	Parameter		C_{max} (µg/ml)	t_{max}** (h)	AUC0-48 (µg.h/ml)	AUC0-inf (µg.h/ml)	$t_{1/2}$ (h)	MRT (h)	Kel (h)
200	Mean	NF	375.5 ± 195.3	1.25	2152.6 ± 768.7	2174.9 ± 772.3	5.4 ± 3.3	6.3 ± 1.7	0.094 ± 0.104
		PM	808.2 ± 770.1*	1.25	3321.7 ± 2931.0*	3686.1 ± 735.5*	7.9 ± 2.5*	10.8 ±3.3*	0.089 ± 0.026
	% Change		115.23	-	54.31	69.48	46.3	71.43	-5.32
	90% CI		0.567 - 1.558	-	0.802 - 1.574	0.614 - 0.825	0.234 - 0.689	0.294 - 0.702	-
400	Mean	NF	1426.2 ± 473.7	4	14506.6 ± 3794.1	14825.3 ± 3960.3	6.07 ± 1.9	11.8 ± 3.5	0.128 ± 0.022
		PM	1634.9 ± 662.7*	4	19096.2 ± 2999.3*	19403.7 ± 3056.4*	9.2 ± 1.7*	9.9 ± 1.3*	0.291 ± 0.050 *
	% Change		14.63	-	31.64	30.88	51.57	16.1	127.34
	90% C.I		0.721 -1.351	-	0.961 - 1.468	0.721 - 1.315	0.697 - 1.562	0.654 - 1.452	-
800	Mean	NF	2765.7 ± 21.5	5	17506.6 ± 3974.1	17625.3 ± 3960.3	6.2 ± 1.7	9.9 ± 1.3	0.088 ± 0.073
		PM	2948.9±1432.5	4	30837.4± 12513.5*	32440.5±13196.7**	9.7 ± 4.2*	13.5 ± 5.1*	0.118 ± 0.026*
	% Change		6.62	-	76.15	84.06	56.45	36.36	34.09
	90% CI		0.598 -1.541	-	0.567 - 1.364	0.654 1.450	0.781 - 1.291	0.687 - 1.368	-

*Statistical difference from corresponding group of normally fed rats ($P < 0.05$). **Median for t_{max}. CI: Confidence interval.

in their diet (González-Hernández et al., 2008). Elevation of maximum concentrations in protein malnourished animals can be due to the decrease in plasma protein levels, mainly, albumin leading to an increase in free metronidazole which has high affinity to albumin (Piskorz et al., 2011). The average MRT and half life were significantly prolonged in all protein malnourished animals in comparison to the corresponding normally fed rats.

This prolongation in MRT and half life time of metronidazole in protein malnourished rats may be as a result of reduction in the renal clearance of metronidazole due to alteration in renal tubular function associated with protein restriction (Lares-Asseff et al., 1992, 1993). This suggestion is consistent with the finding of others that decrease protein intake may lead to decrease excretion of drugs excreted by glomular filtration (Mehta, 1983) and increase reabsorption of the other drugs (Berlinger et al., 1985). Prolongation of $t_{1/2}$ and MRT, decrease in the elimination rate and increase in metronidazole level in protein malnourished animals is considered as a contributing factor increasing the area under time concentration curve.

These changes in all pharmacokinetic parameters of metronidazole due to protein deficiency indicate that there is a risk of accumulation of the drug after repeated doses. The dose of this drug should be reduced and adjusted in malnourished patient even when metronidazole has wide therapeutic margin.

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