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Haematological, Biochemical and Histopathological Alterations Induced by Ivermectin in Donkeys (*Equus asinus*)

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ABSTRACT

The current study was conducted to assess the pharmacotoxic effects of repeated administration of Ivermectin (Intermectin 1%) injection formulation in male donkeys. The various haematological, biochemical parameters and histopathological changes were noted. Two groups each of six of male donkeys were utilized in the current investigation. Following fasting for 48 hours, Ivermectin was administered subcutaneously at the neck region either at the recommended dose level 200 µg/kg (T1) or at 5 times the recommended dose. 1000 µg/kg (T2) body weight for seven successive days to one of the groups, respectively. Ivermectin was found to pose risks of renal and hepato-toxicity in donkeys, since the biochemical parameters of liver function (aspartate aminotransferase "AST" activity, alanine aminotransferase activity "ALT") and kidney function (urea concentration) were severely affected. Changes in biochemical parameters were more intense in donkeys from group T2 than those reported in group T1. Four animals out of six died following treatment in group T2. The level of ALT, AST, and urea were significantly elevated in donkeys from group T2 when compared to the pretreatment values. Post-mortem and histopathological examination ensured biochemical alterations of liver and kidneys. Likewise, some haematological indices: erythrocyte count, leukocyte count, haemoglobin concentration and PCV, were also influenced. In conclusion the repeated administration of Ivermectin injection formulation at five times the recommended dose is fatal to donkeys.

INTRODUCTION

Ivermectin (IVM) was the first Macrocyclic Lactone anthelmintic, introduced as a Veterinary antiparasitic agent in France in 1981. Ivermectin is marketed as mixture of 22, 23 dihydro avermectin B1a (>80%) and 22, 23 dihydro avermectin B1b (<20%) (Fisher and Mrozik, 1989). Ivermectin (Avermectin B1a) is a natural fermentation product derived from the soil dwelling *Streptomyces avermitilis*. Ivermectin and doramectin are currently used in Sudan as an anthelmintic in donkeys with high efficacy (Seri *et al.*, 2004; Seri *et al.*, 2005; Sawsan *et al.*, 2010).

In equines, a variety of adverse reactions have been reported in horses after parenteral administration of Ivermectin at the recommended dosage of 0.2 mg/kg body weight (French *et al*., 1983; Leaning, 1983; Reed, 1983). These reactions have occurred in a small percentage of treated horses and the drug is now sold only as a paste for oral administration. The misuse of drugs is a common practice in animal husbandry in Sudan, and animal owners tend to increase and /or repeat the dose without consultation of veterinarians, hence we thought it would be better to evaluate the safety of Ivermectin injection formulation in donkeys.

The prominent importance of donkeys in the Sudan coupled with rarity and scantiness of data pertaining to various toxic effects of repeated exposure to Ivermectin at sub-acute doses. Hence, the present investigation was undertaken to assess the effect of repeated administration of Ivermectin injection formulation on blood picture, hepatic and renal parameters as well as histopathological changes in donkeys.

MATERIALS AND METHODS

Experimental drug:

The commercial formulation of Ivermectin (1% Intermectin®) was supplied by Interchemie, Holland ®. Two doses of Ivermectin, equal to 200 µg and 1000 µg/kg were injected subcutaneously in this study through repeated-dose tests.

Animals and treatments:

A total number of 12 male donkeys (*Equus asinus*) 4-10 years of age, with body weights of 90-150 kg, was used in the study. Animals obtained from a local market in East Nile Locality, Khartoum, Sudan. The animals were housed per groups (6 each), in different pens at the college of Veterinary Medicine (SUST) farm, Hillat Kuku. Animals were allowed with free access to water and feed. The animals were acclimatized to the farm conditions for two weeks prior to the start of the study. The experimental animals were subjected to 48 hours fasting to induce stress. Then animals of group-T1 were administered with Ivermectin at a dose of 200 µg/kg b. wt., while donkeys in group-T2 were administered with Ivermectin at dose of 1000 µg/kg b. wt. once daily for seven successive days, respectively.

Following each treatment; animals in the two groups were monitored for two hours to report any side and/or adverse effects.

Blood Samples:

Blood samples were collected at predetermined time intervals i.e. -3, -1 (Baseline), 1, 2, 3, 4, 5, 6, 7, 10 and 15 post treatment.

Blood was collected directly from jugular vein using disposable 10 ml syringes (Changzhou Huichun Medical Equipment, China) and immediately transferred into containers (AFCO- DISPO, Jordan) coated with lithium heparin as anti-coagulant, mixed gently and then placed in ice, transported to the laboratory. Two ml of the blood sample was kept for the evaluation of haematological parameters; the remaining amount of the blood was centrifuged (EBA20- Hettich zentrifugen Germany) for 5 minutes 5×1000 round per minute. Plasma

was harvested in labelled eppendorf tubes and kept at -20˚C for biochemical analyses.

Haematological Examination:

The following haematological indices were determined using routine laboratory methods. Packed cell volume (PCV) was determined by the micro haematocrit method described by Schalm *et al*., (1975) and Dacie and Lewis (1984). Erythrocytes (RBC) were counted using the improved Neubauer haemocytometer (Dacie and Lewis, 1984). Haemoglobin concentration (Hb) was determined by method described by Jain (1986).

Biochemical Analysis:

Activities of some enzymes and concentrations of certain biochemical parameters representing liver and kidney functions were determined in the donkeys' blood plasma spectrophotometrically as follows: Aspartate and alanine aminotransferase (AST & ALT) activities were determined according to the method of (Reitman and Frankel, 1957). Albumin, urea, triglycerides, and cholesterol**,** concentrations were determined according to the methods of (Doumas *et al*., 1971; Fawcett and Scott, 1960; Stein and Myers, 1995; Tietz, 1995), respectively. All measurements were done using Jenway 6305 U.V/VIS. spectrophotometer UK.

Histological studies

Lungs, liver, kidneys and spleen from necropsied animals were removed, washed in saline, and fixed in 10% formal saline for histopathological examination. The formalin fixed tissues were processed as per method described by Culling (1974). The sections were stained with Hematoxylin and Eosin (H & E) stains (Luna, 1968). The H & E stained slides were observed under the microscope, and lesions were recorded.

Statistical analysis:

Data obtained from the experiments were expressed as mean ±SD. Significant differences of measurement traits were analysed using student t-test using SPSS version 16 for Windows. A p value < 0.05 was considered statistically significant.

RESULTS

Side and/or adverse effects:

Donkeys treated with five times the recommended dose repeatedly for seven successive days exhibited toxicity signs viz: animal fell down, rolled in the ground with prominent tremors at the peripheral muscles immediately following the administration of the drug. Four animals out of six died following treatment with five times the recommended dose at days 6, 8, 10 and day 11 following the first treatment.

Gross lesions:

At necropsy, congestion in the main visceral organs was the prominent feature in animals. Necrosis in the liver (Figure 1), trabeculations in the spleen (Figure 2), viscous yellow fluids were also observed in kidneys and pericardium. The liver was pale yellow and the kidneys were also pale with sticky yellowish fluid inside. The pericardium contained large amount of yellowish fluid. Haematomas were observed at the injection site in all animals. As a general observation no helminth parasites (larval stages or mature worms) were recovered during necropsy.

Figure 1: Liver necrosis in donkey treated with Ivermectin

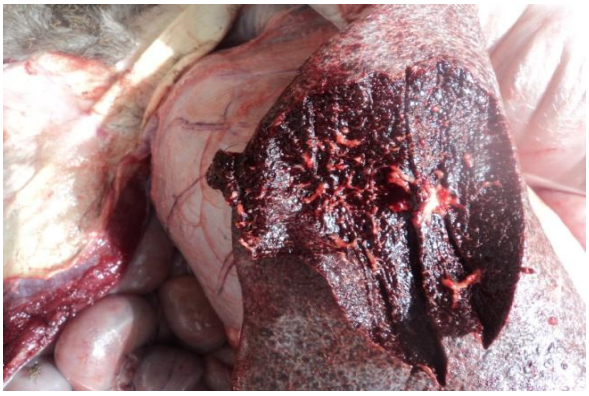

Figure 2: Thick trabeculations in the spleen of donkey treated with Ivermectin

Histopathological changes:

Histopathological changes were observed in all selected organs of Ivermectin-treated animals. In liver of necropsied donkeys, fine and large hepatocyte cytoplasmic vaculation and congestion in centeral vein with siunsodial dilatation were observed in donkeys treated with five times the recommended dose (Figure 3).

Sections from kidneys showed hypercellular glomeruli (Figure 4); glomeruli appear hypercellular tufi, pinkish deposits seen Bowman space (Figure 5). Also selected lung sections showed collapse and emphysema (Figures 6 and 7). Moreover, severe congestion, alveolar odema and thickening of interstitium at certain area with dense cellularity were seen in the lungs of animals exposed to Ivermectin for 7 successive days (Figure 8). The pulmonary arteries showed mural thickening with large numbers of neutrophils seen inside the blood vessels (Figure 9). Large neutrophil seen in blood vessels in lung of donkey treated with Ivermectin (Figure 10).

The spleen showed congestion of the red pulp, with dense dark brown deposits (haemosiderin) (Figure 11). The heart showed degenarative changes in muscl cells, bluisht deposit in muscles interstitial cells, congestion, increase in interstitial cells, muscle cells hyperatrophoid and thickened arterial walls, vessels with dense prominet smooth muscle nuclei.

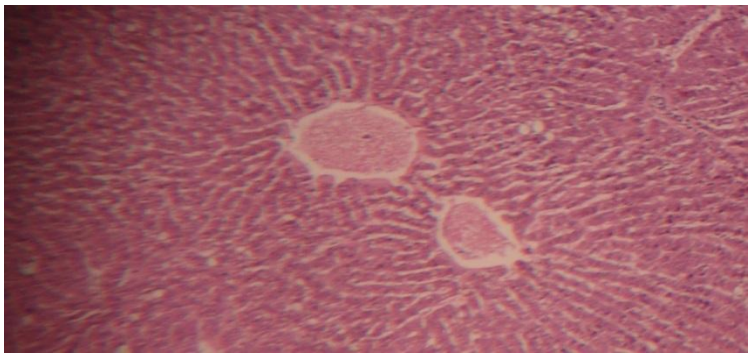

Figure 3: Liver: Fine and large hepatocyte cytoplasmic vaculation. Congection in centeral vein and siunsodial dilatation in animal treated with Ivermectin at five times the recommended dose for seven successive days (H&E 40)

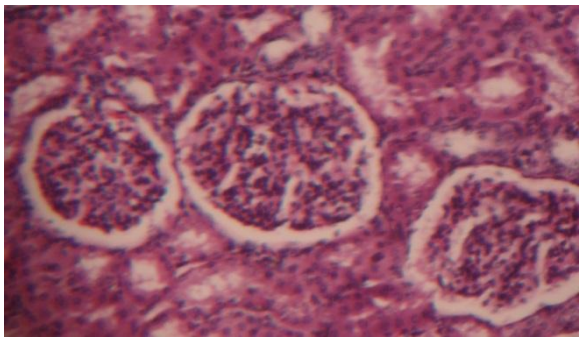

Figure 4: Kidney: hypercellular glomeruli in animal treated with Ivermectin at five times the recommended dose for seven successive days

(H&E 100)

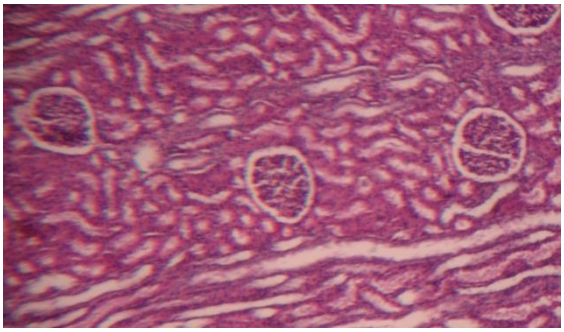

Figure 5: Kidney: glomeruli appear hyper cellular tufi, pinkish deposits seen in Bowman space in animal treated with Ivermectin at five times the recommended dose for seven successive days (H&E 400).

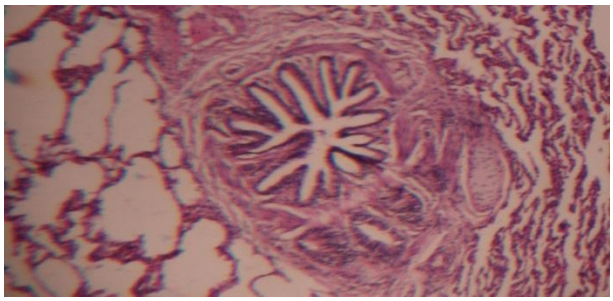

Figure 6: Lung: Collapse and emphysema around a bronchus in animal treated with Ivermectin at five times the recommended dose for seven successive days (H&E 400)

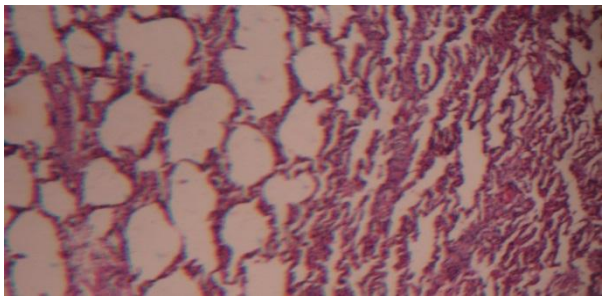

Figure 7: Lung: Alveoler Collapes and emphysema with in animal treated with Ivermectin at five times the recommended dose for seven successive days (H&E 100)

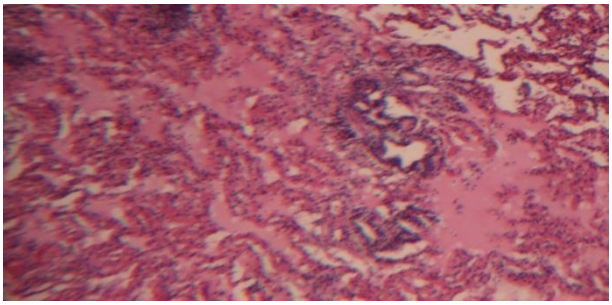

Figure 8: Lung: Sever congestion and alveolar odema, thickening of interstitium at certain area with dense cellularity in animal treated with Ivermectin at five times the recommended dose for seven successive days (H&E 40)

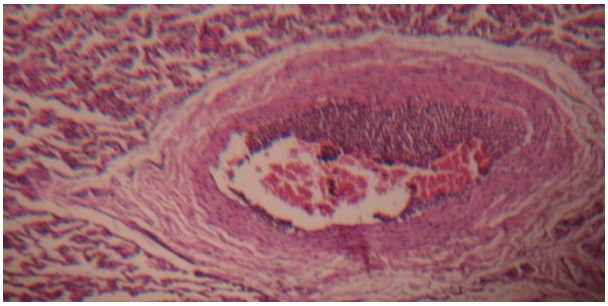

Figure 9: Pulmonary artery: mural thickening and large neutrophils seen blood vessels in animal treated with Ivermectin at five times the recommended dose for seven successive days (H&E100)

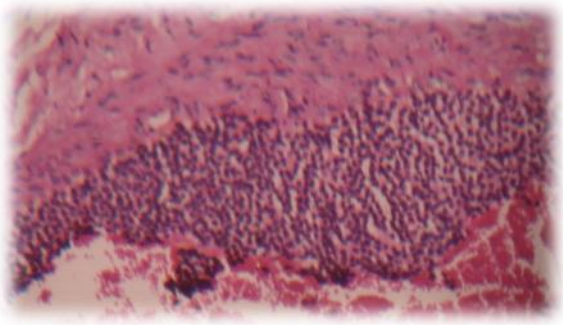

Figure 10: Lung: Large neutrophil seen blood vessels in animal treated with Ivermectin at five times the recommended dose for seven successive days (H&E 100).

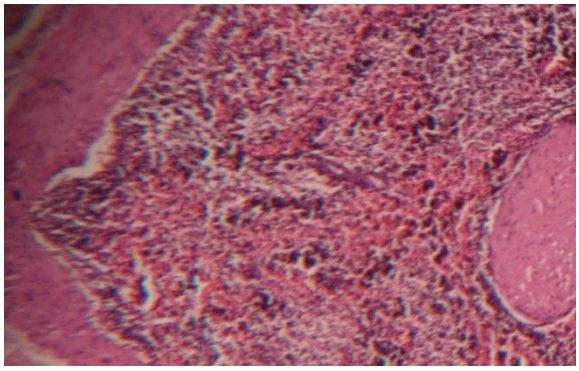

Figure 11: Spleen: Congection of red pulp ,with dense dark brown deposits (haemosiderin) in animal treated with Ivermectin at five times the recommended dose for seven successive days (H&E 400)

Haematological results:

Significant increase (P<0.05) in Hb concentration (P<0.05) was observed in the two treated groups following treatment with Ivermectin for seven successive days. Significant increase (P< 0.05) in PCV was also observed in the first group at day 3, and increased with no significant difference throughout the experiment period in the second group. Significant increase in (P <0.05) in total leukocytes count by day 7 and 10 in the first group, and in the second group there was no significant increase throughout the experiment (Table 1)

Significant decrease (P <0.05) in the total RBCs count was observed in the third day of treatment and continued with significant fluctuation throughout the entire period of the experiment in the first group. In the second group a significant (P<0.05) decrease in RBCs count was observed in the 5^{th} and 6^{th} day following treatment.

Table 1: PCV (%), haemoglobin concentration (g/dl) and Counts of red and white blood cells in blood of male donkeys administered with Ivermectin at different dose levels repeatedly for seven successive days

Parameters	Hb (g/dl)		PCV (%)		RBCs (x10^6 cell/µl)		WBCs (x10^3 cell/µl)	
Groups	T1	T2	T1	T2	T1	T2	T1	T2
Baseline	12.9±1.50	15.3±0.50	26.9±5.05	26.8±1.53	5.6±0.39	4.6±0.22	2.10±0.52	2.17±0.60
1	17.0±2.09*	15.5±1.60	29.3±4.13	28.8±4.83	5.06±0.91	4.6±0.32	2.0±0.40	2.48±0.39
2	16.1±2.80*	16.5±1.14*	27.3±2.33	27.6±1.36	5.03±1.00	4.6±0.17	2.1±0.19	2.46±0.39
3	16.5±2.27*	16.2±1.09	30.1±5.40*	27.5±2.07	4.8±0.71*	4.7±0.23	2.1±0.19	2.70±0.55
4	17.2±1.85*	15.8±.82	28.3±6.08	27.6±1.63	4.6±0.57*	4.6±0.15	2.0±0.21	2.59±0.57
5	17.7±3.06*	15.7±0.95	25.6±7.33	27.6±2.25	6.1±0.61*	4.3±0.23*	3.0±0.13	2.53±0.49
6	15.8 ±1.05*	15.8±0.79	28.6±5.92	30.5±4.80	4.9±0.73*	4.3±0.23*	2.1±0.20	2.69±0.50
7	16.7±3.40*	16.8±0.50*	32.0±7.32	29.6±3.13	5.08±0.38*	4.3±0.28	2.4±0.37 *	2.86±0.70
10	15.1±1.50	15.3±0.44*	28.8±2.94	27.0±4.0	4.5±0.30*	4.3±0.36	2.7±0.24*	3.00±0.73
15	16.4±1.03*	16.4±0.64	27.8±3.11	26.0±1.41	4.6±0.41*	4.2±0.17	2.4±0.36	2.92±0.45

Values were expressed as Mean ±standard deviation

*Means with asterisk within columns were significantly (P<0.05) different with baseline value

Biochemical assessments:

The effect of Ivermectin on certain blood biochemical parameters are summarized in tables (2, 3 and 4). Male donkeys of treated group T1 exhibited a gradual non-significant increase in ALT activity immediately following administration of Ivermectin and continued up to the 4^{th} day where a significant reduction was observed by day 5 and 6. While in the second group (T2) there was non-significant increase in ALT activity up to day 6 where it reached a significant difference in day 10 and up to end of experiment (Table 2). No significant ($P > 0.05$) change was observed in Aspartate aminotransferase activity in the first group, but in the second group significant ($P<0.05$) decrease was observed by the 2^{nd} day and up to end of experiment (Table 2).

Sub-acute poisoning with Ivermectin (group – T2), resulted in a significant increase in albumin concentration in the second and the third day following treatment while in the second group (T2) there was significant ($P<0.05$) increase in the 3^{rd} up to the 5^{th} day in donkeys treated with Ivermectin (Table 3). Significant ($P< 0.05$) increase in in urea concentration in the first group (T1) was observed in the 5^{th} day, and in the second group decreased by the day 4, 5 and 7 in donkey treated with Ivermectin in the second group (T2) (Table 3).

Significant ($P<0.05$) decrease in triglyceride concentration in day 2, 6 and 7 in the first group (T1) in donkey treated with Ivermectin (Table 3). Significant decrease in (P value 0.05) in cholesterol concentration in 5^{th} day in the first group (T1) and in the second in the 5^{th} and 6^{th} day in donkey treated with Ivermectin (Table 3).

Significant ($P<0.05$) decrease in the level of phosphorus was observed at the end of the experiment in day 15 in both treatment groups (Table 4). Significant increase in ($P< 0.05$) sodium concentration by day 5 up to end of the experiment in the first group, while significant ($P<0.05$) increase in sodium concentration in the second treatment group in the 2^{nd} ,4^{th} ,5^{th} and 7^{th} days following the first injection of the drug (Table 4). Significant fluctuation ($P < 0.05$) in potassium concentration in the two treatment groups, with a prominent increase in the 10^{th} and 15^{th} days following the first injection of the drug (Table 4).

Table 2: Plasma Alanine aminotransferase (ALT) and Aspartate aminotransferase activities of male donkeys administered with Ivermectin at different dose levels repeatedly for seven successive days

Parameters	ALT		AST	
Groups	T1	T2	T1	T2
Baseline	33.80±18.16	4.96±5.69	34.23±7.81	26.17±9.91
1	21.81±15.84	4.20±3.44	39.23±23.38	1.08±0.39
2	17.30±10.74	15.42±23.80	33.98±27.85	1.08±0.39*
3	14.31±9.89	2.74±1.25	44.20±28.80	1.16±0.09*
4	20.60±14.48	18.36±24.41	38.34±21.94	1.24±0.39*
5	6.14±4.53*	14.61±12.5	41.27±11.83	1.04±0.34*
6	7.03±1.98*	43.80±21.74*	35.38±15.85	1.20±0.42*
7	9.25±6.36	21.71±13.79	33.34±11.71	1.18±.37*
10	4.30±1.27	19.80±2.05 *	40.70±37.74	1.62±0.17*
15	10.45±7.42	28.78±19.89	49.15±25.44	1.15±0.35

Table 3: Concentration of albumin, urea, triglycerides and cholesterol in plasma of male donkeys administered with Ivermectin at different dose levels repeatedly for seven successive days

Parameters	Albumin (g/dl)		Urea (mmol/l)		Triglycerides (mmol/l)		Cholesterol (mmol/l)	
Groups	T1	T2	T1	T2	T1	T2	T1	T2
Baseline	28.37 ± 5.15	39.56 ± 4.6	9.35± 1.72	13.81 ± 4.03	1.17 ±0.31	0.96 ±0.42	1.87 ±0.40	2.09 ±0.34
1	32.89 ± 5.737	40.79 ± 9.39	10.76 ± 1.56	15 .78 ± 4.83	0.97 ±0.49	1.10 ±0.40	2.05 ±0.80	2.10 ± 0.83
2	36.28 ± 3.80*	45.54 ± 8.9	10.73 ± 1.80	12.21 ± 2.96	0.78 ±0.29*	1.15 ±0.08	1.88 ±0.74	1.96 ±0.84
3	37.30 ± 3.56*	49.68 ± 4.13*	10.71 ± 2.44	9.58 ± 1.96	0.98 ±0.48	1.23±0.35	1.85 ± 0.56	1.80 ± 0.52
4	32.30± 3.25	56.48 ± 15.87	11.86 ± 2.72	9.15 ± 1.57 *	0.83 ±0.24	1.00 ±0.32	1.71 ± 0.82	1.53 ± 0.57
5	27.40 ± 4.36	49.08 ± 10.76*	12.43 ± 2.06 *	8.25 ± 2.17*	1.03 ±0.51	1.15±0.40	1.30 ± 0.31*	1.55 ±0.47*
6	27.04 ± 4.63	44.49 ± 6.57	12.03 ± 3.60	11.51 ± 9.79	0.75 ±0.26*	1.08±0.41	1.75 ± 0.74	1.36 ±0.43*
7	28.69 ± 4.63	38.62 ± 4.33	8.86 ± 3.88	8.160 ± 2.98 *	0.70 ±0.23*	1.50±0.32	1.75 ± 0.66	1.86 ±0.57
10	32.443 ± 3.19	37.66 ± 3.41	8.04 ± 3.39	11.70 ± 1.21	1.22 ±0.41	0.93±0.45	1.56 ±0.68	2.30 ± 1.49
15	28.315 ±0 .90	37.49 ± 2.41	8.06 ± 2.81	10.10 ± 2.83	1.30 ±0.50	1.25±0.49	1.66 ± 0.68	1.20 ±0.28

Table 4: Phosphorus, sodium and potassium level in plasma of male donkeys administered with Ivermectin at different dose levels repeatedly for seven successive days

Parameters	Phosphorus (mmol/l)		Sodium (meq/l)		Potassium (meq/l)	
Groups	T1	T2	T1	T2	T1	T2
Baseline	1.52 ± 0.12	1.20 ±0.36	138.1±7.4	141.5±6.10	3.9±0.47	3.8±0.52
1	1.45 ± 0.49	1.00 ±0.18	129.1±19.6	161.1±725*	3.4±0.74	3.4±0.90
2	1.40 ± 0.37	1.13 ±0.19	141.7±7.2	154.6±13.56	3.3±0.93	3.2±0.61
3	1.71 ± 0.39	1.15 ±0 .43	138.1±15.7	150.3±11.05	4.0±0.95	3.3±0.52*
4	1.36 ±0.70	0.92 ±0.17	163.2±20.3	158.8±5.06*	4.8±0.68*	3.5±2.20
5	1.41 ±0.60	1.65 ±0.98	151.1±10.1	150.0±1.74*	2.5±0.32*	2.5±0.63*
6	1.48 ±0.46	1.56 ±0.84	161.6±6.47*	146.7±3.92	2.0±0.20*	3.7±0.36
7	1.36 ±0.43	2.04 ±0.99	158.5±5.06*	152.8±7.14*	9.5±3.67*	11.8±1.09*
10	1.28 ± 0.46	2.13 ±1.88	158.6±6.35*	133.5±7.44	9.9±1.38*	9.6±2.56*
15	1.02 ±0.29*	0 .75 ±0.49*	156.9±9.56*	139.9±12.2	5.1±2.24	6.7±.2.47

DISCUSSION

A variety of biochemical parameters are measured in toxicity studies, in attempts to evaluate a broad range of physiological and metabolic functions affecting target organ identification and tissue injury assessment (Akhtar *et al*. 2012). Some common biochemical parameters provide better information from pattern recognition, e.g. enzymes like ALT and AST for hepatotoxicity, and urea and creatinine for glomerular function (Evans, 1996).

Signs of toxicity observed in treatment group (T2) following administration of the drugs are in accordance with the results of Lankas and Gordon (1989) in red footed tortoises (*Geochelone carbonaira*) treated with Ivermectin (0.4 mg/kg) single intramuscular injection that showed a state of extreme paresis or flaccid paralysis. One

of the tortoises died within three days of receiving the treatment, a result which in the same line with pattern of death in the current study.

At necropsy, congestion in the main visceral organs was the prominent feature in animals; this result was in agreement with observations of Eissa and Zidan (2009), and Abd-Elhady and Abou-Elghar, (2013).

The histopathological changes in lungs of donkeys treated with five times the recommended dose for seven successive days were in accordance with the observations of Abd-Elhady and Abou-Elghar, (2013), in Albino rats. They reported some necrobiotic changes in the lungs of abamectin-treated rats. Interstitial pneumonia with marked congestion and oedema were observed in lungs of animals exposed to abamectin for 30 days. Moreover, diffuse local haemorrhages associated with atelectasis were seen in the lungs of animals exposed to abamectin for 210 days (Abd-Elhady and Abou-Elghar, 2013).

The histopathological changes observed in the liver of donkeys were in association with the findings of Abd-Elhady and Abou-Elghar, (2013) in Albino rat. They reported marked degenerative changes of hepato-cytes, congestion, and marked diffuse necrosis of hepatic tissue was observed in liver of abamectin-treated animal. Such necrobiotic changes were more intense in the livers of the group treated with abamectin for 210 days. Moreover, fibrosis was observed in the portal triads associated with disruption of sinusoids and marked degenerative changes of hepatocytes along with evidence of marked congestion. The portal tract infiltration by lymphocytes and a focus of dysplasia with cytological atypia were observed in Vertimec (Abamectin) treated male rat's liver at either dose levels used (Eissa and Zidan, 2009). It was postulated that such changes were a prominent response of body tissues facing any injurious impacts (El-Banhawy *et al.,* 1993).

The changes observed in kidneys by Abd-Elhady and Abou-Elghar, (2013) in albino rat; that even the kidneys showed marked necrobiotic changes in abamectin-treated animals as compared to the normal histological examination of renal tissue in the control rats may confirm our results. A marked necrosis of tubular cells, atrophy of the glomeruli, and areas of interstitial infiltration of round cells were also observed in rats.

Concerning the kidney, Vertimec at either dose levels induced interstitial nephritis in male rat's kidney (Eissa and Zidan, 2009). Histological changes in rainbow trout organs showed a direct toxicity of abamectin since degenerative changes in brain and kidney as well as in low level in liver were established (Jencic *et al.,* 2006).

The significant increase in Hb concentration, PCV, and total leukocytes count observed in the current study is not in agreement with the observations of Eissa and Zidan (2009) in Albino rats where he reported that abamectin caused reduction in erythrocyte counts (RBCs), leukocyte counts (WBCs) and haemoglobin concentration. These differences may be attributed to the stress following fasting for 48 hours where donkeys reduced water intake and haemoconcentration is the normal consequence. A significant increase in Hb concentration was observed in camels treated with Ivermectin (Ibrahim *et al*., 1981). A significant increase in PCV was observed in ewes treated with Ivermectin (Shaddad, 1997).

In the second group, a significant ($P<0.05$) decrease in RBCs count was observed in the 5^{th} and 6^{th} day following treatment (Table 3.3), a result that in the same line with

observations of Eissa and Zidan (2009). The obtained results are in accordance with those found by Ali (1990), and Anubama *et al.,* (2001) who stated that avermectins reduced erythrocyte in rabbits and rats.

Although Saddad, (1997) reported no significant increase in total leucocytes, this study showed that, there was significant increase in total leucocytes ($P < 0.05$) by the day 5,7 and 10 in first group, and throughout the experiment in second group of animal this result was contradictory to that obtained by Eissa and Zidan, (2009). A significantly reduced amount of white blood cells could be indicative of immuno-suppression (Schroder *et al.,* 2007). A reduction in erythrocyte counts may be attributed to more than one factor, i.e. the failure to supply the blood circulation with cells from haemohepatic tissues, since the liver has an important role in the regeneration of erythrocyte and the possible destructive effect on erythrocyte by the toxicants.

The significant decrease ($P < 0.05$) in ALT activity observed in the first group disagrees with findings of Eissa and Zidan, (2009) in albino rats. While the significant increase in ALT activity in the second group may be attributed to the increase activity of the liver. No significant change was observed in the first group ($P > 0.05$) pertaining to AST activity, where in the second group significant ($P<0.05$) decrease was observed by the 2nd day and up to end of experiment. These results were not in agreement with that obtained by Abd-Elhady and Abou-Elghar, (2013) in albino rats. Elevation of AST may render the liver to be more susceptible to other pathogen/toxicants (Chamulitrat and Spitzer 1996; Nayak *et al.*, 1996). Aspartate aminotransferase is an important indicator of liver damage in clinical studies.

The results obtained by Abd-Elhady and Abou-Elghar, (2013) showed that *per os* administration of abamectin, at 1/30 LD_{50}, for a period of 210 days (group T2) significantly increased the levels of plasma ALT and AST in treated male rats, compared to the control group. Hsu *et al*., (2001) indicated that the activities of ALT and AST levels were elevated in abamectin-dosed rats in a dose-dependent manner at 1, 3, and 12 h, respectively. Activities of serum enzymes like AST and ALT represent the functional status of the liver (Cremer and Seville, 1982). As certain hepatic damage is considered pathologically irreversible (Helling *et al*., 1995)

The elevation in the liver enzyme activities may be due to liver dysfunction with a consequent reduction in enzyme biosynthesis and altered membrane permeability permitting enzyme leakages into the blood (Mansour and Mossa, 2010). The liver is susceptible to damage because of direct exposure to toxic products. The liver plays a role in the detoxification of metabolic by-products and xenobiotics. In the present study, the increased levels of AST and ALT could be due to hepatotoxicity causing permeability alterations and leakage of lysosomal enzymes enhancing the release of enzymes (Shrivastava *et al*., 1989; Choudhary *et al*., 2003). The elevation of ALT and AST levels in this study suggests probable liver tissue damage due to Ivermectin.

Many reports had elucidated that hepatocellular damage could be correlated with the disturbed enzymes activities. In this respect, liver tissues which were famous for their rich contents of aminotransferases (AST and ALT) suffer markedly from their loss under many pathological conditions (Rodwell, 1983).

There was significant increase ($P > 0.05$) in albumin concentration among the animals in the two groups which treated with Ivermectin. This result was in agreement

with findings of Seri *et al.*, (2006) who reported no significant increase in albumin concentration in donkeys treated with doramectin. Herd and Kociba (1985) reported similar non-significant increase in albumin level in horses treated with Ivermectin intramuscularly. Eissa and Zidan (2009) and Abd-Elhady and Abou-Elghar, (2013), reported a reasonable reduction in albumin concentration in rats following administration of abamectin. Here we could justify the current status by the intoxication of animals with Ivermectin that ended with off-food and consequently shame water drinking and haemconcentration.

The significant increase in urea blood level observed in the two treated groups was also observed by Herd and Kociba (1985) who showed significant increase in urea level 8 days later after Ivermectin injection. This result was also supported with the work of Seri *et al.*, (2006) in donkeys, Mohammed and Samia, (1994) in desert sheep and Shadad (1997) in sheep treated with three times the recommended dose of Ivermectin.
Significant decrease in triglyceride and cholesterol concentration was observed in donkeys after injection of Ivermectin in the first and the second group of animals a result that was in close agreement with that of Eissa and Zidan, (2009) who reported minor non-significant reduction in cholesterol concentration in albino rats treated with 1/10 the LD50 (18.1 mg/kg) of abamectin for thirty successive days.

Our result showed that, there was significant decrease in phosphorus concentration level at the end of the experiment (day 15) in both treated groups. Herd and Kociba (1985) reported significant decrease in inorganic phosphorus level 4 days post treatment with Ivermectin in horses. While Seri and his colleagues (2006) reported significant decrease in inorganic phosphorus level in donkeys treated with doramectin at 100, 200 and 300 μg/kg daily for seven successive days.

The significant increase in sodium and potassium level at the end of current study may be attributed to kidneys dysfunction following administration of the drug for seven successive days as shown in degenerative changes in histopathological sections; as well as it is in the same line with observations of Seri *et al.*, (2006) in donkeys treated with doramectin at the normal dose.

CONCLUSION

The results of this study demonstrated that sub-acute administration of Ivermectin at 1000 μg/kg for seven successive days induces toxic effects on biochemical functions which correlate well with the histopathological changes in the liver, kidneys and lungs. Although the data on donkeys cannot be directly applied to horses, it may be concluded that use of Ivermectin injection may cause hazardous effects at various levels to equines.

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