INTRODUCTION

Donkeys are becoming increasingly important animals in the Sudan given the new socio-economic situation with an increased use of donkeys instead of horses in labour and transportation.

Donkey's main role is that of a beast of burden though in some areas it has traditional role as a drought animal. This use is currently being much expanded and relatively recent developments have improved its efficiency when used in carts (Wilson, 1990).

Nematode infection was the main problem reported in donkeys admitted to veterinary clinics (Ali *et al.*, 2001). Recently several reports documented high prevalence rate with helminths infestation in donkeys in different parts of Sudan (Abakar *et al.*, 2013; Ismail *et al.*, 2013; Adam *et al.*, 2013, Fangama, 2013, Sawsan *et al.*, 2008).

Ivermectin "ivomec" is a potent agent, active against many external and internal parasites in domestic animals. Ivermectin is today elixir in the world of parasite chemotherapy. It is vogue and highly effective with a wide margin of activity and safety.

Avermectins (ivermectin- like drugs) are macrocyclic lactones and share similarities including mechanism of action; these drugs are neurotoxic to the parasite by potentiating glutamate-gated chloride ion in parasites. Paralysis and death of parasites is caused by increase in permeability to chloride ions and hyperpolarization of nerve cells. These drugs also potentiate other chloride cells, including (ones) gated by gamma amino butyric acid (GABA) (Manger, 1991).

Mammals ordinarily are not affected, by the lack of glutamate-gated chloride channels and there is a lower affinity for other mammalian chloride

channels.Because these drugs ordinarily do not penetrate the blood brain barriers, GABA gated channel in the CNS of mammal are not affected (Manger, 1991).

Ivermectin has an excellent efficacy for an important range of gastrointestinal nematodes of equines (Campbell *et al*., 1989). Ivermectin proved to be highly efficacious for the treatment and control of large strongylus (adult) (*Strongylusvulgaris*, *Strongylusedentatous*, and *Triodontophorus* sp.), small strongylus (adult and fourth stage larvae) (*Cyathostomum* sp., *Cylicocyclus* sp., *Cylicostephanus* sp.), pinworms (adult and fourth stage larvae) (*Oxyuris equi*), large round worms (adult) (*Parascarisequourm*), hair worms (adult) (*Trichostrongylusaxei*), large mouth stomach worms (adult) (*Habronemamuscae*), neck thread worms (adult) (Microfilariae) (*Onchocerca* sp.) and (stomach bots) (*Gasterophilus* sp.) in donkeys (Manger, 1991, 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 current study was conducted to evaluate some pharmacotoxic aspects of ivermectin injection formulation at different dose levels in donkeys. It is also aimed to study the combined effect of induced stress and

simultaneous administration of ivermectin injection formulation on some haematological and biochemical parameters of donkeys blood.

Specific objectives are to:

(1) Determine spectrum of specific effects of repeated doses of ivermectin on liver and kidney functions.

(2) Investigate the possible haematological and biochemical changes that may follow administration of ivermectin as injectable formulation at repeated doses.

(3) Determine the changes, if any that may result following administration of a high dose ivermectin to animals subjected to stress.

CHAPTER ONE

Literature Review

1. Ivermectin

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 was introduced to the market as an anti-parasitic drug in 1981 by MSD AG VET. It is a member of the ivermectin family of compounds. The efficacy of ivermectin against nematode and arthropod parasites is unprecedented in potency and breadth of spectrum. Its worldwide acceptance in livestock production and in health care of companion animals has made it a major commercial success. Its efficacy in human onchocerchiasis (River blindness) has made it a promising candidate for the control of one of the most insidious and intercatable tropical diseases (Campbell, 1989)

1.1 Identity

1.1.1 Chemical composition

Ivermectin is a semi-synthetic derivative of avermectin B, which is fermentation product of the actinomyces *Streptomyces avermitils* (Miller *et al.*, 1979). Ivermectin is a mixture of two homologous compounds. Usually contains at least 80% of 22, 23 dihydro avermectin B1a which has an ethyl group C_2H_5 at position 26 carbon and more than 20% of dihydro avermectin B1b, which has a methyl group CH_3 at C26. Both compounds possess similar biological and toxicological activities but the antiparasitic agents IVM B1b marketed as such a mixture (Campbell, 1985).

1.1.2 Chemical name

22, 23-dihydroavermectin B1a

22, 23-dihydro avermectin B1b

1.1.3 Molecular formulae

H_2B1a $C_{48}H_{74}O_{14}$

H_2B1b $C_{47}H_{72}O_{14}$

1.1.4 Molecular weight

H_2B1a 875.1

H_2B1b 861.1

1.1.5 Appearance

Ivermectin is highly lipophilic off-white powder (a) ± 71.5^{o}C that dissolves in most organic solvents with a low solubility in water. It shows strong U.V. light absorption at 238-245 nm (E27, 100.30, 100) a characteristic used in the assay of the drug.

1.1.6Molecular structure

The avermectins structures are closely related complex 16 membered macrocyclic lactones. Although, they share structural features with the anti-bacterial macrolides and the antifungal macrocyclic polyenes, the avermectins are not grouped with those compounds. Avermectins have neither anti-bacterial nor antifungal activities and do not inhibit protein or chitin synthesis as do the other two groups (Fisher and Morzik, 1989; David and Green, 1986). The structure of ivermectin is present in figure (1.1) below.

1.2 Mode of action

The anthelmintic activity of the AVMs was first described in 1979 (Burg *et al.,* 1979). Identifying the mode of action is all the more difficult because the avermectins have been studied in so many different model

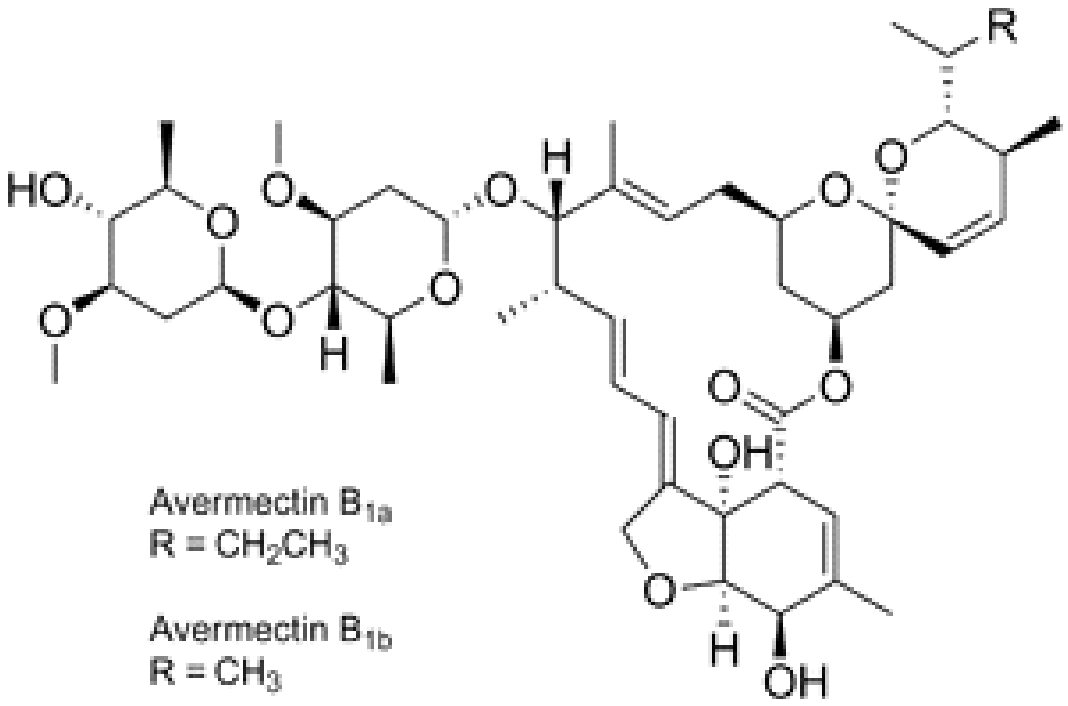

Figure 1.1: Molecular structure of ivermectin

systems in invertebrates, rodents and primates. Different results were obtained for example: direct injection of avermectin into *Ascarissuum* resulted in rapid paralysis that is neither flaccid nor rigid. Incubation of the free living nematodes, *Caenorhobitiselegons* with avermectin causes a low onset rigid paralysis. The problems encountered during the studies for the elucidation of mechanisms which could explain the mode of action of ivermectin have been further confounded by several additional factors. The drug acts at multiple sites, various target species have different sensitivities to the drug, and avermectins have poor solubility in aqueous solution. But the general opinion is the IVM effect was found to be mediated through a specific high affinity receptor. The interaction between IVM and the receptor is thought to lead to the release of gamma amino butyric acid (GABA) from the nerve ending and to enhance the binding of GABA to its receptor. Avermectins stimulates the release of endogenous GABA from the rat cerebral cortex synaptosomes (Pong *et al.*, 1980); a response which appears relatively specific because endogenous glutmaic was not released in these assays. Using rat brain slices from the region of the caudate nucleus, Ishiko *et al.*, (1985) perfused the tissue with avermectin and demonstrated an inhibitory effect of calcium-dependent and potassium stimulated dopamine release. The inhibitory effect of avermectin was completely antagonized by picrotoxin and bacuculline. Experimentally, avermectin blocked transmission at neuromuscular junction in lobster, and synapses between inter-neurons and motor neurons in the nervous system of *Ascarissuum*. It was hypothesized that these responses to avermectins were caused by an increase of membrane permeability to chloride ions (Fritz *et al.*, 1979; Martin, 1985) perhaps due to its interaction with GABA binding sites or by potentiating the release of GABA (Fritz *et al.*, 1979; Urquhart *et al.*, 1988).

Sikariskie (1986) reported that GABA is not found in the mammalian peripheral nervous system. At routine dose ivermectin does not cross the blood brain barrier and is considered safe in mammals. IVMs have been renowned as tremendously successful anthelmintic agents because of their ability to kill parasite without affecting the host organism.

1.3 Toxicology

The compounds' mechanism of toxicity in mammals is unknown but GABA is mammalian nervous system neurotransmitter and effects on GABA may be relevant to their safety in mammals (Lankas and Gordon, 1989).

The fact that much higher concentrations of these compounds are needed in mammals as compared to nematodes to affect neurological function, clearly appears in case of cattle as toxicity and death occurred when the animals were given 40 times the therapeutic dose, but 30 times the normal dose did not produce signs of toxicity (Bartlet, 1985). Also Ivermectin can be administered repeatedly without adverse effects at rates up to 60 μg/kg (10 times the recommended dose level) to collies known to be sensitive to this drug (Fassler *et al.*, 1991). This may be due to the lack of a specific, high affinity site associated with neuronal function or to the relatively poor penetration of these large molecular weight compounds into the C. N. S. (Lankas and Gordon, 1989).

In general, Ivermectin has a good margin of safety, but over –dosage may produce mydriasis (pupillary dilatation), tremor and death (Bartlet, 1985).

1.3.1 Genotoxicity studies

Ivermectin was tested *in vitro* for genotoxic activity in microbial and mammalian cell mutagenesis assays and in human fibroblasts for DNA

damage as measured by unscheduled DNA synthesis. In all instances, ivermectin had no genotoxic activity (Lankas and Gordon, 1989).

1.3.2 Acute toxicity of ivermectin

It was expected that the acute toxicity of ivermectin would be due to its effects in the CNS. This hypothesis has proved correct in acute toxicity studies conducted in a variety of laboratory animal species, although the exact mechanism of neurotoxicity was unknown (Lankas and Gordon, 1989).

No sever clinical sings were seen in dromedary calves injected with 5mg/kg ivermectin while 10mg/kg caused severe depression, ataxia and death within 24 hours (Radwan *et al.*, 1987).

The drug's effects in mice were similar after either oral or intra-peritoneal administration. They consisted of ataxia (uncoordinated movement), hypopnea (abnormally slow respiration rate), and tremors at all dosage levels within approximately 1 hour of treatment (Lankas and Gordon, 1989).

Mydriasis was the most sensitive indicator of toxicity in dogs occurring at all dosage levels. More severe signs of CNS including depression, ataxia (which may prolong in duration and quite severe) and tremors, occurred at doses of 10mg/kg and above (Beasly, 1986; Lankas and Gordon, 1989).

Single episodes of vomiting were recorded in two dogs treated with ivermectin (Fassler *et al.*, 1991). While Calvert and Rawlings (1986) reported cases with vomiting, trembling and lethargy, tachycardia, hypotension, tachypnea, and collapse occasionally develop. A case reported by ILiff-Sizemore*etal.*, (1990) showed that a rehuses monkey (*Rhesusmacque*) developed ataxia and latitudinal abnormalities, following

four intramuscular injections (6 mg/kg) of ivermectin.Blood examination showed a leukocytosis and increase in serum aspartate aminotranferase, lactate dehydrogenase, alkaline phosophatase and total bilirubin.

Seven dogs treated with Ivermectin 200 µg/kg body weight developed severe toxicosis (seizure like activity, recumbancy, non-responsiveness and coma) (Paul *et al.,* 1986)

Frischke and Hunt (1991) reported severe toxic signs, including ataxia and deep come, developed in four to seven -week kittens injected with 1 mg ivermectin used for cattle.

Studies in red footed tortoises (*Geochelonecarbonaira*) treated with ivermectin (0.4 mg/kg) single intramuscular injection showed that they were found in a state of extreme paresis or flaccid paralysis. Although, one of the tortoises died within three days of receiving the treatment,the only consistent post-mortem finding was severe fatty change in the liver (Lankas and Gordon, 1989).

The minimum toxic dose (MTD) in the leopard tortoise (*Geochelonepardalis*) is 0.025 mg/kg; death occurred with as low as 0.3 mg/kg. A dosage of 0.05 mg/kg was found to be safe in red footed tortoises, provided that treatment was not repeated at intervals less than seven days (Teare and Bush, 1983).

1.3.3 Reproductive toxicity

Ivermectin had no adverse effects on spermatogenesis, fertility, or reproduction performance of Beagle dogs when administered orally at (0.6mg/kg) of body weight monthly for eight treatments (Carolyn *et al.*, 1987).

In beef heifers treated with ivermectin (200μg/kg) no difference in conception rate was observed between the treated and control groups (Zagac *et al.,* 1991).

1.3.4 Adverse effects

In a survey by Karns and Luther (1984), it was determined that 366 of 3316 horses developed adverse reactions following use of ivermectin. Almost all of the reactions were of minor to moderate concern; however, one death was reported. Three hundred and thirty two (91% of all reactions)were reported as ventral midline pruritis oedema (10% of all doses). Fifteen (0.45%) were transient injection site swelling and/or stiffness. Eleven horses (0.33%) developed limb oedema. Eyelid oedema was reported in four horses (0.12%). Two horses (0.06%) had fever. Rate and depth of respiration was increased in one horses (0.03%). One horse became disoriented. Three horses (0.09%) developed signs of colic. One horse (0.03%) died few minutes after injection, and one horse (0.03%) became depressed (Karns and Luther, 1984).

In horse adverse reactions may include itching because of effects on microfilariae (Riviere and Papich, 2009). Depression and impairment of vision were the main signs seen following drug administration. All affected horses recovered within 5 days (Merck, 1982). Acute eczema on the injection site developed in two cats when the injection went accidentally intra-dermally (Shneck, 1988).

In humans, post treatment reactions were more common and severe in individuals with Sowda (onchodermatitis); they consisted mainly of musculoskeletal pain, local swellings with pitting oedema, and lymph gland tenderness and enlargement (Baraka *et al.,* 1995). In general, the adverse effects reported with ivermectin in humans are generally consistent with

mild mazotti reaction arising from its effect on the microfilariae. They include fever, pruritus, arthralgia, myalagia, postural hypotension, oedema, lymphadenopathy, gastrointestinal symptoms, sore throat, cough, and headache (Reynolds, 1993).

Data obtained by Eissa and Zidan (2009) revealed that Vertimec (Abamectin) caused reduction in erythrocyte counts (RBCs), leukocyte counts (WBCs) and haemoglobin concentration. These effects were significantly more pronounced in Vertimec treated rats at the high dose. The obtained results are in agreement with those found by (Ali, 1990; Anubama *et al.,* 2001) who stated that avermectins reduced erythrocyte, leukocyte counts and haemoglobin concentration in rabbits and rats.

Vertimec elevated the activity of AST and decreased ALT activity, total protein, albumin, and glucose concentrations in serum of treated rats in a dose-dependent manner, whereas alkaline phosphatase (ALP) activity and cholesterol concentration remained unaltered (Eissa and Zidan, 2009). The aforementioned findings are in coincidence with those reported by (Hsu *et al.*, 2001) who reported that AST was elevated in abamectin-dosed rats in a dose-dependent manner. AST and ALT are considered to be sensitive indicators of hepatocellular damage and within limits can provide a quantitative assessment of the degree of damage sustained by the liver (Peng *et al.,* 2007).

Qualitative and quantitative disturbance of protein synthesis is a consequence of impaired hepatic function (Celia and Wilkinson, 1973). Hypoalbuminemia (decreased albumin) is a liver disorder thought to be a consequence of decreased hepatic synthesis of albumin (Burtis and Ashwood, 1994).

Vertimec (Abamectin) also increased uric acid and creatinine concentrations in serum of treated rats in a dose-dependent manner (Eissa and Zidan, 2009). Uric acid and creatinine are useful in early detuction of nephrotoxicity induced by exogenous compounds. Elevation of uric acid and creatinine concentration in serum of treated male albino rats may be attributed to reduction in glomerular filtration in the kidney and also reflect dysfunction of the kidney tubules (Hayes, 1989; Walmsley and White, 1994).

Portal tract infiltration by lymphocytes and a focus of dysplasia with cytological atypia were observed in Vertimec treated male rat's liver at either dose levels; a remarkable abundance of lymphocytes infiltration in the liver tissues post-drug- administration. 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).

Male rats of both treated groups, T1 (30 mg/kg three times a week for 30 days) and T2 (10 mg/kg once a week for 210 days), exhibited a significant increase in the activity of ALT over those values obtained from the control groups. Subchronic poisoning with abamectin (group – T2), also resulted in a significant increase in the levels of plasma AST, urea, and creatinine compared to the values obtained from the control group. However, the subacute treatment with abamectin (group – T1) did not result in a significant change in the levels of AST, urea, and creatinine when compared to the control group. Data also show a significant decrease in the level of plasma albumin observed in the rats of group I, whereas, subchronic

treatment (group – T2) did not result in a significant change in albumin level compared to the control (Abd-Elhady and Abou-Elghar,2013).

Histopathological changes were observed in all selected organs of abamectin-treated animals. Marked degenerative changes of hepatocytes, congestion, and marked diffuse necrosis of hepatic tissue were observed in liver of abamectin-treated animal. Such necrobiotic changes were more intens in the livers of group T2. 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. 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. A marked necrosis of tubular cells, atrophy of the glomeruli, and areas of interstitial infiltration of round cells were found. However, some necrobiotic changes were observed in the lungs of abamectin-treated rats. Interstitial pneumonia with marked congestion and oedema were observed in lung of animals exposed for 30 days to abamectin. Moreover, diffuse local hemorrhages associated with atelectasis were seen in the lungs of animals exposed to abamectin for 210 days (Abd-Elhady and Abou-Elghar, 2013).

1.4 Pharmacokinetics of ivermectin in animals and humans

Ivermectin is effective against parasites in wide variety of hosts-including cattle, sheep, dogs, swine and horses (Fink and Porras, 1989). Pharmacokinetics of ivermectin is the function of the species in which the compound is studied.

The following summary includes the results of pharmacokinetics and bioavailability studies of this drug in different animal species and in humans.

1.4.1 Horses

The pharmacokinetic behaviour of avermectins is significantly affected by route of administration, drug formulation, and interspecies and inter-individual variation (Mckellar and Benchaoui, 1996).

The higher persistence of doramectin (DRM) compared to that of the ivermectin (IVM) could confer persistent efficiency against equine parasites due to its longer retention time in plasma and excretion into the gastrointestinal tract (Gokbulut *et al*., 2005). The results of IVM obtained by Gokbulut and his colleagues (2005) differed substantially from those previously reported by Scott (1997) in donkeys at same dose rate and administration route. The authors attributed these differences to methods used and/or different feeding regimen, age, sex, pathological status and body size that may cause differences in absorption, disposition and persistence of endectocides in the animals.

In contrast to ruminants, the absorption process in horses is faster after oral versus subcutaneous (SC) administration. The oral route is preferred, as parenteral administration can produce local swelling and other adverse reactions (Anderson, 1984). Plasma concentrations are higher and more rapidly achieved in horses compared to sheep probably because the rumen delays absorption in ruminant species

Perez and his colleagues (2002) conducted study in order to compare plasma disposition kinetic parameters of doramectin (DRM) and ivermectin (IVM) in horses after oral administration. Ten crossbreed adult horses, clinically healthy, weighing 380-470 kg body weight (bw) were selected for the study.Horses were allocated to two groups of five animals to provide an even distribution considering the variables sex, body weight and faecal egg count. Group I, were treated with an oral paste formulation of IVM at 0.2

mg/kg b/w and Group II, were treated with an oral dose of 0.2 mg/kg bw of DRM prepared as paste from the injectable formulation for oral administration. The mean plasma concentrations of DRM and IVM after oral administration in horses were detected until 30 and 20 days, respectively. Both drugs showed similar patterns of absorption and no significant differences were found for peak concentration, the time to peak concentration, or for absorptive half-life. The terminal elimination half-life was significantly ($P<0.05$) longer in the DRM treated group than for the IVM treated group. The differences observed in the elimination half-life explain the longer mean residence time and high values of area under the concentration time curve for the group treated with DRM, which are 30% higher than those of the IVM group (Perez *et al*., 2002).

Ivermectin (IVM- Eqvalan paste, 1.87%) and doramectin (DRM- Dectomax 1%) were each administered orally to donkeys at 200 microgkg(-1) bodyweight. Blood and faecal samples were collected at predetermined times over 30 days and plasmapharmacokinetics and faecalexcretion determined. Maximum plasma concentrations (C_{max}) of IVM (23.6 ngml(-1)) and DRM (33.9 ngml(-1)) were obtained at (t_{max}) 19.2 and 24h, respectively. The area under the concentration curve (AUC) of DRM (228.9 ng.day/ml(-1)) was significantly larger than that of IVM (119.3 ngdayml(-1)) and mean residence time (MRT) was 6.5 days for IVM and 9.1days for DRM. The highest (dry weight) faecal concentrations (9.33 microg/g(-1) - IVM, 12.12 microg/g(-1) - DRM) were detected at 55.9 and 48.0 h, respectively and each compound was detected (0.05 microg/g(-1)) in faeces between 11h and 9 days following oral administration in donkeys (Gokbulut *et al*., 2005).

The higher concentrations found in equine faeces compared to cattle faeces have been attributed to a lower production of more concentrated faeces (Perez *et al*., 2001).

A study was conducted to investigate whether the pharmacokinetics of avermectins or a milbemycin could explain their known or predicted efficacy in the horse. The avermectins, ivermectin (IVM) and doramectin (DRM), and the milbemycin, moxidectin (MXD), were each administered orally to horses at 200 microg/kg bwt. Blood and faecal samples were collected at predetermined times over 80 days (197 days for MXD) and 30 days, respectively, and plasmapharmacokinetics and faecalexcretion determined. Maximum plasma concentrations (C_{max}) (IVM: 21.4 ng/ml; DRM: 21.3 ng/ml; MXD: 30.1 ng/ml) were obtained at (t_{max}) 7.9 h (IVM), 8 h (DRM) and 7.9 h (MXD). The area under the concentration time curve (AUC) of MXD (92.8 ng x day/ml) was significantly larger than that of IVM (46.1 ng x day/ml) but not of DRM (53.3 ng x day/ml) and mean residence time of MXD (17.5 days) was significantly longer than that of either avermectin, while that of DRM (3 days) was significantly longer than that of IVM (2:3 days). The highest (dry weight) faecal concentrations (IVM: 19.5 microg/g; DRM: 20.5 microg/g; MXD: 16.6 microg/g) were detected at 24 h for all molecules and each compound was detected (> or = 0.05 microg/g) in faeces between 8 h and 8 days following administration (Gokbulut *et al.*, 2001).

1.4.2 Camels

In a pilot study Oukessou *et al.*, (1996) reported that the pharmacokinetic parameters of ivermectin after subcutaneous injection of 0.2mg/kg to three camels were reported as follows: peak plasma concentration (C_{max}) was found to be 3.23ng/ml, the time need to reach peak

plasma concentration (C_{max}) was 6±3.46 days. The area under the plasma concentration versus time curve (AUC) was 66.30±11.70ng.day/ml, the mean residence time (MRT) 21.50 days.

1.4.3 Cattle

Commercially, ivermectin is most widely used for treating cattle. Bioavailability studies have been run in these species using injectable formulation (Fink and Porras, 1989). When ivermectin was administered intravenously to six young calves (one bull, five steers) as a bolus dose of 200 μg/kg, the disposition kinetics of ivermectin in cattle could be described by three compartment open model with elimination from the central compartment. Compartment analysis yielded mean parameters as follows: terminal elimination rate constant (B) equal 0.258/day; biological half-life ($t½_B$) equal 2.7 days; apparent volume of distribution of at steady state (V_{dss}) equal 2.4 1/kg. The area under the plasma concentration-time curve (AUC) was 245ng.day|ml. Non-compartmental parameters obtained by utilizing statistical moment theory, mean resident time (MRT), clearance (CL), and V_{dss} were calculated to be 2.8days, 0.79 1/kg and 2.2 l/kg respectively (Wilkinson *et al.,* 1985).

In an independent study, application of a 2-compartement (biexponential) model to the data obtained, reveal a very short distributive phase in cattle K=6 days-1, a biological half-life $t_{½}$ of 2.8days, a very large volume of distribution 1.9 l/kg, and an area under the curve (AUC) of about 700 days.ng/kg (Lo *et al.,* 1985).

Formulation modification, based on the solubility properties of the drug, can have a significant effect on the bioavailability of ivermectin in cattle following subcutaneous administration. For example, changes in solvent composition can be used to achieve prolonged levels. The

bioavailability method was used to compare two experimental formulation labelled (i) and (ii). Formulation (i) is an aqueous micellar solution of ivermectin formulated to overcome its solubility limitations in water. A micelle is formed with a surface –active agent, such as polyxyethylene sorbitan monooleate (polysorbate 80, Tween80), and a co solvent such as glycerol formal in the presence of other substrates, such as benzyl alcohol. Formulation (ii) is modified from (i) by mixing it with prolonged (ng/ml) achieved with aqueous product (i): 84±7 are significantly higher than with modified formulation (ii): 25±14. The former vehicle affords a much greater bioavailability (AUC): 246±46 than the later: 186±81 (ng.hr/ml). The biological half - life (day) of the aqueous micellar product (i): 2.0±0.3 whereas for product (ii): 3.7±0.7 (Lo *et al.*, 1985).

1.4.4 Goats

Pharmacokinetics and mammary excretion of ivermectin were determined in goats following a single subcutaneous administration. Kinetic analysis of plasma and milk levels was performed using a one –compartment model. The maximum plasma and concentration of 6.12ng|ml occurred at 2.85 days. The half-life of 4.03 days was similar to the value in sheep (3.68days). Ivermectin was detected in the milk at the first sampling and thereafter for at least 25 days (Alvinerie *et al.*, 1993).

Bioavailability was lower with percutaneous administration and varied with individuals. Oral administration gave peak plasma concentration (15.85ng/ml) within one day and clearance by 72-120hours. Percutaneous administration gave peak plasma concentration (about 3.8 ng/ml) within two days and clearance by 192 hours. Ivermectin concentration in milk was lower than in plasma (Scott *et al.*, 1990).

1.4.5 Dogs

The results in dogs reveal a significant species difference that ivermectin is eliminated (biotransformation, distribution and excretion) more rapidly in dogs (t½ =1.8 days) than in either cattle or sheep (Lo *et al.*, 1985). This measurement of the terminal half-life in dogs is in accord with results reported by Kojima*et al.*, (1987) in japan, which yielded a $t_{½}$=31.1±6.67hours,maximum concentration curve (AUC) 262±21.9ng.hr/ml.

1.4.6 Sheep

The results in sheep were analogous to those for cattle. Application of a 2-compartment (biexponential model) to the data obtained, reveal a very rapid distributive phase, as in cattle, Kd=10days-1. The volume of distribution =4.6 1|kg which is greater than that in cattle. The biological half-life was again 2.7days, very similar to that obtained in cattle (Lo *et al.*, 1985).

Other workers have also evaluated pharmacokinetics of ivermectin in sheep. In contrast to the data described previously Prichard *et al.*, (1985) reported a larger ivermectin biological half-life in sheep of is 7.2 days. Volume of distribution a steady state of 5.3 l/kg following intravenous administration. Intra abomasal administration resulted in rapid absorption, peak plasma concentration of 60.6ng/ml at 4.4 hour, and 100% bioavailability. However intra ruminal administration produced much lower peak concentration 17.6ng/ml at 23.5 hours and bioavailability 25.1%.

In another study Marriner *et al.*, (1987) reported that the biological half-life in sheep was 61 hours, which is in close agreement with the measurement of Lo and his colleagues (1985).

In Sudan, pharmacokinetics studies were conducted in non-pregnant and pregnant ewes by Shaddad (1997). The results showed significant difference between the non-pregnant and pregnant animal with the pharmacokinetics parameters (t½(Ka) 38.51±4.79 and 11.2±2.74 hours, time to peak concentration 6.58±0.46 and 3.75±0.29 days, C_{max} 36.07±0.88 and 31.52±0.79 ng/ml, V_d 16.29±0.58 and 19.83±1.16 1/kg, AUC 1674.74±21.42 and 1441.59±43.74 ng/ml days . t½(Ke) 21.49±0.59 and 28.45±1.65 days and the clearance 428.61±1.62 and 470±17.07 L/kg for non-pregnant and pregnant ewes respectively).

1.4.7 Humans

Disposition of ivermectin was studied with onchocerchiasis after oral dose 11.11±0.4mg/kg. The mean plasma values for the 9 subjects as follows: half-life 56 .50± 7.01 hours; clearance 142.3 ± 22. 6 L/kg; volume of distribution 9.91± 2.67 L/kg; area under the plasma concentration 1545.3±190.5ng/ml/day; time to reach maximum concentration 4.7±0.5 hours; maximum concentration 38.2±5.8ng/ml (Okonkwo *et al.,* 1993).

In Sudan, Baraka *et al.*, (1996) reported that, plasma pharmacokinetic variables for patients were; maximum plasma concentration 52.0ng/ml; time to achieve maximum concentration 5.2hours; elimination half-life 35.0 hours; and the area under the plasma concentration curve versus time 2852ng.hours/ml.

The manufacturer reported peak plasma concentration of 46.6 ±21.9 ng of ivermectin B1a per ml 4 hours after oral administration of ivermectin (Reynolds, 1993).

1.5 Interaction of ivermectin with other drugs

There were no significant difference between baseline pharmacokinetics parameters and those calculated after ivermectin

treatment, except for an increase in total body clearance of erythromycin in day 4 (Bohlen *et al.,* 1995).

Administration of ivermectin had no effect the pharmacokinetics of albendazole sulphoxide andthere wasno additiveeffect on the parasite (Awadzi *et al.,* 1994).

When ivermectin and diethylcarbamazine (DEC) are given simultaneously in a single dose to persons with *Wuchereriabancrofti* infection, the resulting suppression of microfilariae is more profound and sustained that when either drug is given alone. Thus, co-administration of ivermectin with DEC seems to interfere with the microfilaricidal action of DEC. These finding have implication both for treatment of the individual patient and for community-based drug distribution programmes designed to interrupt transmission of *Wuchereriabancrofti* (Dreyer *et al.,* 1998).

1.6 Metabolism and tissue residues

Ivermectin is used widely as anti-parasite agent in food producing animals. The residual tissue concentration of the therapeutic agent, or tissue residue, is a safety concern to the meat –consuming public. The absorption, distribution, metabolism and excretion of tritium labelled ivermectin have been studied in target food producing species, (cattle, sheep and swine) as well as in laboratory species (rat). Comparative metabolic studies were done in laboratory animals (the rat) and in liver microsomes form various species (Chiu and Lu, 1989). Tissue residues are often assayed by the rough evaluation of total radioactivity in the tissue or body fluid, after the administration of a given dose of radioactive drug to the animal. The total tissue residues include the free drug, its metabolites, and any tissue bound residues, according to their general classification. Dorough (1980) said that the free and bound residues are differentiated through their extractability by

techniques involving exhaustive extraction, denaturation, or solubilisation methods. Lu *et al*., (1987) reported that toxicological evaluation of the free drug residue is possible when the structure and tissue levels of this residue is extremely difficult. Alvinerie *et al.*, (1994) recorded that in cattle the amount of ivermectin elimination in the milk ranged from 0.6 to 3.8% of the dose administered. Taking into consideration the daily permissible dose of ivermectin (60µglkg in total) and the other possible sources of the drug, it was concluded that there was no health risk in the ingestion of milk taken from cows treated with 0.1 ml of commercial ivermectin. Faecal excretion is the major pathway of drug elimination in all species studied. Regardless of whether the drug was administered intrarumnially or subcutaneously to cattle, only 0.5-20% of the dose was eliminated in urine,the remainder appeared in faeces (Jacob *et al.,* 1983). Seven days after the administration of a given dose, recovery of the radioactive substance varied between 62-83%; in cattle, sheep and rats. Highest residual tissue concentration of radioactivity was observed in the brain. Highest residual tissue concentration of radioactivity is present in the liver and fat tissues, while lowest levels are observed in brain.

A sensitive high performance liquid chromatography (HPLC) method revealed the highest residues at the injection site (up to 59 and 2.6 mg/kg at 7 and 14 days were respectively).

Among the other tissue studied, residues at 7days were in the following order: Liver (<50ug/kg)kidney>(25ug/kg) > Muscle and other edible tissues, including the injection site. Withdrawal period for pigs and cattle 21 and 28 days respectively are suggested (Slanina *et al.*, 1989).

Scott and Mackellar (1992) reported that ivermectin was detected in the contents and mucus at all levels of the gastrointestinal tract. High

concentrations of ivermectin were measured in skin, ears and earwax, suggesting that this drug should be effective in the treatment of ectoparasitic infestations, particularly ear mites.

1.7 Use of ivermectin in animals and humans

1.7.1 Use of ivermectin in horses

Eqvalan paste (TM), Zimectrin paste (TM), this is paste, for oral use, containing 1.87% w/v ivermectin. It is available in a prefilled syringe. The syringe soled in international markets is calibrated to deliver the amount of drug needed for 100kg (220Ib) of body weight at 200ug/kg. Eqvalan liquid (TM) this product is clear, ready-to-use liquid for professional administration by stomach tube (nasogastric intubation) or as oral drench. It contains 1.0% ivermectin and various excipients. The recommended dosage is 200ug/kg, and each ml contains enough ivermectin to treat 110Ib (50kg) of body weight. Eqvalan injection (TM) a micellar formulation containing 20mg of ivermectin per ml of sterile aqueous solution (2.0% W/V) intended for intramuscular injection. Manufacturing and distribution of this product were suspended in 1984. Adverse reaction has included some that were sever or even fatal (Campbell *et al.*, 1989).

Ivermectin is given to horses as an oral paste containing 1.87% of the active ingredient at dose of 200ug/kg (Bennet, 1986). It has 95% to 100% efficacy in killing small strongyles, including adults (Yazwinsky *et al.*, 1982a). The drug has been approximately 99% effective adult *Strongylusvulgaris* and adult *Strongylusedentates*. Efficacy of 99% has been shown against *Gastrophilus* spp., *Trichostrongylusaxei* (Torbert *et al.*, 1982). The intramuscular form of ivermectin was reported to be 100% effective in the removal of *Oxyurisequi*and *Parascarisequorum* infection (Yazwinsky*et al.*, 1982b).

1.7.2 Use of ivermectin in camels

Ivermectin given at 0.2mg/kg subcutaneously effectively treats and controls gastrointestinal nematodes and sarcoptic mange mites in camels (Ibrahim *et al.,* 1981; Dafalla *et al*., 1987).

1.7.3 Use of ivermectin in cattle

Ivomec injection is a sterile solution 1.0% ivermectin. The product is indicated for the treatment and control of gastrointestinal nematodes (including hypobiotic Ostretagia fourth stage larvae), lung worms and certain other nematodes, grubs, sucking lice, psorptic and sarcoptic mange mites, sand tampans, and larvae of the old worm fly. It is administered subcutaneously in front of or behind the shoulder at 1.0ml/50kg body weight. Ivomec oral solution for cattle contains 0.4%w/v ivermectin and should be administered at 2.5-ml/50kg body weight using standard dosing equipment. Ivomec cattle paste contains 0.153% w/v ivermectin and is administered orally by depressing the handle of the medigun applicator completely to deliver 23 mg ivermectin per 113.5kg (250Ib) body weight Topical formulation, ivermectin pour- on for cattle contains 0.5%w/v ivermectin and is applied along the top line in a narrow strip extending from the withers to the tail head. The volume of application is 1ml/10kg body weight (Benz *et al.,* 1989).

The influence of chemoprophylaxis with an ivermectin sustained-release bolus in the first grazing season on the resistance of cattle gastrointestinal nematodes during the following grazing season was investigated by Claerebout *et al.,* (1997). Dairy replacement calves were either given one bolus at the start of their first grazing season or left untreated. During the first grazing season gastrointestinal nematode infections were controlled very effectively by the bolus, as shown by the

greater weight gains, the negligible faecal egg counts and the low serum pepsinogen and antibody levels in the treated calves. In contrast, all parameters showed extensive parasite-host contact in the untreated animals. The efficient prophylaxis in the paddocks grazed by the treated animals, compared to moderate infection levels at the end of both first grazing season on the paddocks grazing by untreated animals. No negative of the previous prophylaxis on the clinical condition and the weight gain of the second season grazing animals was observed.

Ivermectin consistently has greater than 99% efficacy against larval and adult stages of *Ostertagia* spp., *Trichostronglus* spp., *Oesophagastomum* spp., *Haemonchus* spp. (Berry *et al*., 1983). *Dictyocaulusviviparous* (Armour *et al*., 1985; Benz *et al*., 1984), Psorptic (Meleney *et al*., 1982) and Sarcoptic (Barth and Preston, 1988) mange mite.

1.7.4 Use of ivermectin in goats

Ivomec or Oramec liquid for goats is the same 0.08%w/v solution of ivermectin as used in sheep, and is to be given 2.5ml/10kg body weight. The product is indicated for the treatment and control of gastrointestinal nematodes and lung worms. In goats, there is insufficient efficacy against nematodes justify use of injectable formulation (ivomec injection). Otherwise recommended in cattle and sheep (Benz *et al.,* 1989).

1.7.5 Use of ivermectin in sheep

Oral formulation (ivomec or oramec) liquid for sheep is a 0.08%w/v solution of ivermectin to be given orally to sheep at 2.5ml/10kg body weight using standard drenching equipment. The oral formulation is for the treatment and control of gastrointestinal nematodes, lung warms, nasal bots and itch mites. Injectable formulation (ivomec) injection the some 1.0-%w/v solution used in cattle is also employed for sheep. The product is

administered subcutaneously at 0.5-ml/25-kg body weight (Menz *et al.*, 1989).

Intermectin in 0.08% solution given as drench to sheep at dosage of 200ug/kg has had efficacies of greater than 99% against adult *Haemonchuscontortus* (Armour *et al*., 1982) including benzimidazole-resistant strains (Wescott and Lea Master, 1982). *Cooperia* spp. *Trichostrongylus* spp. *Ostertagia* (Todd *et al.*, 1984).*Chaperiaovina*, *Nematodirus* spp., *Trichuris* spp. (Armour *et al*., 1982).

1.7.6 Use of Ivermectin in humans

Ivermectin is an anthelmintic used in the treatment of onchocerciasis when it one dose of 3 to 12 mg by month to be given annually to control most cases and such dose is being used in mass treatment programmes in endemic areas. Ivermectin also possess activity other nematodes in man including *Ascarislumbricoides*, *Strongloidessterocoralis*, *Trichuristrichuis*, and *Enterobiusvermicularis* (Reynolds, 1993).

Baraka *et al.*, (1995) from his findings suggested an improvement in the cellular immunity of humans to *Onchocecavlvulus*associated with IVM chemotherapy. Single doses of ivermectin (150ug/kg) resulted in good clinical responses and much goodwill among villagers. Improvements in physical fitness, ability to work, and freedom from musculoskeletal pain were reported at the 3 months follow-up. In areas where Sowda (Onchodermatitis) syndrome is prevalent, medical surveillance for 3days or more should be considered (Baraka *et al.*, 1995).

Ismail *et al.*, (1996) recommended that, new drug or dosing schedule are needed to achieve the goal of killing all filarial parasites.

CHAPTER TWO

Materials and Methods

2.1 Study site

This study was conducted in Khartoum State (Figure2.1), which is located in the semi-arid zone, between latitudes 15° 38′ N and longitudes 32°32′ E. Experimental animals were housed in the farm of the College of Veterinary Medicine (SUST), Hillat Kuku, Khartoum North.

2.2 Experimental animals

For the purpose of the study, a total of 18 healthy donkeys, 4-10 years, weighing 90- 150 kg was purchased from local market in Khartoum North. Upon their arrival animals were subjected to thorough clinical examination to ascertain freedom from clinical disorders. Animals were housed in pens (Figure 2.2) in the farm and allowed with free access to water and calculated amount of feed.

2.3 Experimental drug

Ivermectin 1%(Intermectin, interchemie, Holland) injection formulation was used in this study.

2.4 Experimental design

2.4.1 Experiment 1

In the first experiment, 12 donkeys were used to evaluate the toxicity of repeated daily doses of ivermectin injected subcutaneously for seven successive days. Animals were randomly divided into two groups each of six. Following adaptation period (7 days) animals were subjected to fasting for 48 hours as stress to mimic the situation during disease state. Animals in the first group (T1) received ivermectin injection subcutaneously (SC) once daily at the manufacturer recommended dose 200 μg/kg body weight for

seven successive days. Animals in the second group (T2) received ivermectin injection subcutaneously (SC) once daily at 5 times the manufacturer recommended dose i.e. 1 mg/kg body weight for seven successive days.

Following each injection animals were monitored for 2 hours to report physical side and/or toxic effects, if any.

2.4.2 Experiment II

In the second experiment, another 6 donkeys were used to test the acute toxicity of ivermectin when used as single dose. Following adaptation period (seven days) animals were subjected to fasting for 48 hours as stress to mimic the situation during disease state.Animals then treated with ivermectin injection subcutaneously (SC) as a single dose at 10 times the recommended dose i.e. 2 mg/kg body weight.

Animals were also monitored following drug administration for two hours to report any physical side or adverse effects.

2.5 Collection of blood samples

Blood samples were 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.

Figure 2.1: Location of Khartoum, in the centre of Sudan

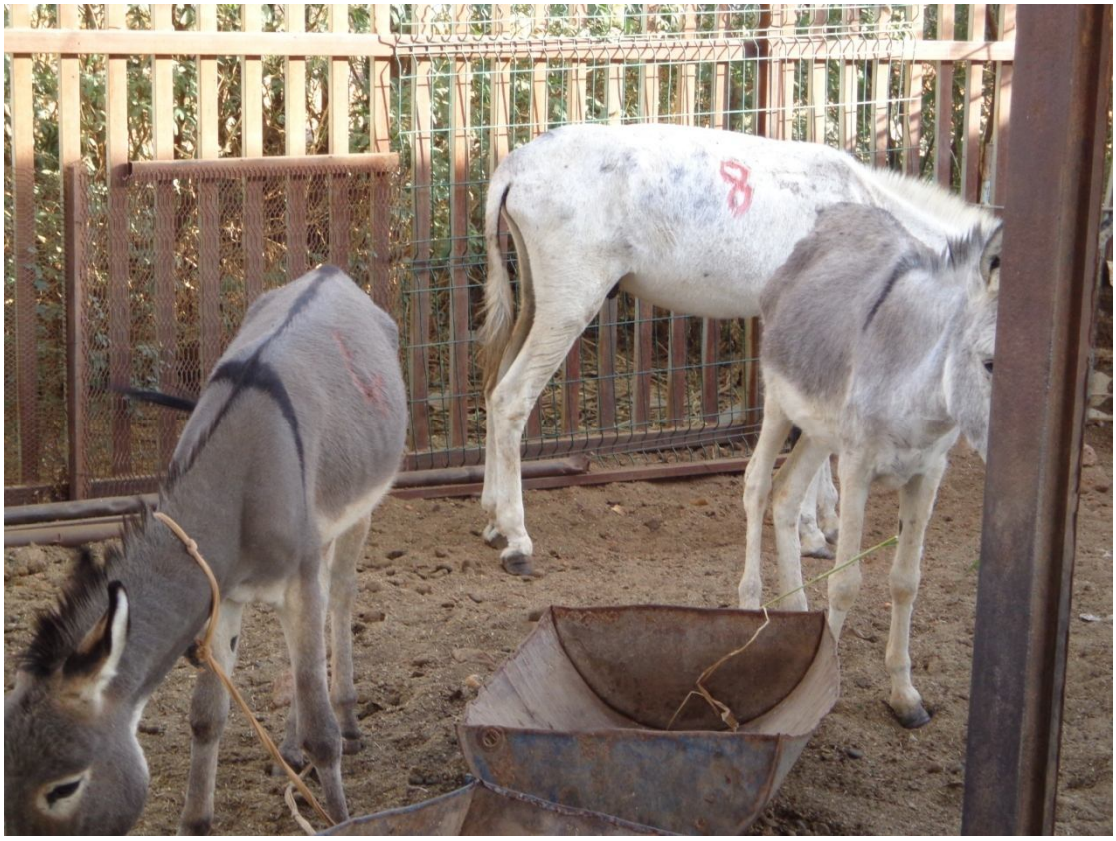

Figure 2.2: Experimental animals housed in pens in the college farm

2.6 Sampling schedule

In the first experiment, following the adaptation period, blood samples were collected at two time points (-4 and -2) before the start of the experiment (baseline values);and then at 1^{st},2^{nd}, 3^{rd}, 4^{th}, 5^{th}, 6^{th}, 7^{th}, 10^{th} and 15^{th} day following the first injection.

In the second experiment, following the adaptation period, blood samples were collected at two occasions (-4 and -2) as baseline value, and following fasting, then at 1^{st}, 4^{th}, 5^{th}, 7^{th}, and 9^{th}day following the treatment.

2.7 Haematological method

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

2.7.1 Haemoglobin concentration

Haemoglobin (Hb) was determined by cyanomethaemoglobin method using Jenway spectrophotometer (Jenway 6305 spectrophotometer, Jenway Ltd. U. K.). The haemoglobin concentration was measured in g/100 ml of blood.

The heparinized blood was mixed gently. Exactly 10µl of blood were pipette (the outside of the tip was whipped with a piece of cotton wool) and then mixed with 2.5ml of Drabkin's solution. Then the mixture left to stand for 10-15 minutes. The intensity of the developed colour was measured using spectrophotometer (Jenway 6305 spectrophotometer, Jenway Ltd. U.K) at wavelength 540 nm.

Hb concentration was calculated using the following formula:

Optical density of sample× Hb concentration in standard = g/dl
Optical density of standard

2.7.2 Packed cell volume (PCV)

Blood was mixed very well; capillary tubes were filled with blood by capillary traction until ¾ of its length then sealed at one end by plasticeal. The sealed end was placed at the outer angle of the microhaematocrite (Hettich-Zentrifugen –Germany) centrifuge. The tubes were centrifuged at 40.000 rpm for 5 minutes then removed and was put on the reader. The reading was recorded as percentage of packed red cells to the total blood volume. PCV% measured in the same days with other parameters.

2.7.3 Total erythrocytes(RBCs) count

The working dilution (hayem) was prepared in eppendorf tubes in suitable volumes to reach a final dilution of 1:200. The dilution was gently put into the improved Neubauer haemocytometer (Neubauer improved – Germany) using microtitre pipette. Then the Neubauer haemocytometer was left for 2-3 minutes to settle the RBCs, counting then made in 4 squares in corner and that which in the middle or centre of 25 squares of RBCs were counted using ×10 eye – and ×40,then multiplied by the haemocytometer correction factor to give the number of the RBCs per cubic mm.

2.7.4 Total Leucocytes (WBCs) count

The working solution (Glacial Acetic Acid) was prepared in Eppendorf tubes in suitable volumes to reach a final dilution of 1:200. Further dilution was made as duplicates of this dilution. Following mixing, diluted blood was gently put into haemocytometer using micropipette to fill the counting chamber. Counting is then made in 4 large squares in corners using 40 objective lens. The counted number was then multiplied by the

haemocytometer correction factor to give the number of WBCs per cubic mm in the sample.

2.8 Biochemical parameters

2.8.1 Total protein

Total protein was analysed by Biuret method (King and Wooton,1956) using a commercial kit(Vitro scient, Egypt). Cupric ions, in alkaline medium, interact with protein peptide bonds resulting in formulation of a coloured complex. The optical density of the developing colour was measured at 546 nm using spectrophotometer (Jenway 6305 UV/Vis, UK).

2.8.2 Albumin

Albumin was detected by colorimetric endpoint method following the modified bromocresol green binding according to Bartholomew and Delany, (1966). In this method Albumin at pH 4.2 bind to bromocresol green and form blue green coloured complex, the intensity of this colour is proportional to the albumin concentration and determined by measuring the increase in the absorbance at 580 nm using spectrophotometer (Jenway 6305 UV/Vis, UK) and commercial kits (Vitro scient, Egypt).

2.8.2 Alanine aminotransferase (ALT)

ALT activity was detected by Kinetic UV method which was first described by Henley and Pollard (1955) and modified by Reitman and Frankel, (1957). In this method the amino group is enzymatically transferred by ALT present in the specimen from alanine to the carbon atom of 2 oxoglutrate yielding pyruvate and L-glutamate. Pyruvate is reduced to lactate by LDH present in the reagent with the simultaneous oxidation of NADH to NAD. The rate of oxidation of NADH is proportional to ALT activity in the specimen, and it was determined by measuring the decrease in

absorbance at 340 nm using spectrophotometer (Jenway 6305 UV/Vis, UK) and commercial kits (Vitro scient, Egypt).

ALT activity was calculated by determining the change in absorbance per minute (ΔA/min) from the linear portion of the reaction curve and the ALT activity was calculated using the following formulae

U/I= 1746× ΔA/min.

2.8.3 Aspartate aminotransferase (AST)

Aspartate aminotransferase (AST or GOT) catalyzes the transfer of the amino group from aspartate to 2-oxoglutarate, forming oxalacetate and glutamate. The catalytic concentration is determined from the rate of decrease of NADH; measured at 340 nm, by means of the malate Dehydrogenase (MDH) coupled reaction according to Reitman and Frankel, (1957).

The working reagent and the instrument were brought into reaction temperature. The plasma was collected by standard procedures. Then the required amounts of plasma, standard solution and distilled water (blank) were prepared in the cuvettes.

The cuvette was mixed and inserted into the spectrophotometer (Jenway 6305- U.V/VIS. spectrophotometer UK). The stopwatch was started.

After 1 minute interval, the initial absorbance was recorded and at 1 minute intervals thereafter for 3 minutes. The difference between consecutive absorbance was calculated, and the average absorbance difference per minute (ΔA /min).

The AST/GOT concentration in the sample was calculated using the following general formula:

U/I= 1746× ΔA/min.

2.8.4 Urea

Urea in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry (Fawcett and Scott, 1960).

The plasma was collected by standard procedures. Then tubes containing the required amounts of plasma, standard solution and distilled water (blank) were prepared.

Spectrophotometer (Jenway 6305- U.V/VIS. spectrophotometer UK) was used; the absorbance (A) of the standard and the sample were Red at 600 nm against the Blank.

The urea concentration in the sample was calculated using the following general formula:

$$\frac{A\ Sample}{A\ Standard} \times C\ Standard \times Sample\ dilution\ factor = C\ Sample.$$

2.8.5 Triglycerides

Triglycerides in the sample originate, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry (Bucoloand David, 1973, and Stein and Myers, 1995).

The plasma was collected by standard procedures. Then, the tubes containing the required amounts of plasma, standard solution and distilled water (blank) were prepared.

The tubes were mixed thoroughly and incubated for 15 minutes at room temperature or for 5 minutes at 37^0C. Spectrophotometer (Jenway 6305- U.V/VIS. spectrophotometer UK) was used; the absorbance (A) of the standard and sample were measured at 500 nm against the Blank.

Triglycerides concentration in the sample was calculated using the following general formula:

A Sample XC Standard = C Sample
A Standard

2.8.6 Cholesterol

Free and esterified cholesterol in the sample originates, by means of the coupled reactions described below, a coloured complex that can be measured by spectrophotometry (Allain *et al*., 1974, and Tietz, 1995).

Spectrophotometer (Jenway 6305- U.V/VIS. spectrophotometer UK) was used, the absorbance (A) of the Standard and the Sample were measured at 500 nm against the Blank. The colour is stable for at least 2 hours.

The Cholesterol concentration in the sample was calculated using the following general formula:

A Sample × C Standard = C Sample.
A Standard

2.8.7 Phosphorus

Inorganic phosphorus reacts with ammonium molybdate in acid medium to form a phospholybdate complex which absorbs light at 600-675 nm. The absorbance at this wavelength is directly proportional to the mount of inorganic phosphorus present in the sample.

The plasma was collected by standard method. Then the tubes containing the required amounts of plasma or standard solution were prepared.

The tubes were mixed and incubated for 15 minutes at room temperature (+15-25ºC). Spectrophotometer (Jenway 6305- U.V/VIS. spectrophotometer UK) was used, the absorbance (A) of the Standard and the Sample were measured at 600-675 nm against the Blank.

The final concentration of phosphorus in the sample was calculated using the following general formula:

Serum inorganic phosphorus mg/dl = A Sample / A Standard ×5

2.8.8 Sodium

The present method is based on reaction of sodium with a selective chromogen producing a chromophore whose absorbance varies directly at the concentration of sodium in test.

The plasma was collected by standard method. Then the tubes containing the required amounts of plasma or standard solution were prepared.

The tubes were mixed and incubated for 5 minutes at room temperature (+15-25ºC). Spectrophotometer (Jenway 6305- U.V/VIS. spectrophotometer UK) was used, the absorbance (A) of the Standard and the Sample were measured at 623 nm (620-640) against the Blank.

The final concentration of sodium in the sample was calculated using the following general formula:

$$\text{Sodium mEq/l} = \frac{A\ \text{Sample}}{A\ \text{Standard}} \times 150$$

2.8.9 Potassium

The amount of potassium is determined by using sodium tetraphenylboron in a specifically prepared mixture to produce a colloidal suspension. The turbidity of which is proportional to concentration of K^+ in the range of 2-7 mEq/L.

The plasma was collected by standard method. Then the tubes containing the required amounts of plasma or standard solution were prepared.

The tubes were mixed and incubated for 5 minutes at room temperature (+15-25ºC). Spectrophotometer (Jenway 6305- U.V/VIS.

spectrophotometer UK) was used, the absorbance (A) of the Standard and the Sample were measured at 623 nm (620-640) against the Blank.

The final concentration of potassium in the sample was calculated using the following general formula:

$$\text{Potassium mEq/l} = \frac{A\text{Sample}}{A\text{ Standard}} \times 5.0$$

2.9 Necropsy and post-mortem examination

Following death of animals, post mortem examination was conducted and animals were examined to identify gross lesions and specimens of the liver, lungs, kidneys, heart, spleen and intestine were fixed in 10% neutral buffered formalin and processed for histopathology.

2.10 Histopathology sections and staining

At necropsy, suitable pieces of liver, kidney, lung, and spleen were removed, washed in saline, and fixed in 10% formalin for histopathological examinations. The formalin fixed tissues were processed by the paraffin wax embedding method of tissue sectioning, as per method described by Culling (1974). A rotatatory microtome was used to cut serial sections to a thickness of 4–5 microns. The sections were stained with Haematoxylin and Eosin (H & E) stains (Luna, 1968; Bancroft *et al.*, 1996). The H & E stained slides were observed under the microscope, and lesions were recorded.

2.11 Statistical analysis

Values obtained were tested for significance using t-test to compare pre-treatment (baseline) with post treatment values for the different parameters tested using SPSS version 16 package for statistical analysis.

CHAPTER THREE

Results

3.1 Experiment I

3.1.1 Side and/or adverse effects

In the group treated with five times the recommended dose (T2), immediately following injection of the drug one animal fell down, rolled in the ground with prominent tremors at the peripheral muscles approximately for 3 minutes, then the animal stood up and continued to feed and drink normally.In the fifth day in the same treatment group (T2) animal number 11 fell down and refused to drink or to eat for a whole day. Next day the animal died. In the seventh day animal number 10 fell down and stopped feeding for a whole day. In the eighth day animal number 9 fell down and died within 24 hours, while animal number 12 died after 3 days following recumbancy of the animal.

Generally four animals out of six in T2 group died following treatment with five times the recommended dose at days 6, 8, 10 and day 11 following the first treatment.

3.1.2 Gross lesions

At necropsy, congestion in the main visceral organs (Figure 3.1) was the prominent feature in animals. Necrosis in the liver (Figure 3.2), and trabeculations in the spleen (Figure 3.3), 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.

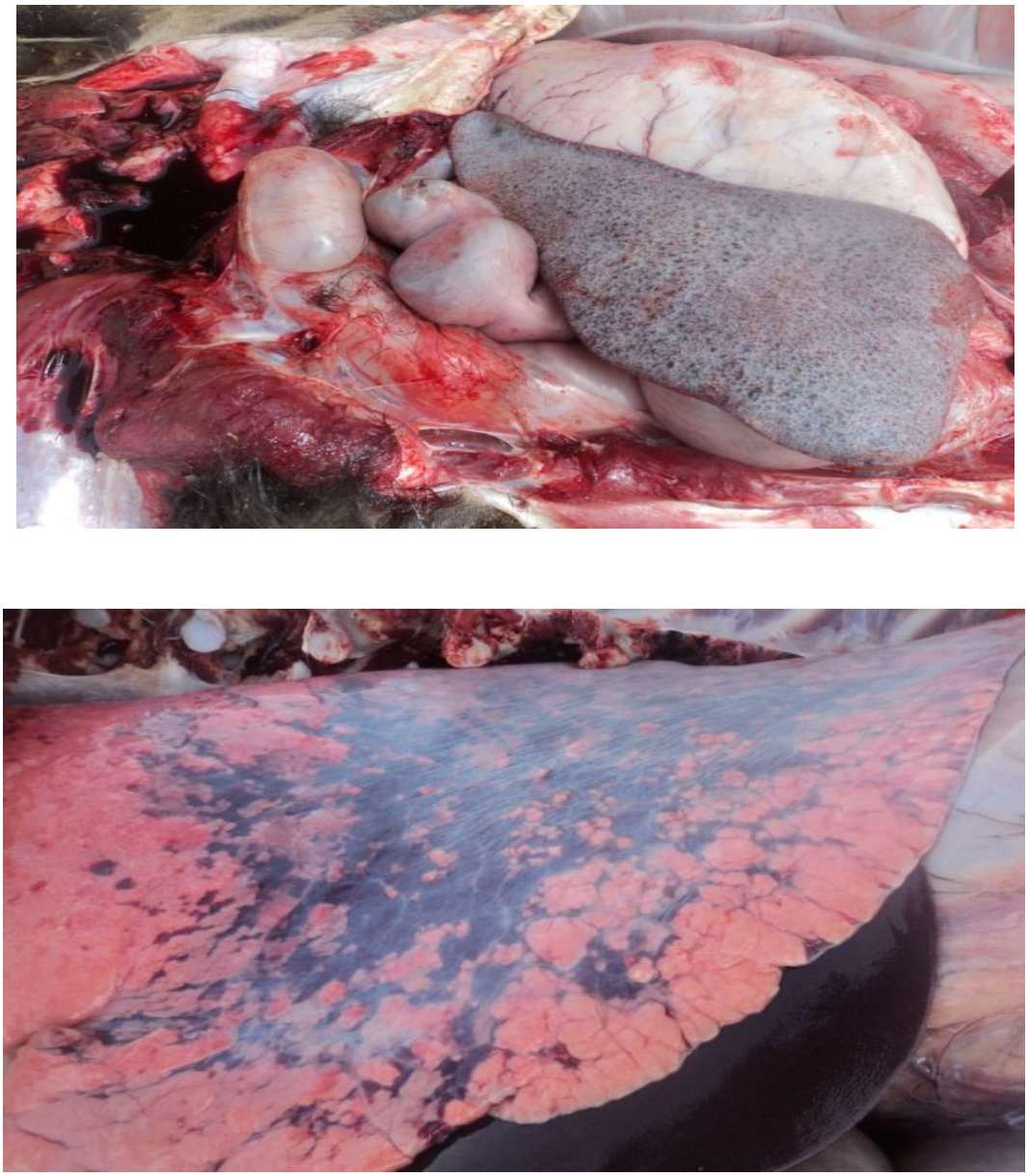

Figure 3.1: Congestion in the internal organs in donkeys treated with different doses of ivermectin

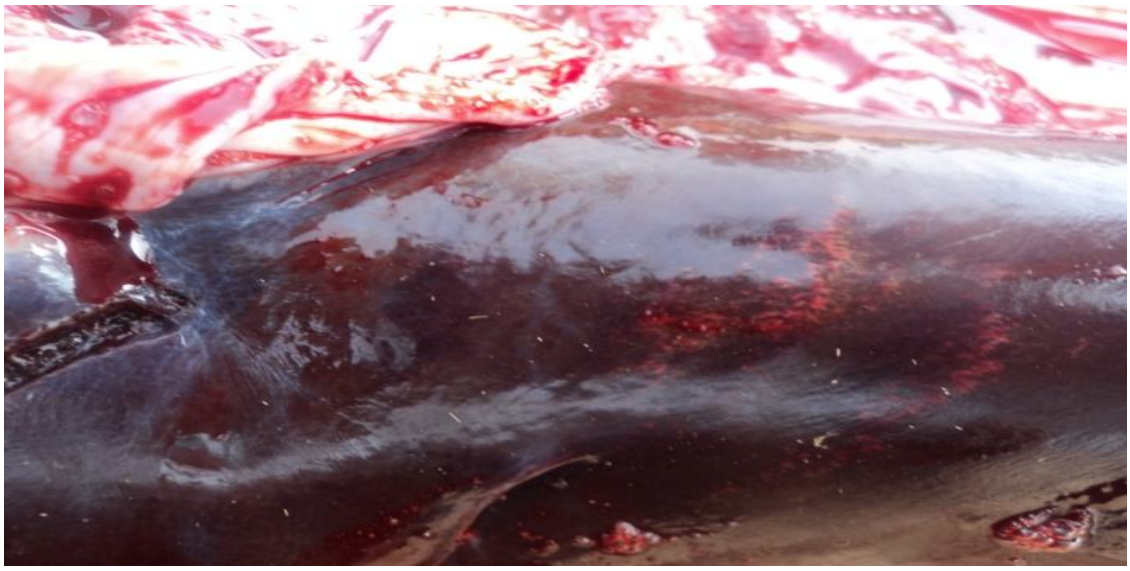

Figure 3.2: Liver necrosis in donkey treated with repadeid doses ivermectin

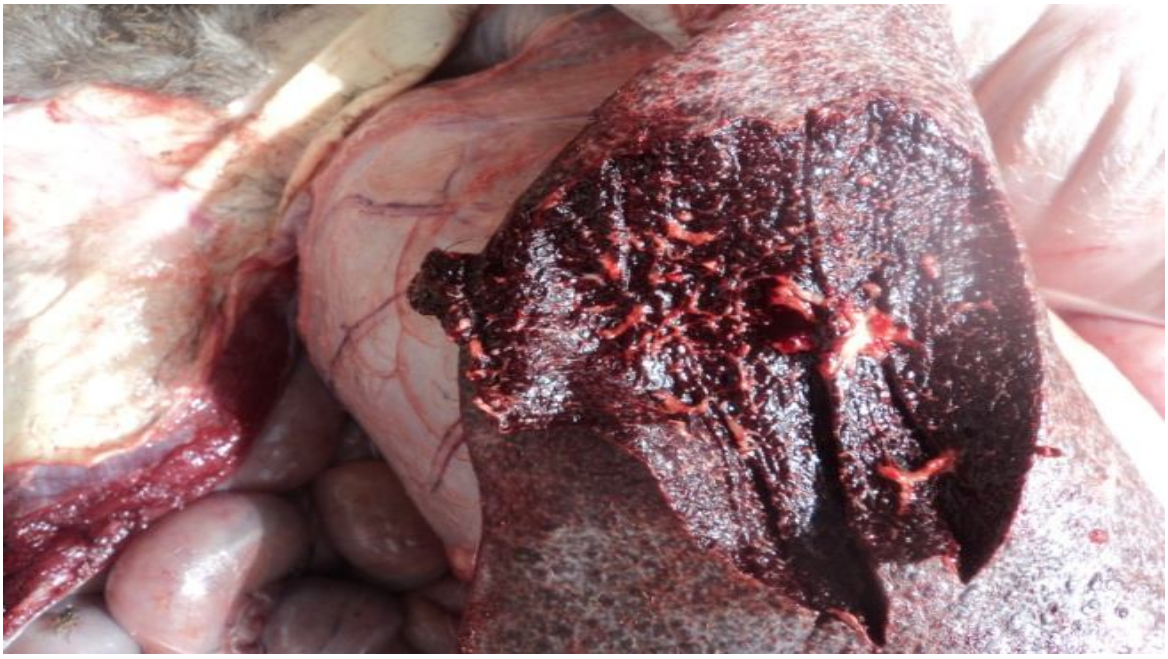

Figure 3.3:Thick trabeculations in the spleen of donkey treated with repadeid doses ivermectin

3.1.3 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 (Figure 3.4) were observed in ivermectin treated donkeys (T2).

Kidneys showed hypercellular glomeruli (Figure 3.5); glomeruli appear hypercellular tufi, pinkish deposits seen Bowman space (Figure 3.6).

The lungs showedcollapse and emphysema (Figure 3.7, 3.8); severe congestion, alveolar odema and thickening of interstitium at certain area with dense cellularity (Figure 3.9). The pulmonary arteries showed mural thickening with large numbers of neutrophils seen inside the blood vessels (3.10). Large neutrophil were seen in blood vessels in donkey treated with ivermectin (Figure 3.11)

Spleen showedcongestion of the red pulp ,with dense dark brown deposits (haemosiderin) (Figure 3.12)

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.

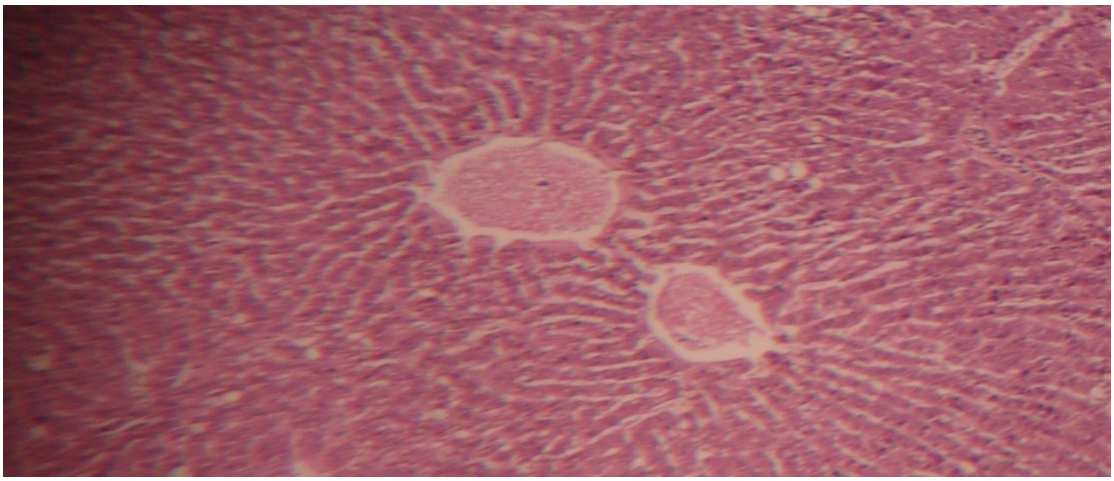

Figure 3.4: Liver tissue:fine and large hepatocyte cytoplasmic vaculation. Congection in centeral vein and siunsodial dilatation in animal treated with ivermectin at five times the recommended dose for seven successive days (H&E 40)

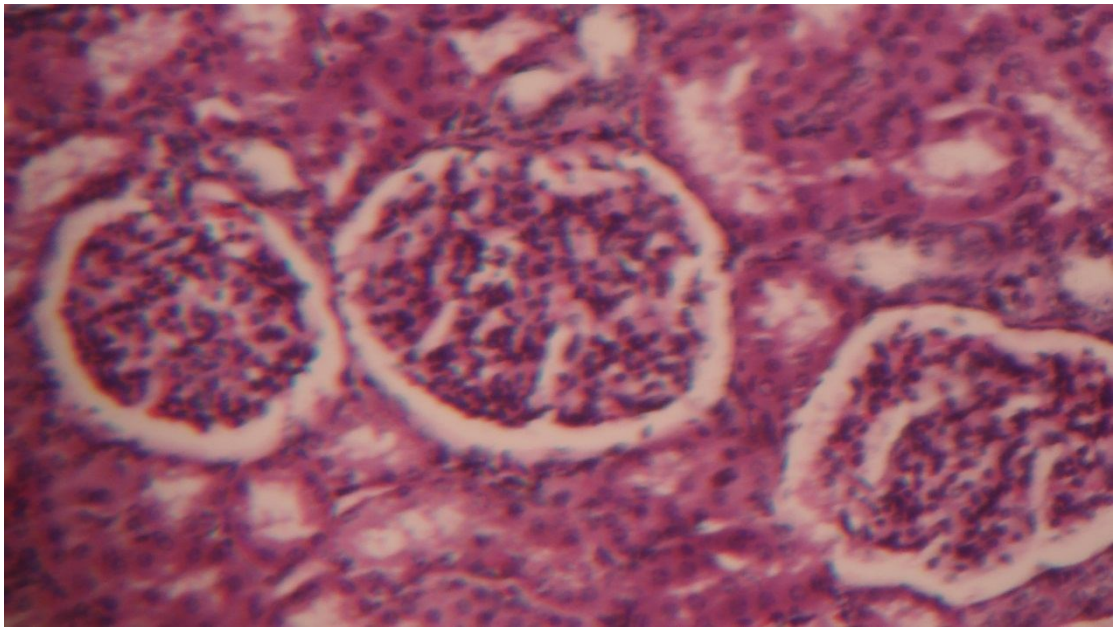

Figure 3:5 Kidney tissue: hypercellular glomeruli in animal treated with ivermectin at five times the recommended dose for seven successive days (H&E 100)

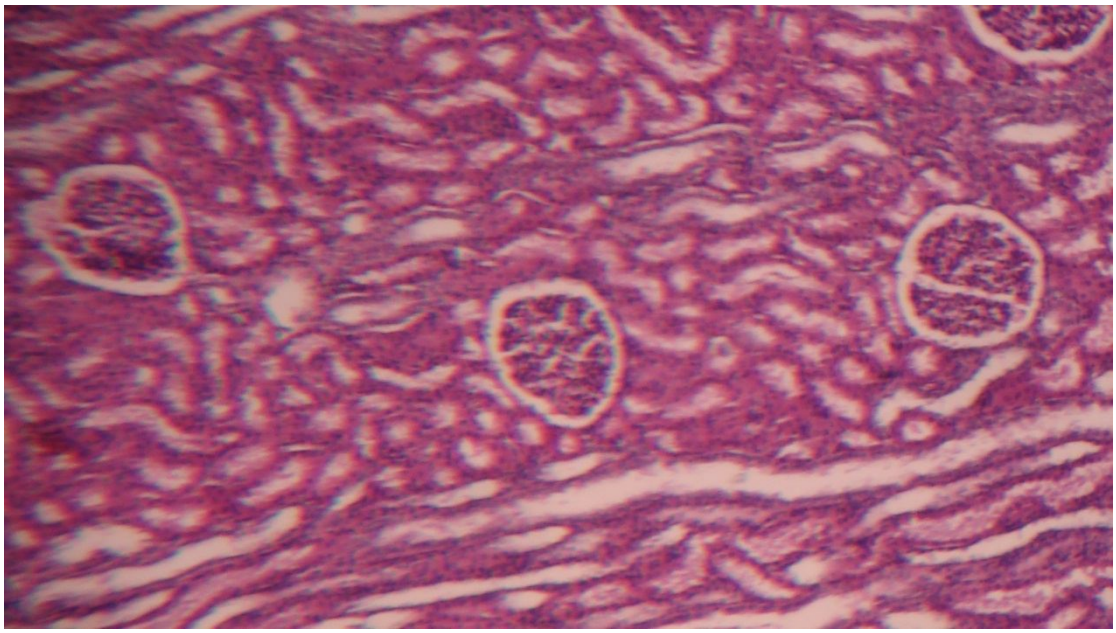

Figure 3.6: Kidney tissue: glomeruli appear hyper cellular tufi, pinkish deposits seen in Bowman space in animal treated with ivermectin at five times the recommended dose for seven successive days (H&E 400).

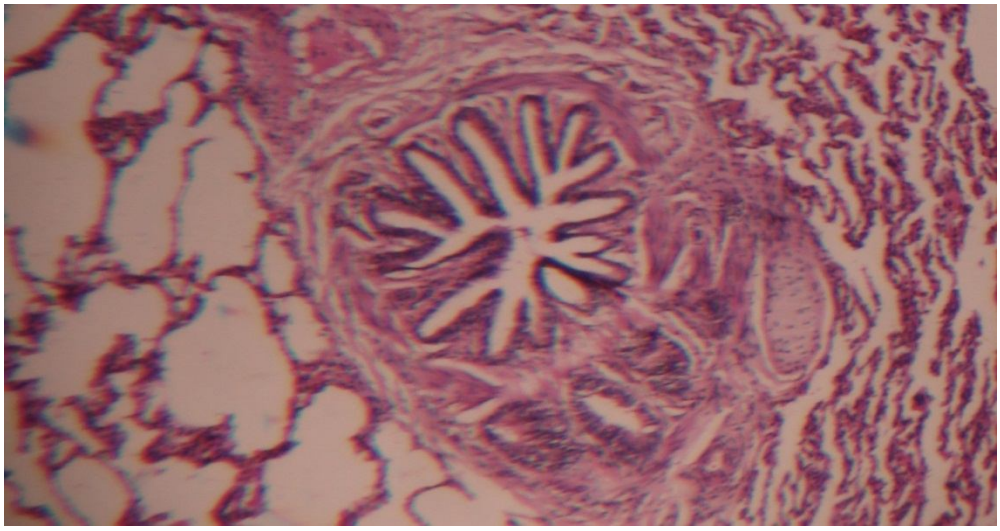

Figure 3.7: Lung tissue: collapse and emphysema around a bronchus in animal treated with ivermectin at five times the recommended dose for seven successive days (H&E 400)

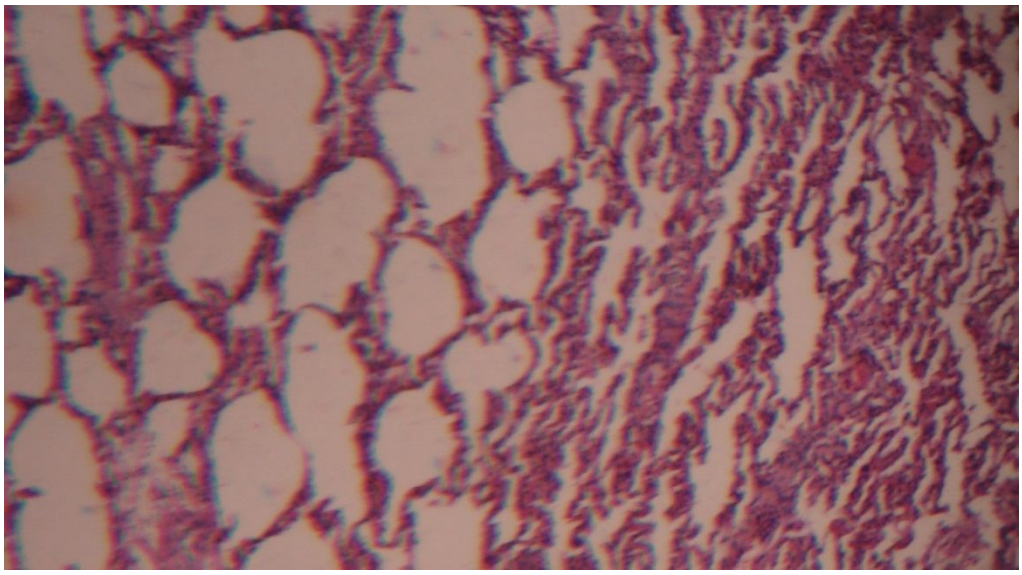

Figure 3.8: Lung tissue:alveoler Collapes and emphysema with in animal treated with ivermectin at five times the recommended dose for seven successive days (H&E 100)

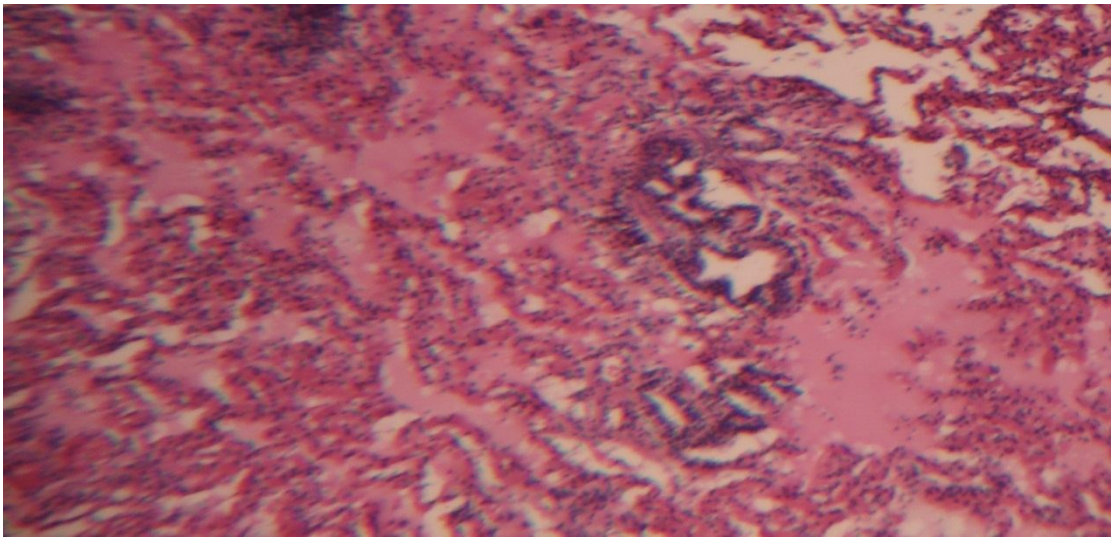

Figure 3.9: lung tissue: sever congestion and alveolar odema, thickening of interstitium at certain area with dense cellularity in animal treated with ivermectin at five times the recommended dose for seven successive days (H&E 40)

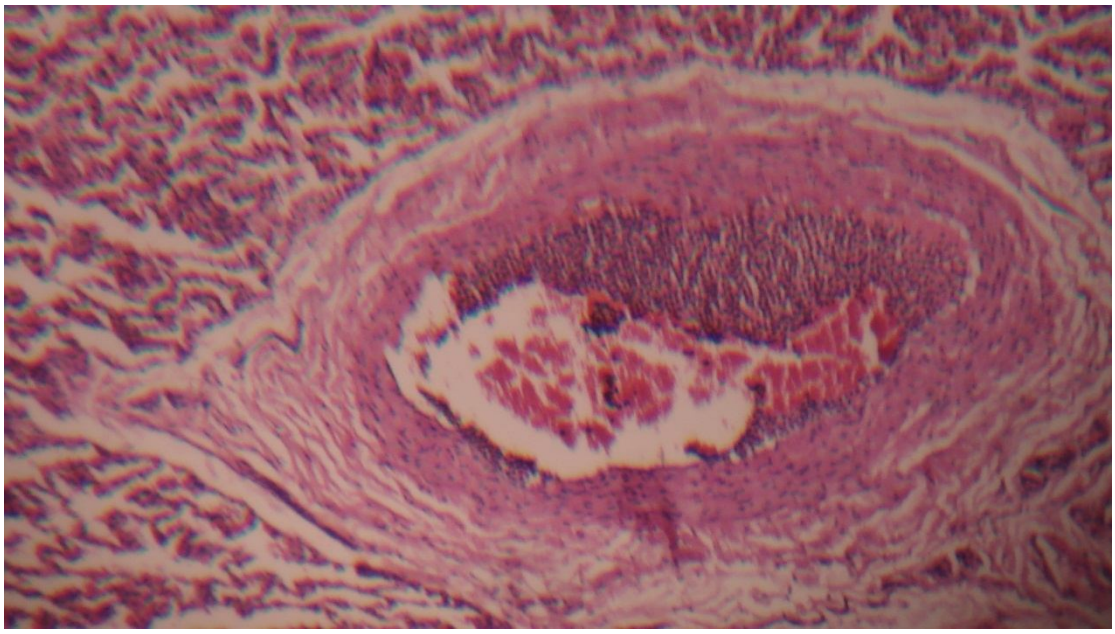

Figure 3.10: Pulmonary artery tissue: mural thickening and large neutrophils were seen inblood vessels in animal treated with ivermectin at five times the recommended dose for seven successive days (H&E100)

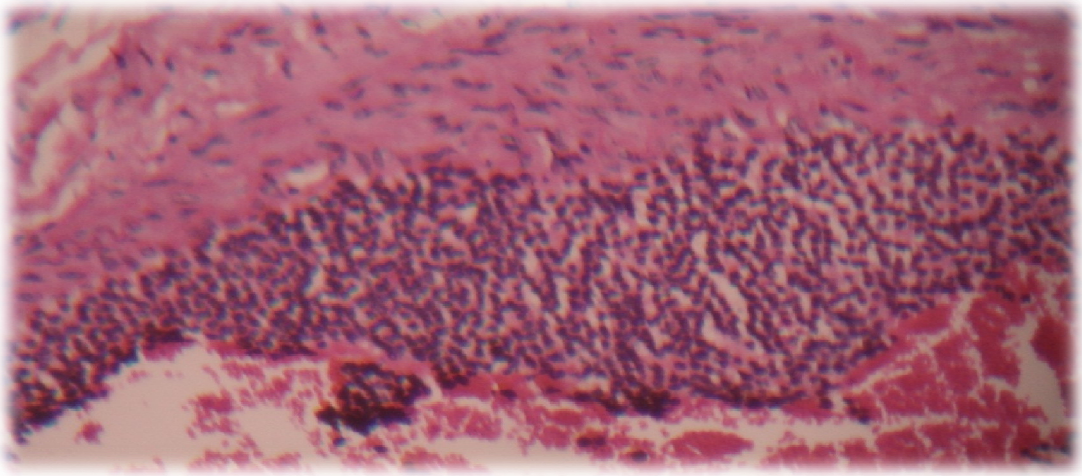

Figure 3.11: lung tissue: large neutrophil wereseen inblood vessels in animal treated with ivermectin at five times the recommended dose for seven successive days (H&E 100).

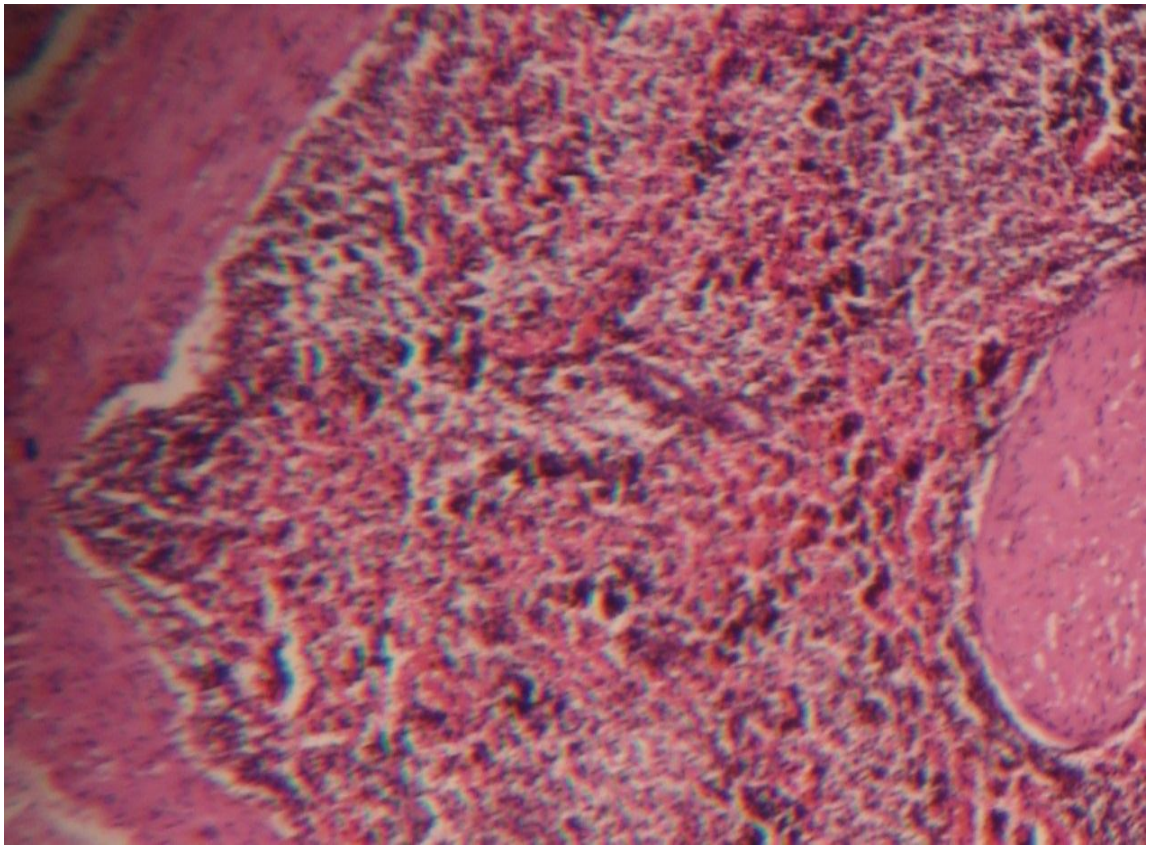

Figure 3.12: Spleen tissue: congection of red pulp ,with dense dark brown deposits (haemosiderin) in animal treated with ivermectin at five times the recommended dose for seven successive days (H&E 400)

3.1.4 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 (Table 3.1).Significant increase ($P< 0.05$) in PCV was also observed in the first group at day 3, andincreased with no significant difference throughout the experiment period in the second group (Table 3.2).

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 experimentin 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 3.3).

Significant increase ($P <0.05$) in total leukocytescount by the day 7 and 10 in the first group, and in the second group there was no significant increase throughout the experiment (Table 3.4).

Table 3.1: Haemoglbin concentration (g/dl) in donkeys treated with ivermectin injection at different dose levels for seven successive days

Days	T1	P value	T2	P value
Baseline	12.9±1.50		15.3±0.50	
1	17.0±2.09*	0.008	15.5±1.60	
2	16.1±2.8*	0.032	16.5±1.14*	0.042
3	16.5±2.27*	0.027	16.2±1.09	
4	17.2±1.85*	0.007	15.8±.82	
5	17.7±3.06*	0.01	15.7±0.95	
6	15.8 ±1.05*	0.019	15.8±0.79	
7	16.7±3.4*	0.049	16.8±0.50*	0.001
10	15.1±1.5		15.3±0.44*	0.005
15	16.4±1.03*	0.021	16.4±0.64	

Values were expressed as Mean ±standard deviation

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

Table3.2: Packed cell volume (%) in donkeys treated with ivermectin injection at different dose levels for seven successive days

Days	T1	P value	T2	P value
Base line	26.9±5.05		26.8±1.53	
1	29.3±4.13		28.8±4.83	
2	27.3±2.33		27.6±1.36	
3	30.1±5.40*	0.021	27.5±2.07	
4	28.3±6.08		27.6±1.63	
5	25.6±7.33		27.6±2.25	
6	28.6±5.92		30.5±4.80	
7	32.0±7.32		29.6±3.13	
10	28.8±2.94		27.0±4.0	
15	27.8±3.11		26.0±1.41	

Values were expressed as Mean ±standard deviation

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

Table 3.3: Total erythrocytes (x10^6 cell/µl) count in donkeys treated with ivermectin injection at different dose levels for seven successive days

Days	T1	P value	T2	P value
Baseline	5.6±0.39		4.6±0.22	
1	5.06±0.91		4.6±0.32	
2	5.03±1.00		4.6±0.17	
3	4.8±0.71*	0.019	4.7±0.23	
4	4.6±0.57*	0.004	4.6±0.15	
5	6.1±0.61*	0.009	4.3±0.23*	0.024
6	4.9±0.73*	0.038	4.3±0.23*	0.041
7	5.08±0.38*	0.038	4.3±0.28	
10	4.5±0.30*	0.003	4.3±0.36	
15	4.6±0.41*	0.009	4.2±0.17	

Values were expressed as Mean ±standard deviation

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

Table 3.4: Total leucocytes (x10^3 cell/µl) count in donkeys treated with ivermectin injection at different dose levels for seven successive days

Days	T1	P value	T2	P value
Baseline	2.10±0.52		2.17±0.60	
1	2.0±0.40		2.48±0.39	
2	2.1±0.19		2.46±0.39	
3	2.1±0.19		2.70±0.55	
4	2.0±0.21		2.59±0.57	
5	3.0±0.13		2.53±0.49	
6	2.1±0.20		2.69±0.50	
7	2.4±0.37 *	0.01	2.86±0.70	
10	2.7±0.24*	0.019	3.00±0.73	
15	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

3.1.5 Blood biochemical parameters

In the first group (T1), there was significant increase ($P < 0.05$) in albumin concentration in the secondand the third day following treatment while in the second group(T2) there was significant ($P<0.05$) increasein the 3^{rd} up to the 5^{th} day in donkeys treated with ivermectin (Table 3.5)

In the first group (T1) a gradual non-significant increase in ALT activity was observed 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 as in day 10 and up to end of experiment (Table 3.6).

No significant ($P > 0.05$) change was observed in the first group in Aspartate aminotransferase, but in the second group significant ($P<0.05$) decrease was observed by the 2^{nd}day and up to end of experiment (table 3.7).

Significant ($P< 0.05$) increase in in urea concentration in the 5^{th} day in the first group (T1), and in the second group decreased by the day 4, 5 and 7 in donkey treated with ivermectinin the second group (T2) (Table 3.8).

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 3.9).

Significant decrease in (P value 0.05) incholesterol 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 3.10).

Significant ($P<0.05$) decrease in the level of phosphorus at the end of the experiment in day 15 in both treatment groups (table 3.11).

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 3.12).

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 3.13).

Table 3.5: Albumin (g/l) concentration in donkeys treated with ivermectin injection at different dose levels for seven successive days

Days	T1	P value	T2	P value
baseline	28.37± 5.15		39.56 ± 4.6	
1	32.89 ± 5.737		40.79 ± 9.39	
2	36.28 ± 3.80*	0.022	45.54 ± 8.9	
3	37.30 ± 3.56*	0.008	49.68 ± 4.13*	0.001
4	32.30± 3.25		56.48 ± 15.87	
5	27.40 ± 4.36		49.08 ± 10.76*	0.027
6	27.04 ± 4.63		44.49 ± 6.57	
7	28.69 ± 4.63		38.62 ± 4.33	
10	32.443 ± 3.19		37.66 ± 3.41	
15	28.315 ±0 .90		37.49 ± 2.41	

Values were expressed as Mean ±standard deviation

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

Table 3.6: Alanine aminotransferase (U/l) activity in donkeys treated with ivermectin injection at different dose levels for seven successive days

Days	T1	P value	T2	P value
Baseline	33.80±18.16		4.96±5.69	
1	21.81±15.84		4.20±3.44	
2	17.30±10.74		15.42±23.80	
3	14.31±9.89		2.74±1.25	
4	20.60±14.48		18.36±24.41	
5	6.14±4.53*	0.019	14.61±12.5	
6	7.03±1.98*	0.015	43.80±21.74*	0.011
7	9.25±6.36		21.71±13.79	
10	4.30±1.27		19.80±2.05 *	0.001
15	10.45±7.42		28.78±19.89	

Values were expressed as Mean ±standard deviation

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

Table 3.7: Aspartate aminotransferase (U/I) activity in donkeys treated with ivermectin injection at different dose levels for seven successive days

Days	T1	P value	T2	P value
Baseline	34.23±7.81		26.17±9.91	
1	39.23±23.38		1.08±0.39	
2	33.98±27.85		1.08±0.39*	0.005
3	44.20±28.80		1.16±0.09*	0.005
4	38.34±21.94		1.24±0.39*	0.005
5	41.27±11.83		1.04±0.34*	0.005
6	35.38±15.85		1.20±0.42*	0.005
7	33.34±11.71		1.18±.37*	0.005
10	40.70±37.74		1.62±0.17*	0.017
15	49.15±25.44		1.15±0.35	0.096

Values were expressed as Mean ±standard deviation

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

Table 3.8: Urea (mmol/l) concentration in donkeys treated with ivermectin injection at different dose levels for seven successive days

Days	T1	P value	T2	P value
Baseline	9.35± 1.72		13.81 ± 4.03	
1	10.76 ± 1.56		15 .78 ± 4.83	
2	10.73 ± 1.80		12.21 ± 2.96	
3	10.71 ± 2.44		9.58 ± 1.96	
4	11.86 ± 2.72		9.15 ± 1.57 *	0.023
5	12.43 ± 2.06 *	0.045	8.25 ± 2.17*	0.007
6	12.03 ± 3.60		11.51 ± 9.79	
7	8.86 ± 3.88		8.16 ± 2.98*	0.027
10	8.04 ± 3.39		11.70 ± 1.21	
15	8.06 ± 2.81		10.10 ± 2.83	

Values were expressed as Mean ±standard deviation

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

Table 3.9: Triglycerides (mmol/l) concentration in donkeys treated with ivermectin injection at different dose levels for seven successive days

Days	T1	P value	T2	P value
Baseline	1.17 ±0.31		0.96 ±0.42	
1	0.97 ±0.49		1.10 ±0.40	
2	0.78 ±0.29*	0.003	1.15 ±0.08	
3	0.98 ±0.48		1.23±0.35	
4	0.83 ±0.24		1.00 ±0.32	
5	1.03 ±0.51		1.15±0.40	
6	0.75 ±0.26*	0.023	1.08±0.41	
7	0.70 ±0.23*	0.014	1.50±0.32	
10	1.22 ±0.41		0.93±0.45	
15	1.30 ±0.50		1.25±0.49	

Values were expressed as Mean ±standard deviation

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

Table 3.10: Cholesterol (mmol/l) concentration in donkeys treated with ivermectin injection at different dose levels for seven successive days

Days	T1	P value	T2	P value
Baseline	1.87 ±0.40		2.09 ±0.34	
1	2.05 ±0.80		2.10 ± 0.83	
2	1.88 ±0.74		1.96 ±0.84	
3	1.85 ± 0.56		1.80 ± 0.52	
4	1.71 ± 0.82		1.53 ± 0.57	
5	1.30 ± 0.31*	0.028	1.55 ±0.47*	0.048
6	1.75 ± 0.74		1.36 ±0.43*	0.037
7	1.75 ± 0.66		1.86 ±0.57	
10	1.56 ±0.68		2.30 ± 1.49	
15	1.66 ± 0.68		1.20 ±0.28	

Values were expressed as Mean ±standard deviation

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

Table 3.11: Phosphorous (mmol/l) level in donkeys treated with ivermectin injection at different dose levels for seven successive days

Days	T1	P value	T2	P value
Baseline	1.52 ± 0.12		1.20 ±0.36	
1	1.45 ± 0.49		1.00 ±0.18	
2	1.40 ± 0.37		1.13 ±0.19	
3	1.71 ± 0.39		1.15 ±0 .43	
4	1.36 ±0.70		0.92 ±0.17	
5	1.41 ±0.60		1.65 ±0.98	
6	1.48 ±0.46		1.56 ±0.84	
7	1.36 ±0.43		2.04 ±0.99	
10	1.28 ± 0.46		2.13 ±1.88	
15	1.02 ±0.29*	0.027	0 .75 ±0.49*	0.024

Values were expressed as Mean ±standard deviation

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

Table 3.12: Sodium (mEq/l) level in donkeys treated with ivermectin injection at different dose levels for seven successive days

Days	T1	P value	T2	P value
Baseline	138.1±7.4		141.5±6.10	
1	129.1±19.6		161.1±725*	0.004
2	141.7±7.2		154.6±13.56	
3	138.1±15.7		150.3±11.05	
4	163.2±20.3		158.8±5.06*	0.003
5	151.1±10.1		150.0±1.74*	0.017
6	161.6±6.47*	0.003	146.7±3.92	
7	158.5±5.06*	0.003	152.8±7.14*	0.03
10	158.6±6.35*	0.004	133.5±7.44	0.071
15	156.9±9.56*	0.04	139.9±12.2	

Values were expressed as Mean ±standard deviation

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

Table 3.13: Potassium (mEq/l) level in donkeys treated with ivermectin injection at different dose level for seven successive days

Days	T1	P value	T2	P value
Baseline	3.9±0.47		3.8±0.52	
1	3.4±0.74		3.4±0.90	
2	3.3±0.93		3.2±0.61	
3	4.0±0.95		3.3±0.52*	0.000
4	4.8±0.68*	0.005	3.5±2.20	
5	2.5±0.32*	0.002	2.5±0.63*	0.015
6	2.0±0.20*	0.000	3.7±0.36	
7	9.5±3.67*	0.018	11.8±1.09*	0.000
10	9.9±1.38*	0.002	9.6±2.56*	0.048
15	5.1±2.24		6.7±.2.47	

Values were expressed as Mean ±standard deviation

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

3.2 Experiment II

3.2.1 Side and or adverse effects

In the second experiment where animals were treated once with 10 times the recommended dose the animals tend to be ataxic and start to circle and within 48 hours of treatment signs of intoxication started to appear viz: inability to move, salivation, and food rejection. Animal no. 16 died 72 hours following treatment, during such period the animal rejected feed. Animal no. 15 died 13 days following treatment during such period the animal tends to be ataxic, with tremors in all muscles.

3.2.2 Haematological changes

Non-significant increase in Hb concentration started immediately following fasting and treatment and continued up to the end of the experiment (Table 3.14).

Significant increase in PCV value observed only following fasting. While slight non-significant increase in PCV was observed following treatment at the 4^{th} day up to the end of the experiment (Table 3.14).

Table 3.14: Effect of ivermectin Hb (g/dl) and PCV (%) in the blood of donkeys treated with ivermectin injection at 10 times the recommended dose compared to a baseline value

Day	Haemoglobin (g/dl)		PCV (%)	
	Mean ±SD	P value	Mean ±SD	P value
Baseline	8.16±1.86		23.50±1.51	
Fasting	9.27±2.58	0.3	28.83±2.14*	0.001
1	10.02±3.20	0.14	21.67±5.47	0.24
4	10.18±3.94	0.11	23.60±8.14	0.94
5	9.90±4.42	0.32	25.40±5.32	0.48
7	9.25±3.41	0.11	25.00±5.70	0.59
9	9.23±3.82	0.11	23.75±8.10	0.94

3.2.3 Biochemical changes

No significant ($P>0.05$) increase in total proteins concentration was observed following fasting and up to the 5^{th} day following treatment and non-significant decrease was observed at the 7^{th} day of treatment (Table 3.15). The same picture of total proteins was observed in albumin concentration with no significant increase (4^{th} and 5^{th} days) and no significant decrease (7^{th} day) following treatment (Table 3.15).

Significant ($P<0.05$) decrease in ALT activity was observed in the first day following treatment and then the activity increased significantly ($P<0.05$) at the 4^{th} and 5^{th} day following treatment and another significant decrease was observed at the 9^{th} day following treatment (Table 3.16).

The Increase in AST concentration started following treatment to reach significance level at the 4^{th} day of treatment, and continued to be higher up to the end of the experiment (Table 3.16).

While only significant ($P<0.05$) increase in urea concentration was observed during the fasting period, no significant ($P>0.05$) increase in urea concentration was observed in treated animals during the whole period of the experiment (Table 3.17).

No significant change in triglycerides and cholesterol concentration was observed following treatment up to the end of the experiment (Table 3.18).

No significant fluctuation in phosphorus concentration was observed during the course of the experiment (Table 3.19).

No significant change was observed in sodium and potassium concentration following treatment, while slight non-significant decrease was the only change during the course of the experiment (Table 3.20).

Table 3.15: Total protein (g/l) and albumin (g/l) concentration in donkeys treated with ivermectin injection at 10 times the recommended dose

Days	Total protein		Albumin	
	Mean ±SD	P value	Mean ±SD	P value
Baseline	53.64±10.9		27.79±3.93	
Fasting	60.82±13.4	0.04	30.29±3.69	0.59
1	53.74±13.9	0.95	26.18±7.02	0.28
4	58.59±8.4	0.08	28.67±8.21	0.85
5	57.62±10.5	0.06	32.67±10.84	0.51
7	47.00±12.1	0.35	26.91±8.35	0.60
9	54.88±11.1	0.40	29.56±8.60	0.87

Table 3.16: ALT and AST (IU) activity in donkeys treated with ivermectin injection at 10 times the recommended dose

Days	ALT		AST	
	Mean ±SD	P value	Mean ±SD	P value
Baseline	12.50±2.97		21.28±9.41	
Fasting	10.87±1.86	0.72	27.38±12.50	0.32
1	8.43±1.45*	0.02	31.10±13.4	0.13
4	30.90±11.86*	0.01	62.34±11.2*	0.0008
5	28.60±10.22*	0.01	31.16±23.2	0.30
7	10.60±9.41	0.87	35.05±15.9	0.16
9	6.25±1.75*	0.04	50.80±15.9	0.08

*Means with asterisk in the same column are significantly different with baseline value

Table 3.17: Urea (mmol/l) concentration in donkeys treated with ivermectin injection at 10 times the recommended dose

Days	Urea	
	Mean ±SD	P value
Baseline	8.84±3.05	
Fasting	13.38±4.76*	0.04
1	12.23±3.88	0.22
4	11.66±3.07	0.10
5	10.68±2.84	0.11
7	10.44±2.10	0.14
9	11.45±2.95	0.20

*Means with asterisk in the same column are significantly different with baseline value

Table 3.18: Triglycerides and cholesterol (mmol/l) concentration in donkeys treated with ivermectin injection at 10 times the recommended dose

Days	Triglycerides		Cholesterol	
	Mean ±SD	P value	Mean ±SD	P value
Baseline	1.24±0.42		1.83±0.56	
Fasting	1.25±0.76	0.72	2.10±0.89	0.59
1	1.30±0.94	0.64	1.83±0.51	0.91
4	2.16±1.62	0.22	2.18±0.54	0.44
5	2.10±1.07	0.14	2.64±1.31	0.23
7	2.28±0.99	0.09	1.86±1.06	1.00
9	1.78±0.54	0.31	2.05±0.73	0.86

Table 3.19: Phosphorus (mmol/l) level in donkeys treated with ivermectin injection at 10 times the recommended dose

Days	Phosphorus	
	Mean ±SD	P value
Baseline	1.24±0.32	
Fasting	2.13±0.46*	0.02
1	1.93±0.84	0.12
4	1.46±0.50	0.31
5	1.12±0.51	0.79
7	1.48±0.97	0.43
9	0.90±0.14	0.31

*Means with asterisk in the same column are significantly different with baseline value

Table 3.20: Sodium and potassium (meq) level in donkeys treated with ivermectin injection at 10 times the recommended dose

Days	Sodium		Potassium	
	Mean ±SD	P value	Mean ±SD	P value
Baseline	155.8±9.25		4.18±0.74	
Fasting	166.0±0.61	0.12	4.55±1.40	0.61
1	110.0±42.0	0.09	6.00±0.30	0.13
4	146.9±22.4	0.40	4.46±1.08	0.84
5	135.9±31.8	0.30	4.56±1.39	0.76
7	139.1±32.0	0.24	4.48±1.20	0.83
9	149.9±14.**7**	0.52	4.23±1.44	0.85

Chapter four

Discussion

The current study was conducted to evaluate some pharmacotoxic effects of ivermectin injection formulation at different dose levels in donkeys, It is also aimed to study the combined effect of induced stress and simultaneous administration of ivermectin as injection formulation on some haematological and biochemical parameters of donkeys blood.

4.1 Experiment I

In this study two groups of male donkeys each of six animals were first subjected to fasting for 48 hours and then either treated with ivermectin injection formulation at the recommended dose (T1) or with five times the recommended dose (T2) for seven successive days. Different pharmacotoxic effects of ivermectin were evaluated.

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 (*Geochelonecarbonaira*) 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 a result that 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 hepatocytes, 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 group 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, 2209). 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 werereduced water intake and haemoconcentration was the normal consequence. A significant increase in Hb concentration was observed in camels treated with ivermectin (Ibrahim *etal*., 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), Thes results in the same line with observations of Eissa and Zidan (2009). The obtained results are in accordance with those found by (Ali, 1990; 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.

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 same water drinking and haemconcentration.

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 are 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 LD50, 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 (Choudhary *et al*., 2003; Shrivastava *et al*., 1989). 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 & ALT) suffer markedly from their loss under many pathological conditions (Rodwell, 1983).

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 is 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.

4.2 Experiment II

In the second experiment, animals were subjected to fasting for 48 hours and then treated with a single subcutaneous dose of ivermectin equal to 10 times the recommended dose.

Non-significant increase in Hb concentration started immediately following fasting and treatment and continued up to the end of the experiment.Significant increase in PCV value observed only following fasting. While slight non-significant increase in PCV was observed following treatment at the 4^{th} day up to the end of the experiment, this result may attributed to haem-concentration following fasting and partially agrees with the results of Shaddad, (1997) who observed similar result in ewes.

Liver is often the primary target for the toxic effects of xenobiotics. It is known that the detoxification of the toxic materials which enter the body occurs mainly in the liver (Balistreri and Shaw, 1987). Therefore, liver can be used as an index for the toxicity of xenobiotics. Hence, the activities of some enzymes and levels of certain biochemical parameters representing liver function, i.e. AST, ALT, albumin, triglycerides and cholesterol were determined in treated donkeys.

Elevation of AST, a cytosolic enzyme of the hepatocytes, reflects the increase of plasma membrane permeability resulting from the damage of hepatocytes (Plaa and Hewitt, 1982) and is used to detect liver damage

(Klaassen and Eaton, 1991). The alteration in serum levels of alanine aminotransferase (ALT) may be indicative of internal organs damage especially in liver (Kaneko *et al.*, 1997).

Data obtained in the current study illustrated that ivermectin elevated the activity of AST and decreased ALT activity, in plasma of treated donkeys in a dose-dependent manner, whereas total protein, albumin, triglycerides and cholesterol concentration remained unaltered.

The Increase in AST concentration started following treatment to reach significance level at the 4^{th} day of treatment, and continued to be higher up to the end of the experiment. The decrease in ALT activity is in the same line with the observations of Eissa and Zidan, (2009).

Urea is useful in early deduction of nephrotoxicity induced by exogenous compounds. This parameter is used as index of renal damage in living organisms (Coles, 1986). Elevation of urea concentration in plasma of treated male donkeys may be attributed to reduction in glomerular filtration in the kidney and also reflect dysfunction of the kidney tubules (Hayes, 1989; Walmsley and White, 1994).

No significant increase in urea concentration was observed in treated animals during the whole period of the experiment.This result was supported with the work of Seri *et al.*, (2006) in donkeys, Mohammed and Samia, (1994) in desert sheep and Shaddad, (1997) in ewes received three times recommended dose of ivermectin.

The increase of urea concentration is a demonstration of impaired kidney function since the organ primarily excretes urea in the urine. The increase in urea due to ivermectinadministration was also correlated closely with the histopathological changes in the kidney. Marked haemorrhage, congestion, and other degenerative changes were seen in the kidneys.

No significant fluctuation in phosphorus, sodium and potassium level was observed during the course of the experiment.

It has already been noted that, whereas application of AVM markedly affects muscle input resistance, the drug produces only small changes in resting potential. This indicates that the drug acts to increase the permeability to ions whose equilibrium potentials are close to the resting potential. Fritz *et al.*, (1979) suggested that AVM indeed increases the permeability to Cl- and not K+. The data, however, cannot totally eliminate the possibility that a small increase in K+ conductance may also occur.Here the non-significant change in phosphorus, sodium and potassium is in the same line with this hypothesis.

Chapter Five

Conclusion and recommendations

5.1 Conclusion

The results of this study demonstrate that sub-acute administration of ivermectin at five times the recommended dose for seven successive days induced toxic effects on biochemical functions which correlate well with the histopathological changes in the lungs, liver, kidneys, spleen and heartof donkeys. Although the data on donkeys cannot be directly applied to horses, it may be concluded that use of ivermectinat the above mentioned regimen may cause hazardous effects at various levels to equine species.

5.2 Recommendations

Following results obtained in the current study we recommend the following:

1. The use of repeated doses of ivermectin injection formulation could be of risk in donkeys.
2. Doses equal to or higher than five times the recommended dose of ivermectin is toxic in donkeys.
3. Further studies with donkeys infested with helminths parasites are required.

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