Toxicological and pathological studies of Ivermectin on male albino rats

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Abstract: Ivermectin (IVM), natural fermentation product derived from the soil bacterium *Streptomyces avermitilis*, is a broad spectrum anthelmintic, insecticide and acaricide. The present study was designed to detect the effect of therapeutic and double therapeutic dose of ivermectin on liver and kidney function parameters, sperm count and abnormalities as well as, histopathological alterations on liver, kidney and tests. Thirty male white albino rats were equally divided into three groups, group (A): control, group (b): have therapeutic dose of ivermectin (0.2 mg/kg BW/SC), group (C): have double therapeutic dose of ivermectin (0.4 mg/kg BW/SC). The rats were injected once weekly for eight weeks. In both treated groups a significant increase in aspartate aminotransferase (AST), alanine aminotransferase (ALT), urea, uric acid and creatinine were recorded. Albumin and total protein were significantly decreased than that of the control group. Moreover, significant decrease in total sperm count with significant increase in sperm abnormality was also demonstrated. Various pathological changes in liver, kidney and tests were also detected. The severity of these changes varied from mild to severe changes according to the dose as histopathological changes were more severe in male rats injected with double therapeutic dose than that injected with the therapeutic one. Following to the present results, the administration of either therapeutic or double therapeutic dose of ivermectin produced alterations in some biochemical parameters which correlated with histopathological changes as well as, it has deleterious effect on male fertility. Consequently, it could be concluded that, it is not preferable to use ivermectin particularly at double therapeutic dose mostly to breading males.
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1. Introduction:

Ivermectin, (22, 23-dihydroavermectin B) a semisynthetic macrocyclic lactone, is a macrolide antibiotic produced from *streptomyces avermitilis* firstly isolated from soil sample in Japan. Ivermectin is acricide belong to the avermactine family **(Daurio, *et al.*, 1987 and Chavasse *et al.*, 1992)**. Avermectin are a class of chemicals that have a novel mode of action against all gastrointestinal and lung nematodes, arthropod and certain ectoparasites of various animal species **(Roberson, 1988 and Roder, 1998)**. Although, the antiparasitic activity of ivermectin is well known, there is lack in literatures of its effect on the host. The veterinarian used ivermectin as routine treatment against nematodes while in human; it is considered the first drug of choice for treatment of *onchocerciasis ascariasis* and *enterobiasis* **(Gonzalez, *et al.*, 2012)**. Ivermectin can diffuse to all tissue compartments except the central nervous system after being taken orally or in other ways **(Lankas, *et al.*, 1989)**. **Hopkins, *et al.* (1990)** reported that there is negligible penetration of ivermectin through blood brain barrier in some species.

The undesirable efficacy of ivermectin depends on the dose and duration of administration **(Prichard, 1985)**. Ivermectin overdose may be produced variable side effects ranging from mild to extremely severe **(Epstein and Hollingsworth, 2013)**. The most dominant clinical symptoms of ivermectin poisoning in domestic and wild animals are CNS depression and sometimes coma frequently resulting in death **(Trailovic and Nedejkovic, 2011)**. In addition to, the neural effects of ivermectin, it has marked effect on some liver function parameters at either therapeutic or toxic dose of ivermectin to albino rats **(Ashang, 2009)**. Degenerative changes in brain and kidney as well as in liver were established in rainbow trout organs that indicate a direct toxicity of abamectin **(Jencic, *et al.*, 2006)**. Although permanent liver damage is not revealed immediately, intoxication of abamectin may affect the function of hepatocytes **(Hsu, *et al.* 2001)**. Despite there is several researches on the toxic effects of ivermectin, there is a lack of studied on repeated subcutaneous injection of ivermectin in therapeutic and double therapeutic dose.

Chemotherapy of parasitic infestations with a wide spectrum of activity and minimal side effects are the main objects. However, the commonly used anthelmintics cause many side effects on different organs of treated animals (**Rossef, 1974**). Hence, the present investigation was undertaken to assess the changes in histological, biochemical parameters of

liver and kidneys as well as fertility of male albino rats that had been given therapeutic and double therapeutic doses of ivermectin once weekly for eight weeks.

2. Material and methods:

Experimental animals:

Thirty mature albino rats weighting from 150-200 g were maintained under standard laboratory conditions at an ambient temperature of 25±2°C with 55-64% relative humidity and 12h light dark cycle. They were allowed free access to a standard pellet diet and water *ad libitum*. Rats were kept under the same hygienic and environmental conditions during the entire experiment period

Experiment design:

The study was conducted in Faculty of Veterinary Medicine, Benha University. Thirty male albino rats were randomly divided into three equal groups of ten per each. The first group was kept as a control without any treatment. The second group was injected subcutaneously with therapeutic dose of ivermectin (TD, 0.2 mg /kg BW), while the third group injected subcutaneously with double therapeutic dose of ivermectin (DTD, 0.4 mg/kg BW) **(Naoman, 2012**). The animals were injected once weekly for eight weeks.

Samples:

Blood samples:

Blood samples were collected from the Middle canthus of the eye from all groups at 24h, and 7-days post last injection. Blood samples were centrifuged at 3000 r.p.m. for 10 minutes to separate a clear serum. Collected serum samples were subjected to biochemical analysis of liver function parameters (AST, ALT, Total protein, Total globulins, and Albumin) and kidney function parameters (urea, uric acid, creatinine). All biochemical parameters were analyzed by commercially available kits method. Each parameter was done following manufacturer's instructions. Total protein was determined by colorimetric method according to Weichselbaum **(1946))**. Albumin was determined by colorimetric method according to **Doumas (1971)**. Total globulins were calculated as follow: Globulins=Total protein-Albumin (**Al-Joofy., et al 2005)**. Serum globulin was calculated using mathematic method as the concentration of total serum protein minus serum albumin gives the concentrations of serum globulin **Luxton, (1999)**. Serum level of AST, ALT and alkaline phosphates were determined calorimetrically according to **Reitman and Frankel (1957)**. Creatinine was determined according to **Henry et al., (1974)**. Urea was determined according to **Patton and Crouch (1977)**.

Epididymal sperm count:

Epididymal spermatozoa were counted by a modified method of **Yokoi, *et al*. (2003)**. The epididymis was minced in 5ml of saline, placed in centrifuge for 10 minutes and incubated at room temperature for 2 minutes. The supernatant fluid was diluted 1:100 with a solution containing 5g $NaHCO_3$, 1ml formalin (35%) and 25mg eosin per 100ml distilled water. About 10µl of the diluted semen was transferred to each counting chamber of the improved Neubaur haemocytometer (Deep 1/10 mm LABART, Munich, Germany) and was allowed to stand for 5 min for counting under a light microscope at x200 magnification.

Assay for sperm abnormalities:

The method modified by **Evans and Maxwell (1987)** was used for determination of the percentage of morphologically abnormal spermatozoa. A total of 300 sperm cells were counted on each slide under light microscope at x 400 magnification.

Histopathological studies

Sections were taken from testes, liver, and kidney immediately after each killing of the rats. These samples were fixed in 10% buffered neutral formalin solution. Then after proper fixation, the samples were dehydrated in ascending grades of ethyl alcohol (50-100%), then cleared in xylol, embedded in paraffin and finely blocking occurred. These samples were sectioned at 5µm in thickness and stained with hematoxylin and eosin (H&E) for microscopical examination **(Bancroft and Stevens, 1990)**. During necropsy examination, autoclaved scissors and disposable forceps were used for sampling.

Statistical Analysis

Data was statistically analyzed by ANOVA with post-hock Duncan multiple comparison test using statistical software program (SPSS for Windows version 20, USA). Differences were considered significant at $P < 0.05$.

3. Results:

Biochemical effect:

The biochemical results obtained in the present study are summarized in table (1). Ivermectin in therapeutic and double therapeutic dose induced significant increase ($P < 0.05$) in AST, ALT, urea, uric acid and creatinine in samples collected at either 24h or 7-days post last injection of ivermectin in comparison to control group. Significant decrease ($P < 0.05$) in total protein, albumin and globulin in comparison to the control group at 24h and 7-days post last drug injection was also demonstrated. However, these parameters were gradually decreased by time but remain significantly increased ($P < 0.05$) than the control group.

Assay for sperm count and abnormalities:

Significant decrease (P <0.05) in sperm count in both treated groups comparing to control group was detected at 24h post last injection. Interestingly, various sperm abnormalities such as head, middle piece and tail abnormalities were observed in both treated groups at both 24h and 7-days post last injection. However, at 24h post last injection the degree of sperm abnormalities was significantly (P <0.05) higher than the control group. Furthermore, the severity of the deleterious effect of ivermectin on either sperm count or abnormalities was gradually decreased at 7-days post the last injection comparing to the control group.

Histopathological examination:

Various histopathological changes were demonstrated in liver, kidney and testes obtained at 24h and 7-days post last injection of either therapeutic (0.2mg/Kg BW) or double therapeutic (0.4/kg BW) doses of ivermectin once weekly for eight weeks.

Rats treated with therapeutic dose of ivermectin:

A. Liver:

The investigated liver obtained at 24h post last injection of ivermectin revealed congestion of hepatic blood vessels and blood sinusoids in association with vacuolated cytoplasm of hepatocytes that characterized by multiple variably sized discrete empty vacuoles that distend the cell cytoplasm (Fig. 1A).

Meanwhile, the commonly hepatic changes in livers obtained at 7-days post last injection of TD of ivermectin (0.2 mg/kg BW) represented mainly in congestion of the central veins and blood sinusoids with activation of Von Kupffer's cells (Fig. 1B). Additionally, mild degenerative changes in the form of vacuolar degeneration in hepatocytes were also demonstrated in some cases.

B. Kidney:

The microscopical examination of kidneys acquired at 24h post last injection of TD of ivermectin revealed congestion of the renal blood vessels and intertubular capillaries. The renal cortex showed hypercellularity of glomerular tufts with proliferation of the lining endothelial cells of glomerular capillaries. Moreover, the Bowman's capsule was completely filled with glomerular tuft with absence of the subcapsular space. Moreover, accumulation of homogenous structureless eosinophilic fluid in Bowman's space was also observed (Fig. 1C). The lining epithelium of the convoluted tubules in the renal cortex showed vacuolar and hydropic degeneration. However, individual epithelial cells were shrunken with pyknosis of the nuclei
Furthermore. mild cystic dilatation of some renal tubules was also noticed.

Concerning the investigated kidneys obtained at 7-days after last injection of TD of ivermectin showed mild congestion of the renal blood vessels and intertubular capillaries. Additionally, the lining epithelium of the convoluted tubules was mostly appeared vacuolated as well as vacuolation of the endothelial lining of the glomerular tuft (Fig. 1D).

C. Testes:

The microscopical examination of testes obtained at 24h post last injection of TD of ivermectin revealed congestion of testicular blood vessels. Furthermore, destruction of the lining epithelium of seminiferous tubules was demonstrated. Accumulation of edematous fluid in the interstitial tissue was also demonstrated (Fig. 1E). The epididymes and ductus deferens has no pathological changes except congestion of blood vessels.

Interestingly, testes of rats got at 7-days post last injection revealed congestion of testicular blood vessels in association with severe degeneration of the lining epithelium of seminiferous tubules (Fig. 1F) as well as less spermatogenesis were also demonstrated.

The epididymal changes were restricted only on slight congestion of the blood vessels, while the ductus deferens has no pathological changes.

2. Rats treated with double therapeutic dose of ivermectin:

A) Liver:

The liver obtained at 24h post last injection of DTD of ivermectin showed congestion of the hepatic blood vessels and blood sinusoids. Additionally, Proliferation of the billiary epithelium and newly formed bile ductules in association with fibrous connective proliferation around the portal area admixed with few leukocytic cellular infiltrations were seen (Fig. 2A). Moreover, perivascular mononuclear leukocytic cellular infiltration mainly lymphocytes was also observed in some cases. Occasionally, focal areas of coagulative necrosis of hepatocytes characterized by loss of cellular details with pyknosis or absence of nuclei were detected in the hepatic parenchyma (Fig. 2B).

In the meantime, the livers of treated rats obtained at 7-days post last injection of DTD of ivermectin (0.4mg/kg BW) showed congestion of the hepatic blood vessels and blood sinusoids with degenerative changes in the hepatocytes represented mainly in hydropic degeneration characterized by swollen, pale, vacuolated cytoplasm were noticed.

B) Kidney:

The investigated kidneys obtained at 24h post last DTD of ivermectin revealed congestion of renal blood vessels and inter-tubular capillaries. Multifocally, the cortical interstitium, predominantly around cortical blood vessels and glomeruli was occasionally expanded by edema admixed with

inflammatory cells mainly lymphocytes and fewer macrophages (Fig. 2C). In addition to severe vacuolation of the lining epithelium of the renal tubules as well as vacuolation of endothelial lining the glomerular tufts was detected in association with mononuclear leukocytic cellular infiltration in the interstitial tissue. Additionally, desquamation of the lining epithelium of the renal tubules with destruction of the basement membrane of some renal tubules was also observed in some cases.

The investigated kidneys obtained at 7-days post last injection of DTD of ivermectin revealed congestion of the renal blood vessels and intertubular capillaries. Moreover, peri-glomerular leukocytic cellular infiltration mainly lymphocytes was also observed. Vacuolar and hydropic degeneration of the lining epithelium of some convoluted tubules. Additionally, lytic necrotic changes of the cortical renal tubules that characterized by loss of renal tubules and replaced by erythrocytes and few mononuclear leukocytes into the free space were observed in some cases (Fig. 2D).

C) Testes:

The histopathological testicular changes appeared after 24h of last injection of DTD of ivermectin were congestion of testicular blood vessels with perivascular hemorrhage. However, degenerative changes of germ cells lining seminiferous tubules characterized by swollen pale vacuolated cytoplasm with less spermatogenesis was also observed (Fig. 2E). The epididymes and ductus deferens showed absence of any pathological changes.

Meanwhile, the commonly occurred lesions in the testes at 7-days after last injection of DTD of ivermectin were congestion of testicular blood vessels. Furthermore, marked degeneration of the lining epithelium of seminiferous tubules was noticed as well as necrotic tissues and dead sperms were seen in the lumen of some seminiferous tubules (Fig., 2F). Degenerated tubules frequently exhibited incomplete spermatogenesis and absence of spermatozoa in their lumen. Moreover, Activation in the interstitial leydig cells was also seen.

The epididymal changes included only congestion of the blood vessels and edema, while the ductus deferens has no pathological changes.

Table (1): Effect of subcutaneous injection of therapeutic dose (0.2mg/Kg BW) and double therapeutic dose (0.4mg/kg BW) of ivermectin once weekly for 8 weeks on liver and kidney functions test post 24h and 7-days post last injection of the drug

Parameters	killings time /treatments					
	24h post last injection			7 day post last injection		
	control	TD	DTD	Control	TD	DTD
ALT	48.60 ±1.2^{c}	89.80 ±1.306^{b}	96.70 ±2.196^{a}	48.40 ±1.21^{c}	76.80 ±2.38^{a}	86.60 ±2.47^{b}
AST	133.40±1.78^{c}	202.40±3.11^{b}	297.90±5.21^{a}	133.60±1.71^{c}	180.40±3.83^{b}	227.90±3.98^{a}
Total protein	8.42 ±0.15^{a}	6.07 ±0.33^{b}	6.39 ±0.16^{b}	8.46 ±0.16^{a}	5.75 ±0.24^{b}	6.17 ±0.10^{c}
Albumin	3.94 ±0.04^{a}	3.15 ±0.14^{b}	3.22 ±0.05^{b}	3.96 ±0.04^{a}	2.86 ±0.07^{b}	3.09 ±^{0}.18^{b}
Globulin	4.47 ±0.17^{a}	2.92 ±0.348^{b}	3.17 ±0.18^{b}	4.49 ±0.19^{a}	2.14 ±0.28^{b}	2.66 ±0.20^{c}
A/G ration	0.89 ±0.04^{a}	1.25 ±0.19^{a}	1.05 ±0.07^{a}	0.90 ±0.051^{b}	1.34 ±0.09^{b}	1.16 ±^{0}.19^{a}
Urea	33.90 ±1.23^{c}	61.20±2.55^{b}	76.70 ±3.60^{a}	33.70 ±1.18^{c}	58.20 ±2.24^{a}	66.30 ±4.29^{b}
Creatinine	0.62 ±0.04^{c}	0.99 ±0.03^{b}	1.46 ±0.17^{a}	0.61 ±0.04^{b}	0.792 ±0.03^{a}	0.85 ±^{0}.01^{a}
Uric acid	4.08 ±0.07^{c}	5.92 ±0.05^{b}	6.99 ±0.04^{a}	4.08 ±0.07^{c}	5.83 ±0.23^{b}	6.41 ±0.20^{a}

Table (2): Effect of subcutaneous injection of therapeutic dose (0.2mg/Kg BW) and double therapeutic dose (0.4mg/kg BW) of ivermectin once weekly for eight weeks on sperm count and sperm abnormality at 24h and 7-days post last injection of the drug

Killings time/parameters				
Treatments	24h post last injection		7 day post last injection	
	Sperm count (10^{6}/ml)	Sperm Abnormalities (%)	Sperm count (10^{6}/ml)	Sperm Abnormalities (%)
Control	6.90±0.04^{a}	7.20±0.35^{c}	7.02±0.09^{a}	6.60±0.33^{c}
TD	3.0±0.02^{b}	22.10±0.54^{b}	6.58±0.09^{b}	8.20±0.38^{b}
DTD	2.34±0.03^{c}	22.10±0.54^{b}	3.35±0.02^{c}	12.40±0.61b^{a}

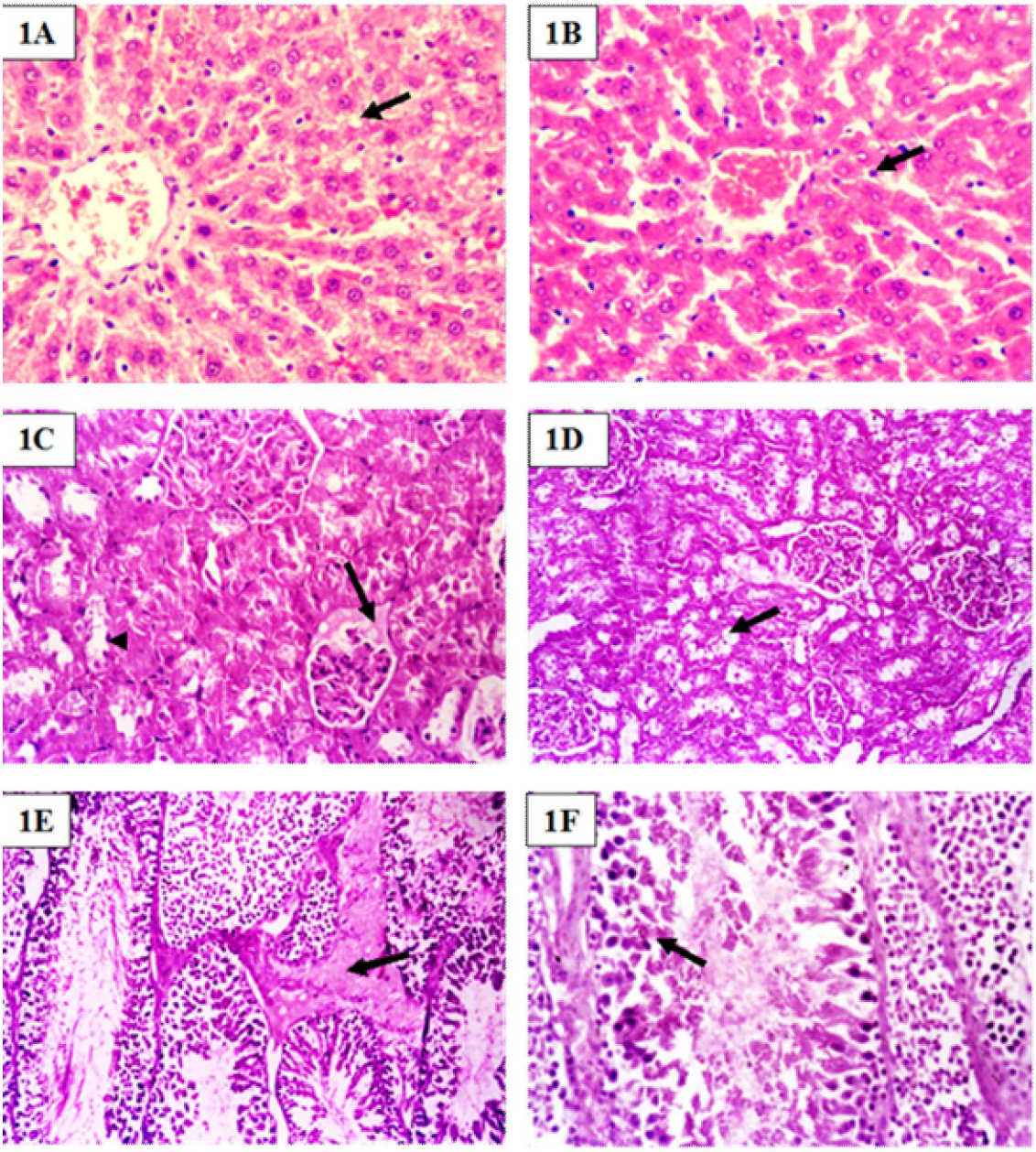


Figure 1: TD-group at 24h and 7-days post last dose of drug injection

Male rat at 24h (A, C and E) and 7-days (B, D and F) post last S/C injection of therapeutic dose of Ivermectin (0.2mg/kg BW), (A) liver at 24h post last drug injection showed congestion of central vein and blood sinusoids in combination with cytoplasmic vacuolization (arrow) of the hepatocytes, (B) liver at 7-days post last injection showed congestion of central vein with activation of Von kupffer's cells (arrow), (C) kidney at 24h post last injection showed degeneration of the lining epithelium of renal tubules (arrow head) with accumulation of edematous fluid in Bowman's space (arrow). Note also hypercellularity of the glomerular tuft (x200), (D) kidney at 7-days post last injection showed vacuolar degeneration of the lining epithelium of the convoluted tubules in the renal cortex (arrow, x100), (E) testes at 24h post last injection showed mild degeneration of the lining epithelium of semineferous tubules with accumulation of edematous fluid in the interstitial tissue (arrow), (F) testes at 7-days post last injection showed destruction of the lining epithelium of semineferous tubules with less spermatogenesis (arrow). H&E stain x 400.

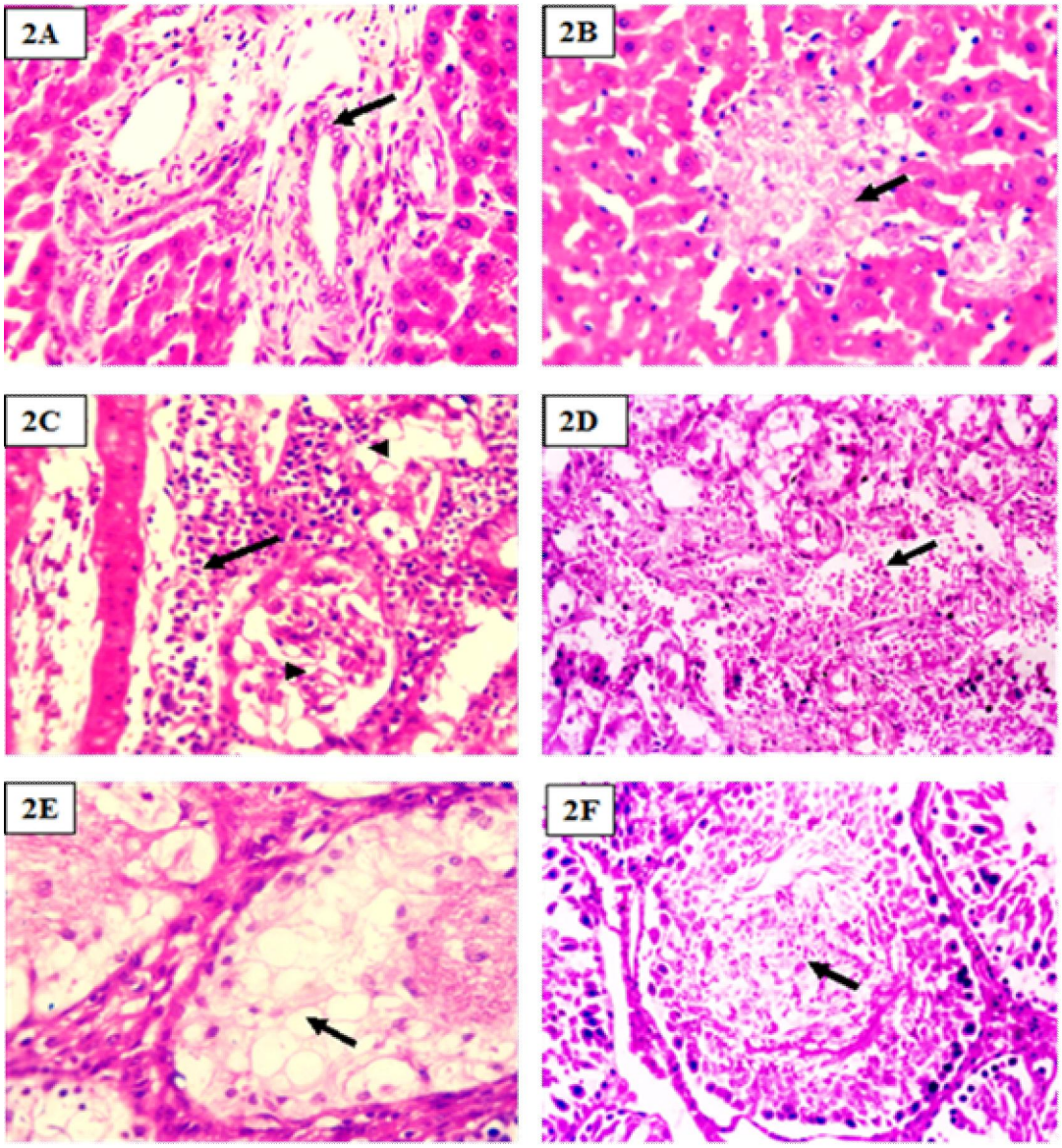
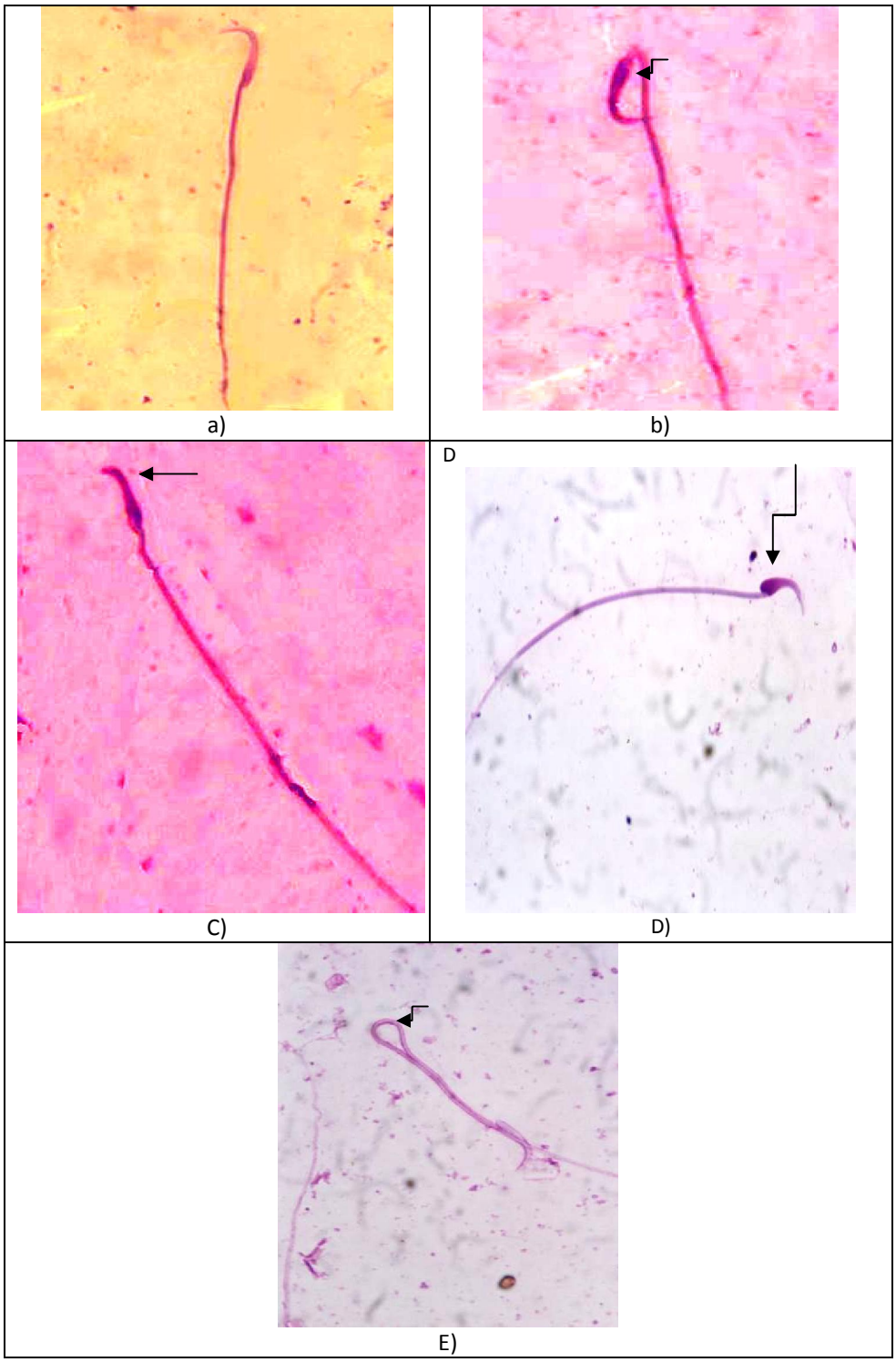


Figure 2: DTD-group at 24h and 7-day post dose of drug injection

Male rat at 24h (A, B, C and E) and 7-days (D and F) post last S/C injection of double therapeutic dose of Ivermectin (0.4mg/kg BW), (A) liver at 24h post last injection showed Proliferation of the billiary epithelium and newly formed bile ductules in association with fibrous connective proliferation around the portal area admixed with few leukocytic cellular infiltrations (arrow), (B) liver at 24h post last injection showed focal areas of coagulative necrosis of hepatocytes characterized by loss of cellular details with pyknosis or absence of nuclei (arrow), (C) kidney at 24h post last injection showed perivascular edema admixed with leukocytic cellular infiltration (black arrow) as well as in the interstitial tissues. Note also vacuolation of the lining epithelium of renal tubules and glomerular endothelium (arrow head), (D) kidney at 7-days post last injection showed lytic necrotic changes of the cortical renal tubules that characterized by loss of renal tubules and replaced by erythrocytes and few mononuclear leukocytes, note also vacuolar and hydropic degeneration of the lining epithelium of the convoluted renal tubules (arrow, x200), (E) testes at 24h post last injection showed degeneration of spermatogonia cells lining seminiferous tubules characterized by swollen, pale, vacuolated cytoplasm with less spermatogenesis (arrow), (F) testes at 7-days post last injection showed severe degeneraion of the lining epithelium of seminiferous tubules in combination with necrotic tissues and dead sperms in the lumen of some seminiferous tubules (arrow). H&E stain x 400.



a) Normal sperm
b) The rats treated with ivermectin in double therapeutic dose showing abnormal sperm with bent mid piece (arrow)
c) The rats treated with ivermectin in double therapeutic dose showing abnormal shape of head with short hock (arrow).
d) The rats treated with ivermectin in double therapeutic dose showing abnormal sperm with enlarged head (arrow)
e) The rats treated with ivermectin in double therapeutic dose showing abnormal sperm with bent tail (arrow).

Figure 3: Sperm abnormality

4. Discussion:

Ivermectin are frequently used to control parasitic infestations in many animal species. The previous studies showed that ivermectin are recommended for field use because of their ease of administration and significant larvicidal activity **(Sharma and siddiqui, 1996).** and arthropods by interfering with neural signals within the parasite conveyed by the neurotransmitter gamma amino butyric acid (GABA), while cestodes and trematodes which don't rely on GABA mediated neural transmission are not affected **(Campbell, et al., 1983 and Egerton, et al., 1980).** However, higher dose of ivermectin produce various CNS abnormalities in experimental and target animals **(Campbell and Benz, 1984 and Arundel, 1985).** Because of the potent antiparasitic activity of ivermectin, its therapeutic dosage is low and range from 0.2 to 0.3 mg/Kg BW in most animal species. The maximum tolerated dose of ivermectin in cattle is 5 times than the usual therapeutic dose **(Button, et al., 1988).** However, signs of ivermectin over dose in man are local irritation, vomition, hypotension, hypothermia, transient asthenia and dizziness **(Hall, et al., 1985).**

Various biochemical parameters are calculated to assess a broad range of physiological and metabolic functions affecting target organ identification and tissue injury evaluation **(Akhtar, et al., 2012)**. Mixtures of some common biochemical parameters provide superior information from pattern detection including enzymes such as ALT and AST for hepatotoxicity, while urea and creatinine for glomerular function **(Evans, 1996).** Therefore, the present study was designed to investigate the effect of either therapeutic or double therapeutic doses of ivermectin on the changes in histological and biochemical parameters of liver and kidneys as well as its deleterious effect on male fertility of albino rats.

The results of the present study showed that ivermectin induced significant increase in liver function enzymes (AST and ALT) at 24h, and 7-days post last injection of ivermectin compared with control group, which is dose dependent that confirm the hepatotoxicity of ivermectin. AST is an important indicator of liver damage in clinical studies. The elevation in the level of liver enzymes (ALT and AST) could be attributed to hepatotoxicity resulting in increase in the permeability of hepatic cell membrane or may be its rupture, leading to leakage of lysosomal enzymes that enhance the release of hepatic enzymes into the blood stream **(Shrivastava, *et al*., 1989, Choudhary, *et al*., 2003)**. Various studies reported that increased transaminase activities as result of hepatocellular damage as in dying or damaged cells, these enzymes leak into the blood stream (**Coles, 1974**, **Roy *et al.* 1992, Choudhary *et al*., 2003, Mansour and Mossa, 2010)**, that are in agreement with the findings of the present study. Additionally, previous studies demonstrated that the electrophilic and oxidative nature of ivermectin might be responsible for several biochemical changes (**Sutherland and Campbell, 1990, and Bloomquist, 2003)**. However, many investigators reported that hepatic and renal changes usually following administration of many anthelminitics **(Brown, *et al*., 1972, Paton, 1976, El-Ashmawey, 1991)**. In the current work, the changes in the biochemical parameters of the liver are in correlation with the hepatic histopathological changes of treated rats in the same study. The hepatic pathological alterations demonstrated in the present study such as congestion, enlargement and dilatation of sinusoids, degenerative and necrotic changes might be attributed to the reduction in free radical (O-2) scavenger formation which is agreement with the findings of **Yavasoglu, *et al*., (2006)**. Moreover, it has been reported that ivermectin accumulated in the liver and fat, where it is metabolized **(Chiu and Lu, 1989)** and it was distributed to different tissues, so long term of administration may be induced some deleterious effect in different organs **(Lifschitz, *et al*., 2000)**. Previous studies have shown the long persistence of unwanted residue of this drug in animal tissues and fluids **(Crooks, *et al*., 2000)**. Additionally, ivermectin have long elimination time in animal for at least two months **(Scott and Mckellar, 1992 and Alvinerie, *et al*., 1993)**. Consequently, liver contain highest and most persistence residues of ivermectin, these residues are responsible for the harmful effect of ivermectin on the hepatic cells (**Slantna, *et al*., 1989)**.

The present study demonstrated that the level of urea, uric acid and creatinine were significantly increased in serum samples collected at 24h and 7-days post last injection of ivermectin. Regardless to the route of administration more than 98% of ivermectin dose was excreted in the feces, while the remainder is being excreted in the urine **(Campbell and Benz, 1984)**. However, the increase in the level of urea and creatinine following ivermectin injection was matched with the findings of **Ali, *et al*. (1988), Ghaniem and Hassan, (1992)** and **Ragab, (1994)**. This elevation may be due to direct effect of ivermectin or their metabolites on the renal tissue. The elevation in creatinine and uric acid concentrations in treated animals with ivermectin may be attributed to the reduction in glomerular filtration in the kidney, as well as dysfunction of the renal tubules **(Walmsley and White, 1994)**. However, the increase in kidney function parameters (urea, uric acid and creatinine) are entirely matched with the histopathological changes in the kidney observed in the current study as congestion, hemorrhage, and other degenerative

changes. On the other hand, xenobiotics are including drugs inducing inflammatory response in the kidney (**Arise, *et al*., 2012)**, that matched with the results of the current work.

Meanwhile, the elevation in kidney biochemical parameters is correlated with increased in protein catabolism, as creatinine is the end product of protein catabolism. Furthermore, the decrease in total protein in the present study indicates their metabolic utilization that could be due to enhanced catabolism **(Swamy, *et al*., 1992)**. Additionally, it has been mentioned that, the changes in the level of protein could be due to either an increased catabolism of the biomolecules to meet the enhanced energy demand of animals under stress or their reduced synthesis due to impaired tissue function **(Ivanova-Chemishanska, 1982).**

In the current work, all investigated semen parameters indicate dramatic changes represented by significant decrease in the sperm count as well as increase in the sperm abnormalities followed ivermectin injection in dose dependant manner. These findings are in agreement with the findings of **Onakpa, *et al.* (2010)**. The deleterious effect of ivermectin on sperm could be due to the oxidant nature of certain neurotransmitter metabolites which triggered by ivermectin **(Shoeb, 2013)**. Additionally, treatment with ivermectin lead to lack of spermatogenesis stimulating hormone (SSH) resulting in decrease in sperm concentration and lack of active testosterone, consequently, increase in sperm abnormalities occurred which varies from abnormalities in head as enlarged head, abnormal middle piece and abnormal tail which may be coiled, bent tail tail as mentioned by other auther **(leaning *et al*., 1983, Onakpa, *et al*., 2010)**. Moreover, Low levels of sperms concentration after ivermectin injection may be due to effect of ivermectin in a dose dependent manner on muscle fiber of male reproductive system leading to decrease in the total force of ejaculation **(, Burg et *al*., 1979)**. Furthermore, **Jacob, *et al*. (1983)** found that the testicular fat retains high concentrations of ivermectin residue that reach its peak concentration until 12h post treatment. After that, ivermectin residues depletes relatively slower than the other tissues. Accordingly, this ensures a persistence harmful effect of ivermectin on the reproductive organs function. However, **Orgebin–Crist, *et al*. (1976)** mentioned that the composition of the epididymal fluid may be affecting the mechanism necessary for sperm maturation, transport and storage. In the present study, various histopathological changes such as necrotic changes in the seminiferous tubules with decrease in spermatogenesis were observed in rats treated with double therapeutic dose of ivermectin. Consequently, these histopathological lesions are probably associated with adverse effects on male fertility.

Conclusion:

The results of this work demonstrated that subcutaneous injection of either therapeutic or double therapeutic dose of ivermectin once weekly for eight weeks induced biochemical changes in liver, kidney and tests which correlate with the histopathological changes in these investigated tissues., Accordingly, it could be concluded that use of ivermectin induce hazardous effects in dose and time dependent especially in male animals mainly that used for breeding.

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