Pathological studies on effects of ivermectin on male and female rabbits

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A B S T R A C T

Ivermectin considered natural fermentation product derived from soil bacterium *streptomyces avermitis* and it is abroad spectrum insecticide, acaricide and anthelimintic. This studies carried out in order to investigate the histopathological effects of therapeutic and double therapeutic dose of ivermectin on mature male rabbits as well as effect of therapeutic dose of same drug on pregnant female rabbits. Forty mature rabbits were used in this study (30 males and 10 pregnant female). Male rabbits received therapeutic and double therapeutic doses of ivermectin, while female rabbits received therapeutic dose. The histopathological results revealed mild to moderate reversible degenerative changes in examined organs of male rabbits received ivermectin weekly for 4weeks meanwhile, this degeneration become more severe and extended to complete necrosis in some organs after longer period and higher doses of ivermectin administration. As well as, pregnant females suffered from abortion in some cases with fetal death. We concluded that ivermectin has adverse effect on male rabbits that received either therapeutic or double therapeutic doses that varied from mild degenerative changes to complete necrosis of spermatogenic cells with complete absence of sperms. Meanwhile, female genital system was severely affected that showing severe degeneration and hemorrhage in uterus as well as atritic follicles and degenerated ova in ovaries. So, caution must be considered for ivermectin administration especially during breeding season and for pregnant females.

Keywords: ivermectin, pathology, male rabbits, pregnant female rabbits.

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1. INTRODUCTION

Avermectins were firstly found in the fermentation broth of a soil microorganism, Streptomyces avermitilis. After conducting numerous bioassays, 8 natural avermectin components, namely A1a, A1b, A2a, A2b, B1a, B1b, B2a, and B2b, were discovered. Compounds of the B series of avermectins were found to be extremely effective against helminthes and arthropods, and ivermectin (22, 23-dihydro-avermectin B1) was released for use in animals and humans in 1981 (Shoop et al., 1995). The chemical structure of ivermectin is composed of a mixture of two compound 22, 23 dihydroavermectin B1a (H2B1a), and 22, 23 dihydroavermectin B1b (H2B1b) (Steel, 1993). Meanwhile, Lifschitz et al. (2000) stated that ivermectin considered an acaricide and antithelminitic drug of semisynthetic derivative from avermectin B1, produced from streptomyces avermitilis cultures, and ivermectinis well tolerated drug with no apparent side effect in mammals at pharmacological doses. Ivermectin is used in human for treatment of Onchocerciasis (river blindness), and also it is effective against strongylodisis; Ascariasis; Trichuriasis; Filariais; Enterobiasis; and Scabies (Bonerjee et al., 2009). Avermectin(AVM) products are macrocyclic lactone derived from the soil microorganism Streptomyces avermitilis. They are primarily used in veterinary medicine for the treatment and prevention of parasitic diseases in animals and as plant protection agents in the agricultural sector (Castanha Zanoli et al., 2012). Additionally, Trailovic and Varagic (2007) added that most prominent recorded clinical sings of (IVM) poisoning in domestic and wild animals appeared in the form of C.N.S depression, coma and may be ended by death. Ming et al. (2013) stated that ivermectin induced pathological changes as neuronal degeneration and necrosis on pigeon brain tissues after sub chronic exposure to different doses of AVM at different periods. Also Al-Jassim et al. (2015) reported that repeated administration of

different doses of ivermectin induced pathological changes in hepatic tissue of female rabbits as vacuolation of hepatocytes and fibrosis. The severity of lesion depending on dose of administration. As well as, therapeutic and double therapeutic doses of ivermectin in male rats revealed significant decrease in total sperm count and mortality in addition to various pathological changes in liver, kidneys and testis including congestion of blood vessels also degenerative changes as vacuolar and hydropic degeneration or even necrosis were also observed and this pathological changes were associated with significant changes in liver and kidney functions. (Elzoghby et al., 2015). Hence, the present study was carried to investigate the pathological effects of therapeutic and double therapeutic dose of ivermectin on rabbits.

2. MATERIAL AND METHODS

2.1. Experimental design:

This study was done in faculty of veterinary medicine, Benha University. *Male rabbits:* thirty male White New Zealand rabbits were randomly divided into 6 groups (five animals for each group) as following: Group1: received weakly 0.4 mg ivermectin /k.g b.wt S.C for 4 weeks (Arise et al., 2012**)**. Group 2: received weakly 0.4 mg ivermectin /k.g b.wt S.C for 8 weeks. Group 3: received weekly 0.8 mg ivermectin /k.g b.wt S.C for 4 weeks. Group 4**:** received weekly 0.8mg ivermectin /k.g b.wt S.C for 8 weeks. Group 5**:** control male received weekly 0.5 cm physiological saline for 4 weeks. Group 6**:** control male received weekly 1cm physiological saline for 8 weeks. Slaughter of group 1, 3, 5 after 4 weeks and 2, 4, 6 after 8 weeks and sample were collected from genital organ (testes, epididymis and seminal vesicle), heart, liver, kidney, spleen and brain for histopathological examination. While female rabbits: ten pregnant White New Zealand female rabbits were divided in two groups (5 in each group) as following: Group 7: received daily 0.4 mg ivermectin /k.g B.wt S/C form 6^{th}- 28^{th} day of pregnancy. Group8: control female received 0.5 cm physiological saline from 6^{th} -28^{th} of pregnancy. Aborted females were slaughtered at day of abortion while non-aborted females were slaughtered at 28^{th} of pregnancy and samples were collected from ovaries, uteri, oviducts, heart, liver, kidney, spleen and brain for histopathological examination

2.2. Histopathological examination

The collected specimens preserved in neutral buffered formalin. After proper fixation, the tissue specimens were trimmed, washed in running tap water, dehydrated in different ascending grades of ethyl alcohol, cleared in xylene and embedded in paraffin. The paraffin embedding block were sectioned at 5 µm thickness and stained with the following stains: 1-hematoxylin and eosin (H&E stain) Harri's Alum hematoxylin (Banchroft et al., 1996). 2-Crossman trichrome stain Crossman (Crossman, 1937). 3-Prussian blue stain (Pearse, 1968).

3. RESULTS

3.1. Macroscopic results

Testes appeared smaller in size than normal also congestion of testicular blood vessels, Liver showed enlargement in the size with bright coloration and necrotic foci appeared on their surface after in group 4. Meanwhile, Kidney appeared congested on cut sections than control, Brain showed congestion of their blood vessels and spleen smaller in size. But, Heart was enlarged and flabby. Additionally, Ovaries and oviducts were apparently normal while, uterus showed death of all feti of females aborted at day 21^{th} of pregnancy and uterus of non-aborted females showed hemorrhagic areas on uterine surface while produced feti were stunted growth and some feti were died with severe subcutaneous hemorrhage (Fig. 13).

3.2. Microscopic results

3.2.1. Testes

There are thickening of testicular capsule which is mild in therapeutic dose after 4 and 8 weeks but severe in double therapeutic dose and associated with hyalinization and perivascular mononuclear leucocytic cellular infiltration in capsular blood vessels after 8 weeks of double therapeutic dose. Additionally, mild congestion of testicular blood vessels at therapeutic dose for 4 weeks which become severe at double therapeutic dose after 4 weeks. Moreover, edema observed in both therapeutic and double therapeutic dose. Additionally, degeneration and vacuolation of spermatogenic cells in therapeutic dose after 4 and 8 weeks were noticed. These lesions were associated with necrosis of the epithelial cell lining the seminiferous tubules with pyknotic nuclei and formation of few number of sperm giant cells (Fig. 1) in therapeutic dose after 8 weeks. Meanwhile, the same degenerative changes also observed in double therapeutic dose associated with complete necrosis and complete absence of spermatogensis in most of seminiferous tubules (Fig. 2) with high number of sperm giant cells. The testicular lesions were in

direct relationship with dose and period of administration.

3.2.2. Epididymis

Epididymis showed degeneration and necrosis in epithelial cell of epididymal tubules and some epididymal sperms in therapeutic dose after 4 and 8 weeks and hyalinization of some sperms in lumen of these tubules associated with mononuclear leucocytic cellular infiltration were seen in double therapeutic dose (Fig. 3).

3.2.3. Liver

Liver revealed congestion of central vain, portal vain with sinusoidal dilatation in different degrees that becomes severe in double therapeutic dose. Additionally, thrombosis in portal blood vessels were also observed in rabbits received double therapeutic dose for 8 weeks and hyalinized wall of portal blood vessels are seen in pregnant females received ivermectin from day 6^{th} till day 28^{th} of pregnancy. Various degrees of degenerative changes in hepatocytes are observed which ranged from mild vacuolar, mild hydropic degeneration and fatty changes (Fig. 4) in either male or female rabbits received therapeutic dose to severe degree of degeneration in double therapeutic dose which extend to form focal necrotic area in hepatic parenchyma after 8 weeks from these dose. Moreover, hyperplasia of epithelial cell lining bile duct with periductal mononuclear leucocytic cellular infiltration and fibrosis were observed in all rabbits received ivermectin either in therapeutic or double therapeutic doses. Fibrous connective tissue takes greenish coloration with Crossman's trichrome stain (Fig. 5) and the severity of lesion increased according to dose and period of exposure. Eosinophilic debris in lumen of bile duct was also noticed (Fig. 6).

3.2.4. Kidneys

Kidneys showed congestion of renal blood vessels that ranged from mild to severe depending on dose of drug administration. Additionally, the glomerular tufts are shrinkage (Fig.7) and become completely degenerated in case of female received daily ivermectin at therapeutic dose from day 6^{th} till day 28^{th} of pregnancy. Moreover, eosinophilic homogenous substance in the Bowman's space was noticed in daily therapeutic dose and after 8 weeks of double therapeutic dose administration (Fig. 8). In addition to, the renal tubules showed severe degree of degeneration as vacuolation of cytoplasm of affected renal tubules in males and females received therapeutic dose while double therapeutic dose induced degeneration, necrosis and desquamation of affected epithelium. It noticed that hyaline casts found in case of long period of drug administration in both therapeutic and double therapeutic doses. Additionally, some renal tubules showed cystic dilatation in daily therapeutic dose of ivermectin. Also, interstitial mononuclear leucocytic cellular infiltrations were seen in double therapeutic dose.

3.2.5. Brain

Brain was suffered from degenerative changes in neurons in form of tygrolysis, satillatosis and neuronophagia (Fig.9) with meningitis in male received therapeutic dose for 8 weeks (Fig.10). These degenerative changes accompined with focal mononuclear leucocytic cellular infiltration and prevascular cuffing with area of malacia among brain substance. Additionally, long administration of ivermectin either in females that treated daily till day 28^{th} of pregnancy or in males treated for 8 weeks with double therapeutic dose showed vesiculation of brain substance. The lesions in brain were directly related to dose and frequency of administration.

3.2.6. Heart

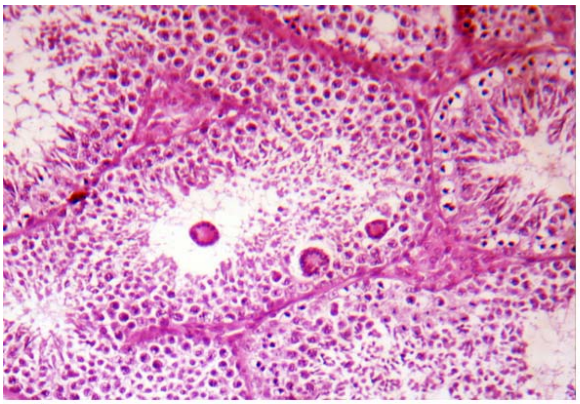
Heart showed severe congestion of myocardial blood vessels and intermuscular blood capillaries in all cases either therapeutic or double therapeutic doses of ivermectin injection. These congestion accompined with thrombus formation in case of therapeutic dose for 4 weeks and in intermuscular hemorrhage in double therapeutic dose of ivermectin treatment. Meanwhile, the myocardial muscles are suffering from degenerative changes in the form of vacuolar degeneration in sarcoplasm of affected muscle fiber, other myocardial muscle fiber showing hyalinization of their cytoplasm this hyalinization become more severe and associated with few macrophagal aggregations in double therapeutic dose of ivermectin.

3.2.7. Spleen

Spleen showed thickening in splenic capsule with lymphoid depletion in white pulp associated with hemosidrosis in therapeutic dose (Fig. 11 &12). Additionally, double therapeutic dose showed same lesions with high severity and sub capsular leuocytic cellular infiltration and vacuolation in wall of splenic arteriole were also noticed.

3.2.8. Ovaries

Ovaries showed severe congestion of ovarian blood vessels, multiple degenerated and atritic follicles were seen scattered on the ovarian stroma and presence of degenerated ova in the follicular lumen of mature graffian follicles (Fig. 14).



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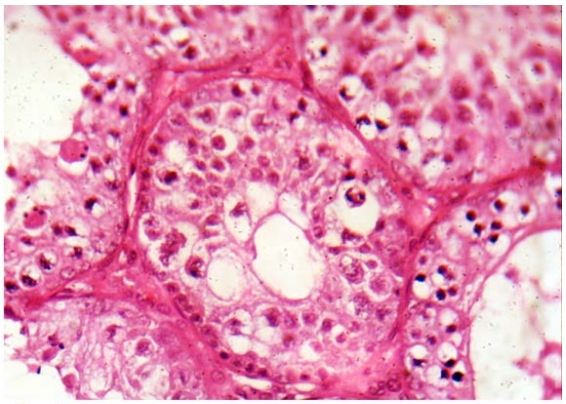

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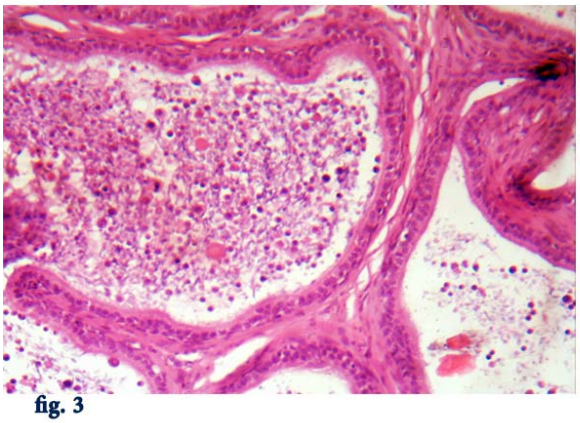

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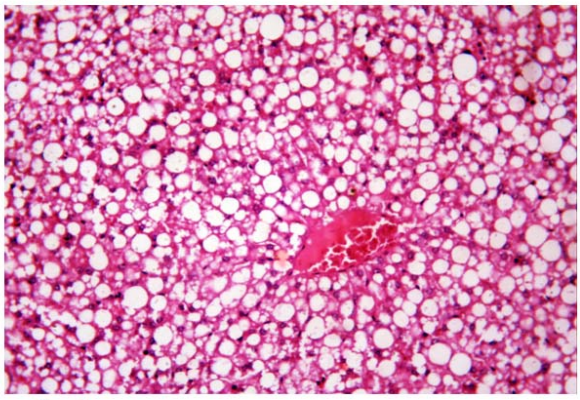

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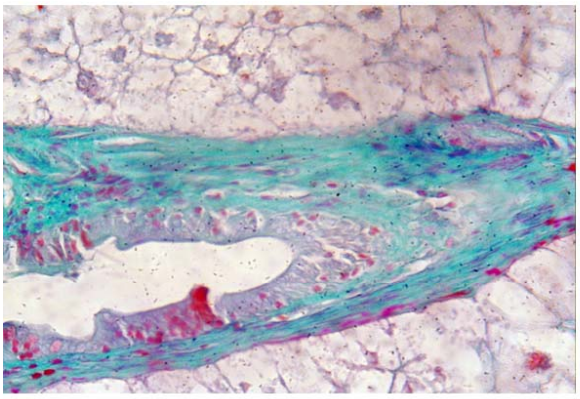

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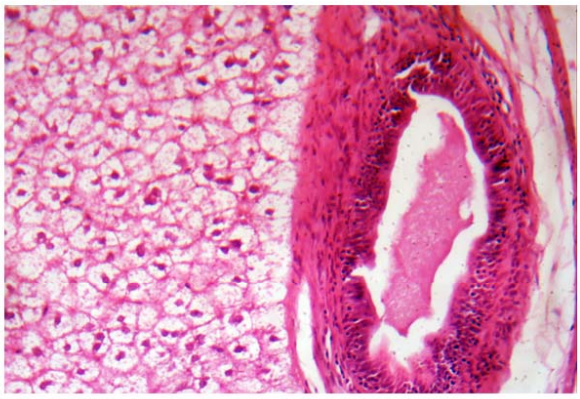

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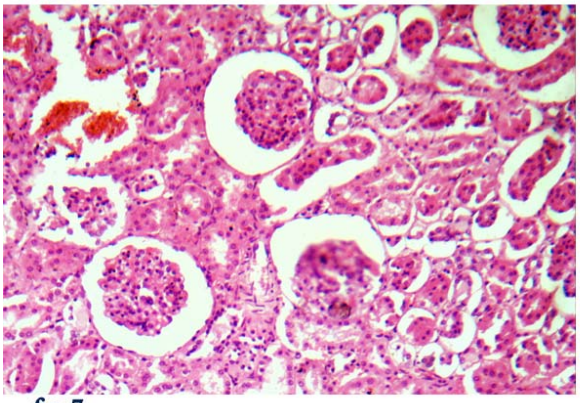

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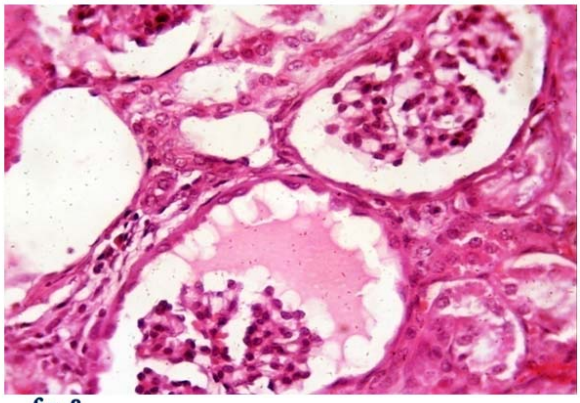

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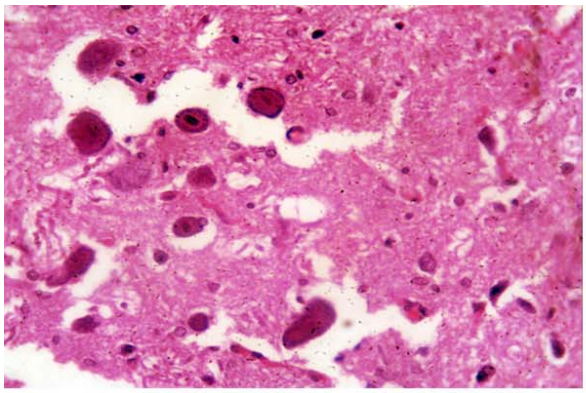

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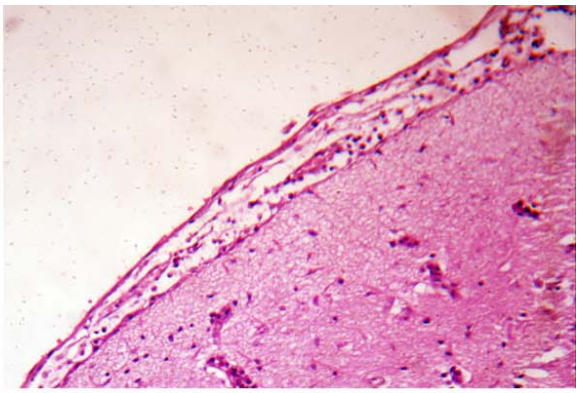

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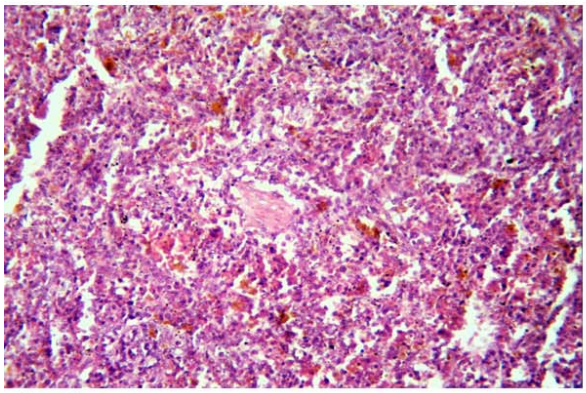

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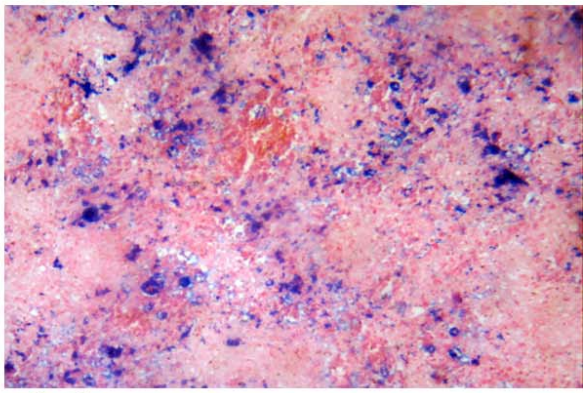

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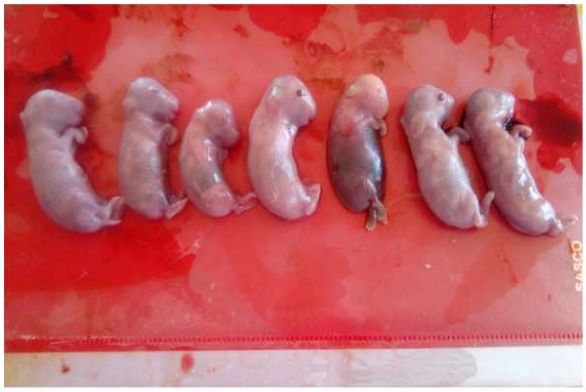

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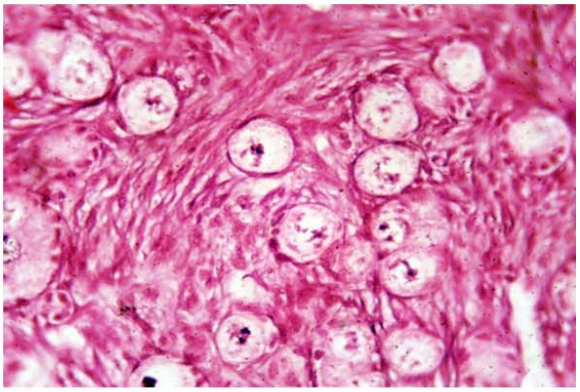

fig. 14

Fig.1 Testis of male rabbit received (0.8mg/kg b.wt) ivermectin for 4weeks showing presence of multiple sperm giant cells (H&E x100). Fig.2 Testes of male rabbit received (0.4mg/kg b.wt) ivermectin for 8weeks showing vacuolation in the cytoplasm of spermatogenic cells with pyknotic nucleus. H&Ex400). Fig3 Epididymis of male rabbit received (0.4mg/kg b.wt) ivermectin for 8weeks showing accumulation of necrotic sperms (black arrow) and some of them hyalinized together with mononuclear leucocytic infiltration (blue color) in the epididymal tubules. (H&E x200). Fig. 4 Liver of pregnant does that received daily ivermectin at dose 0.4mg/k.g b.wt S.C and slaughtered at 28th day of pregnancy showing congestion of central vain and showing severe degree of fatty changes in most hepatocytes. (H&Ex100). Fig.5: Bile duct of male rabbit received (0.4mg/kg b.wt) ivermectin for 8 weeks showing mild periductal fibrosis which take green coloration (Crossman trichrome x400). Fig.6: liver of male rabbit received (0.4mg/kg b.wt) ivermectin for 8weeks showing mild hyperplasia (increasing of the lining epithelium) with eosinophilic homogenous substance in the bile duct lumen with mild periductal fibrosis (H&E x400). Fig.7: The kidneys of male rabbit received (0.4mg/kg b.wt) ivermectin for 4weeks showing shrinkage of the glomerular tuft. (H&E x100). Fig.8L Kidney of pregnant does that received daily ivermectin at dose 0.4mg/k.g b.wt S.C and slaughtered at 28th day of pregnancy showing shrinkage and segmentation of glomerular tuft with presence of eosinophilic homogenous substance in the Bowman's space of the glomeruli (H&E x400). Fig.9: Brain of pregnant does that received daily ivermectin at dose 0.4mg/k.g b.wt S.C and slaughtered at 28th day of pregnancy showing tygrolysis of the neuron as neuron become rounded, swollen, with pyknotic nucleus and loss of dendrites (H&E x400).

Fig.10: Brain of male rabbit received (0.4mg/kg b.wt) ivermectin for 8 weeks showing meningitis (black arrow) which represented by congestion and dilatation of meningeal blood vessels with mononuclear cellular infiltration (H&E x100). Fig11: Spleen of male rabbit received (0.4 mg/kg b.wt) ivermectin for 8weeks showing amorphous golden yellow color of hemosidrine pigment among splenic substance. (H&E x200). Fig.12: Spleen of male rabbit received (0.4mg/kg b.wt) ivermectin for 8 weeks showing mild haemosidrosis which taken bluish coloration (Prussian blue x200). Fig.13: Feti of pregnant does that received daily ivermectin at dose 0.4mg/k.g b.wt S.C and slaughtered at 28th day of pregnancy showing stunted growth. Fig.14: Ovary of pregnant does that received daily ivermectin at dose 0.4mg/k.g b.wt S.C and slaughtered at 28th day of pregnancy showing multiple degenerated and atritic follicles scattered on the ovarian stroma (H&E x100).

Also, focal mononuclear leucocytic cellular in filtration were detected.

3.2.9. Uterus

Uteri showed severe congestion of endometrial blood vessels with thickening and hyalinization of their blood vessel wall in aborted females. Severe degree of hemorrhage is noticed in uteri of both aborted and non-aborted females that appeared either diffuse sub mucosal, peri-glandular or intermuscular hemorrhages (Fig. 15). Additionally, the endometrial glands showed degeneration and desquamation of their lining epithelium with presence of eosinophilic debris in their lumniae (Fig. 16). Also, scattered foci of necrosis seen all over the endometrium.

4. DISCUSSION

Avermectins and milbemycins (A/M) which considered as macrocyclic lactones are the largest selling anthelmintic in the world. They are widely used in veterinary medicine for the treatment of gastro-intestinal nematode as well as ecto-parasite infestations (Vercruysse and Rew, 2002). Macrocyclic lactones (MLs) are potent parasiticides widely used for control of internal and external parasites in domestic animals and livestock. These compounds are hydrophobic molecules characterized by a broad spectrum of activity with remarkable long lasting efficacy. Also, a wide distribution, long residence time, and extensive elimination in milk during lactation (Hennessy and Alvinerie, 2002). Testes in our results grossly were decrease in testicular size with congestion of testicular blood vessels this results are completely agreed with Zaied (2004). while microscopical thickening of testicular capsule were partially agreed with Elbetieha and Da'as (2003) who found moderate fibrosis around the seminiferous tubules, and with El-Assal (2015) who mentioned thick testicular capsule of rabbits treated with doramectin. The fibrous connective tissue proliferation that causes thick testicular capsule may be returned to response of testes toward chronic injury that resulted from ivermectin treatment. The congestion of testicular blood vessels and edema were agreed with Abd-Elhady and Abou-Elghar (2013); Elbetieha and Da'as (2003); Elzoghby et al. (2015) and El-Assal (2015). The degenerative changes of testes in our results were agreed with Abd-Elhady and Abou-Elghar (2013); El-Nahas and El-Ashmawy (2008); Elzoghby et al. (2015) and El-Assal (2015). The bad effect of ivermectin on testicular tissue may be returned to retention of high concentration of ivermectin residues in testicular fat and their depletion are slower than other tissues according to Jacob et al. (1983). And may be returned to certain neurotransmitter metabolites that triggered by ivermectin as previously mentioned by Shoeb (2013). The epididymal lesions were disagreed with El-Assal (2015) who found mild hyperplasia of epithelial cell lining epididymal tubules after 4 and 8 weeks from therapeutic dose of doramectin injection to male rabbits. Additionally, seminal vesicle showed degenerative changes as mild hyperplasia associated with hemorrhage in both therapeutic and double therapeutic dose and this result partially agreed with Elzoghby et al. (2015) who found hemorrhage in case of ivermectin injection but in testicular tissue.

The degenerative changes of liver were completely agreed with Elzoghby et al. (2015); Ismail et al. (2013); Waleed (2010) Al-Jassim et al. (2015) and Abd-Elhady and Abou-Elghar (2013). Necrotic changes of hepatocytes were completely agreed with Dadarkar et al. (2007) and Waleed (2010). Meanwhile, partially agreed with Arise et al. (2012) who showed same result when used ivermectin together with albendazole and with Abd-Elhady and Abou-Elghar (2013) who showed diffuse necrosis in liver of rats treated with abamectin.

Vacuolation of hepatocytes may be attributed to disturbance in membrane function and cellular protein of hepatocytes that resulted from ivermectin detoxification in these hepatocytes according to Arise et al. (2012). Additionally, severity of lesion increased by increasing the drug residues in hepatocytes and these residues responsible for bad effect of ivermectin on hepatic tissue according to Slantna et al. (1989). So increasing dose of drug or by long period of administration the degeneration extended from vacuolar to hydropic and finally induce severe fatty

changes. The repeated ivermectin injection leads to production of free radicals that responsible for liver damage and these free radicals predominantly accumulated in liver according to Nayak et al. (1996). The liver is susceptible to damage because of direct exposure to toxic products from ivermectin metabolism duo to liver plays a role in the detoxification of xenobiotics (Choudhary et al., 2003). These explain presence of necrotic areas in hepatic parenchyma. The biliary lesions in our results were in a complete agreement with Elzoghby et al. (2015) and Al-Jassim et al. (2015). While these lesions are in a partial agreement with Abd-Elhady and Abou-Elghar (2013) who found fibrosis in hepatic parenchyma in high doses of abamectin treatment. Periductal mononuclear leucocytic cellular infiltration and fibrosis may be the hepatic response toward hepatic injury resulted from drug metabolites in liver. The congestion of renal blood vessels with glomerular lesions were agreed with Elzoghby et al. (2015) and Abd-Elhady and Abou-Elghar (2013). Our results in renal tubules were agreed with Abd-Elhady and Abou-Elghar (2013); Elzoghby et al. (2015); Waleed (2010) and Eissa and Zidan (2009). The lesions of brain were in a complete agreement with Shoeb (2013) and Ming et al. (2013) and partial agreed with Arise et al. (2012) who found loss of distinct lamination of brain tissue with increasing polymorphonuclear cells in rats received ivermectin together with albendazole. The neurotoxic effect of ivermectin returned to its action on Gamma amino butyric acid (GABA) receptors so it effect on energy production of neurons that lead to anaerobic respiration for energy production so lactic acid accumulated in neurons causing acidosis and cell damage associated with inflammation according to Leo et al. (1996). Meanwhile, the lesions of heart were completely agreed with Waleed (2010) and Ismail et al. (2013). The splenic lesions were completely agreed with Ismail et al. (2013). Small size feti produced from treated female were agreed with Lankas et al. (1989). The ovarian and uterine lesions in our results were partially agreed with Al-Hizab and Mostafa (2010) who showed congestion of ovarian blood vessels. Also, endometrial edema was seen after 3weeks of double therapeutic dose injection of doramectin while after long period of higher doses of doramectin administration ovaries showed degeneration of oocytes of growing follicles, complete necrosis of some mature follicles and excessive atritic follicles. While uterus showed endometrial edema, degeneration, atrophy of the endometrial glands with mononuclear leucocytic infiltration in case of double and triple therapeutic doses of doramectin injection to guinea pigs. The above mentioned pathological lesions in the genital organs of female rabbits were in line with some previous reports on Avermectin (Cousens et al., 1997). The all above mentioned pathological results in different organs of either male or female rabbits are in line with Lifschitz et al. (2000) who reported that ivermectin was distributed in different tissues, so long term of administration may induce some deleterious effects in different organs.

5. CONCLUSIONS

Ivermectin has adverse effect on male rabbits that received either therapeutic or double therapeutic doses that varied from mild degenerative changes to complete necrosis of spermatogenic cells with complete absence of sperms. Meanwhile, female genital system was severely affected that showing severe degeneration and hemorrhage in uterus and atritic follicles and degenerated ova in ovaries. Additionally, ivermectin induced mild reversible pathological changes in parenchymatous organs of rabbits at therapeutic dose for short period of administration. Additionally, the repeated administration of either therapeutic or double therapeutic doses of ivermectin induced severe degenerative changes and necrosis in some parenchymatous organs. So, caution must be considered for ivermectin administration especially during breeding season and for pregnant females.

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