

EFFECT OF DIFFERENT DOSES OF IVERMECTIN ON BIOMARKER HORMONE OF ADRENAL GLAND AND HISTOLOGY OF IT IN LOCAL FEMALE RABBITS

Bushra F. HASAN

University of Basrah, Iraq

Shaymaa Z.AL RUMAIDH

University of Thi-Qar, Iraq

Abdulrazzak N. KHUDAIR

University of Basrah, Iraq

Nawras A. ALWAN¹

University of Basrah, Iraq

Abstract

Ivermectin (IVM) is a lipophilic anthelmintic drug widely used for the control of internal and external parasites in both human and veterinary medicine. This study aimed to investigate the effect of different dose of Ivermectin (IVM) on the Cortisol hormones (functionally and histologically) of twenty four of mature female rabbits in basrah. Animals were randomly divided into four groups (six for each). The first group was left without treatment (control group), second, third and fourth group treated weekly by injection s/c ivermectin for 8 week at dose (0.5mg, 1mg and 2 mg / kg.bw) respectively .Results conducted to increase significant in cortisol concentration in fourth group ,while the first and second and third group did not affected when compared with fourth group or within them .The histopathological changes as a results of ivermectin treatment in adrenal there is representative by vacuolation of zona glomerulosa and zona fasciculata in group fourth and less effect in other treatment. We concluded high dose of ivermectin and prolong period may affected on adrenal gland function.

Keywords: Ivermectin, Cortisol, Adrenal Tissue, Female Rabbits.

 <http://dx.doi.org/10.47832/2717-8234.12.6>

 ¹ nawras.alwan@uobasrah.edu.iq

Introduction

Ivermectin (IVM) is an anthelmintic drug widely used to control both internal and external parasites in humans and animals (Makhlouf *et al.*, 2020). IVM is a widely used FDA-approved broad-spectrum antiparasitic drug used also to treat pest insects and was found to be especially effective to decrease *P. vivax* transmission (Hotson 2020). However, IVM exhibits a broad spectrum of activity against gastrointestinal and lung nematodes as well as against ectoparasites of clinical relevance in domestic animals (Suarez *et al.*, 2013).

Additionally, there are different gaps regard European Food Safety Authority (EFSA 2016) [ding mammalian endocrinal toxicology which are not properly addressed]. Several study investigation the effect of ivermectin histologically on different tissues Mahmoud *et al.* (2017) reported 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).

Literature reviews: Ivermectin (fig. 1) overdose could cause a combination of clinical side effects ranging from mild to extremely severe (Epstein and Hollingsworth, 2013). The most dominant clinical symptoms of IVM poisoning in domestic and wild animals are CNS depression and sometimes coma, frequently resulting in death (Trailovic and Nedeljkovic, 2011). So, the use of ivermectin must be done with caution (Hutchinson *et al.*, 2009).

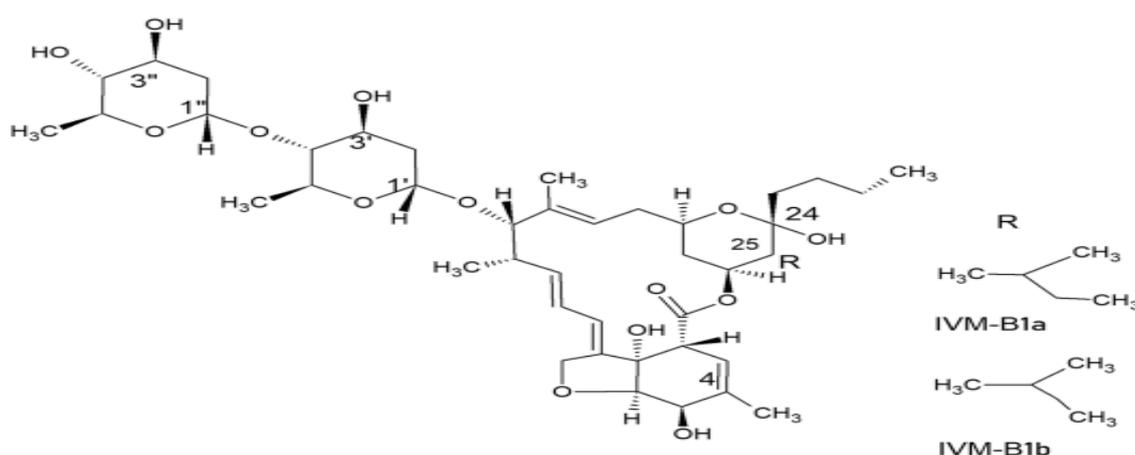


Fig. (1): Ivermectin B1a (IVMB1a) and B1b (IVM-B1b) (Archives of Toxicology)

The anticancer and the antiviral activity of IVM are well known. Thus recently, ivermectin was found to have an anti-cancer effect on human colon cancer and lung carcinoma (Diao *et al.*, 2019). Given the widespread of COVID-19 pandemic caused by Coronavirus 2 (SARS-CoV-2), researchers are constantly striving to find a suitable drug to treat this malady. (Gonçalves *et al.*, 2020) has concluded from the review of many realized works that IVM can inhibit the viral replication of SARS-CoV-2 (fig. 2).

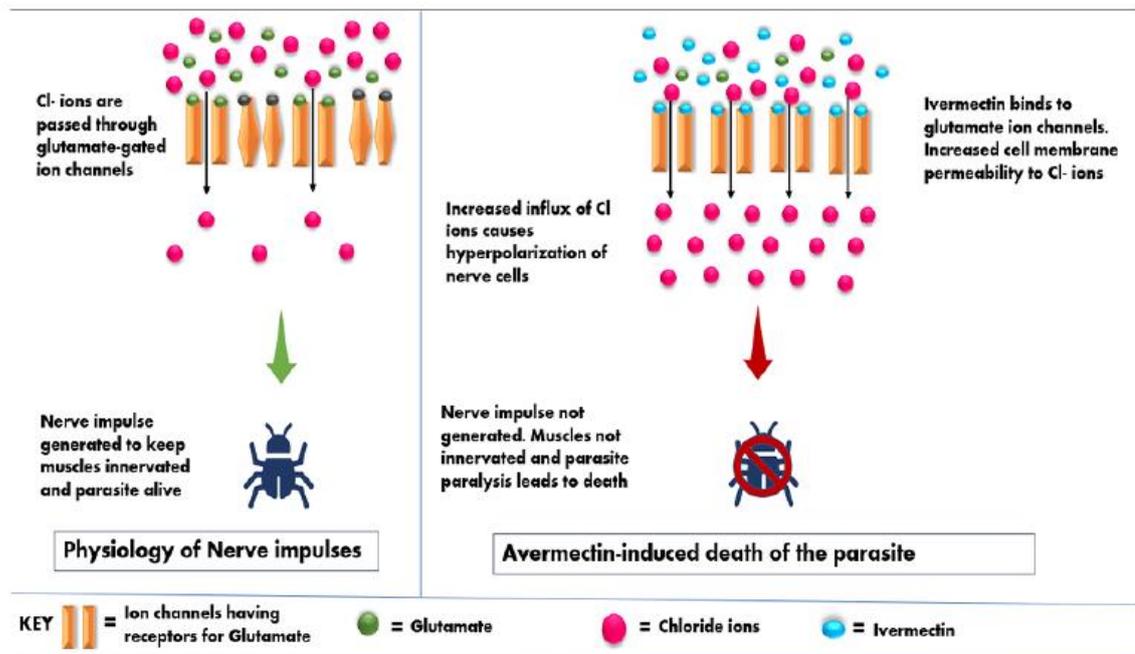


Fig. (2): Mode of action of ivermectin (Muhammed *et al.*, 2022)

Commercially as an antiparasitic agent in both veterinary medicine and human (Burkhart, 2000). Ivermectin acts to enhance the release of GABA at presynaptic neuron. It act as an inhibitory neurotransmitter and block the post synaptic stimulation of the adjacent neuron in nematodes or the muscle fiber in arthropods by stimulating the release of GABA and caused paralysis of the parasite and eventual death (Plumb, 2008; Yovany *et al.*, 2010). These harmful effects arise from avermectins targeting GABA and glutamate-gated chloride channels present both in the parasites and the host animals Muhammed *et al.*, (2022) for this reason the world health organization was listed ivermectin as most important essential medicine needed in a basic health system (WHO, 2013).

Various studies have indicated the ivermectin to have toxic effects on animals (Yas-Natan *et al.*, 2003 and Guizelin *et al.*, 2020). Hopper *et al.* (2002) recorded a case of collie dogs experiencing severe illness presented to the clinic were found to be suffering from ivermectin to toxicity. Other studies conducted on cows indicated therapeutic doses of ivermectin to induce hormonal changes, ultimately affecting the reproductive cycle of cows (Sadek and Shaheen 2015). Other studies have also revealed the administration of ivermectin even at therapeutic doses to cause reproductive, hepato-renal, sexual, and behavioural abnormalities in animals [Parisi, 2019; Nicolas *et al.*, 2020 and Ahmed *et al.*, 2020]. At present, there is limited research available describing the role of ivermectin as endocrine disruptors.

AIM OF STUDY: Study the effects of different doses of ivermectin on cortisol hormone and histological study of adrenal gland in non-infected mature female rabbits.

Materials and Methods

Rabbits Housing and Management: This study done on 24 Healthy mature female rabbit (*Lepus cuniculus*) weighed 1250-1800 grams body weight from Basrah market. All rabbit were kept for two week in the animal house of the College of Veterinary Medicine / University of Basrah, for acclimatization. They were maintained on unrestricted supplies of food that consist of alfa alfa, concentrated pellets and water *ad libitum*.

Experimental Design: Twenty four healthy non pregnant female domestic rabbits were divided into 4 groups (6 rabbits/group). The first group treated S/c 0.9 % normal saline which

considered as a control ,while the second , third and fourth group were treated with (0.5 mg, 1mg and 2mg /kg .BW.) ivermectin respectively. The treatment were given S/c and weekly for 8 week, in the end of the experiment. blood samples were collected from the heart then was put in test tube without anticoagulant, centrifugated to collect serum and kept in ependrof tubes and stored at -20°C, for hormonal analysis.

Histological study: After sacrificed by all animals, harvested adrenals gland and kept in 10% formalin for histological examination so, dehydration was done by passing in ascending concentration of ethanol; the glands were infiltrated by xylene and then embedded in paraffin wax. The section of embedded adrenal glands were done (5micron thick) by using microtome and after that put on glass slides that applied with by albumin mayer for fixed the section of gland ,dehydrated at hot plate overnight and finally staining by hematoxylin –eosin stain to examined by light microscope (Luna, 1968).

Results

Effect of different dose of ivermectin on cortisol hormone level in female rabbit:

According to the results is table, there is no significant differences between group2 and group 3 which administrated with ivermectin when compared with control or with group 4. Whereas group 4 has a high dose of administration of ivermectin (2mg/kg.bw) causes a increase significant difference ($p < 0.05$) compared with group 2 (0.5 mg/kg bw) and group 3(1 mg/kg.bw) and with control group. just high dose of ivermectin that injection S/C caused increased significantly.

Table 1- Effect of different dose of ivermectin on cortisol hormone level in female rabbit (means±SE)

GROUP	CORTISOL LEVELS
G1 CONTROL	4.34±3.15 b
G2 (0.5 mg IVM)	4.80±3.07 b
G3 (1mg IVM)	7.90±1.93 b
G4 (2mg IVM)	13.46±3.36 a
LSD	9.123

Different letters denote significant differences ($p < 0.05$) between groups

Histological changes on adrenal gland: Section of adrenal gland of female rabbit figure (1) as control shows normal structure of adrenal cortex (zona glomerulus and zona fasciculata). Figure (2): adrenal gland of female rabbit injection (0.5gm/kg.bw)s/c weekly for 8 week. Show vaculation of zona glomerulta and minimum vaculation of zona fasciculata. Figure (3) adrenal gland of female rabbit injection (1gm/kg.bw) s/c weekly for 8 week. Show moderated vacuolation of zona glomerulosa. Figure (4) adrenal gland of female rabbit injection (2gm/kg bw) s/c weekly for 8 week. Show severs vacuolation of zona glomerulta and vaculation of zona fasciculata.

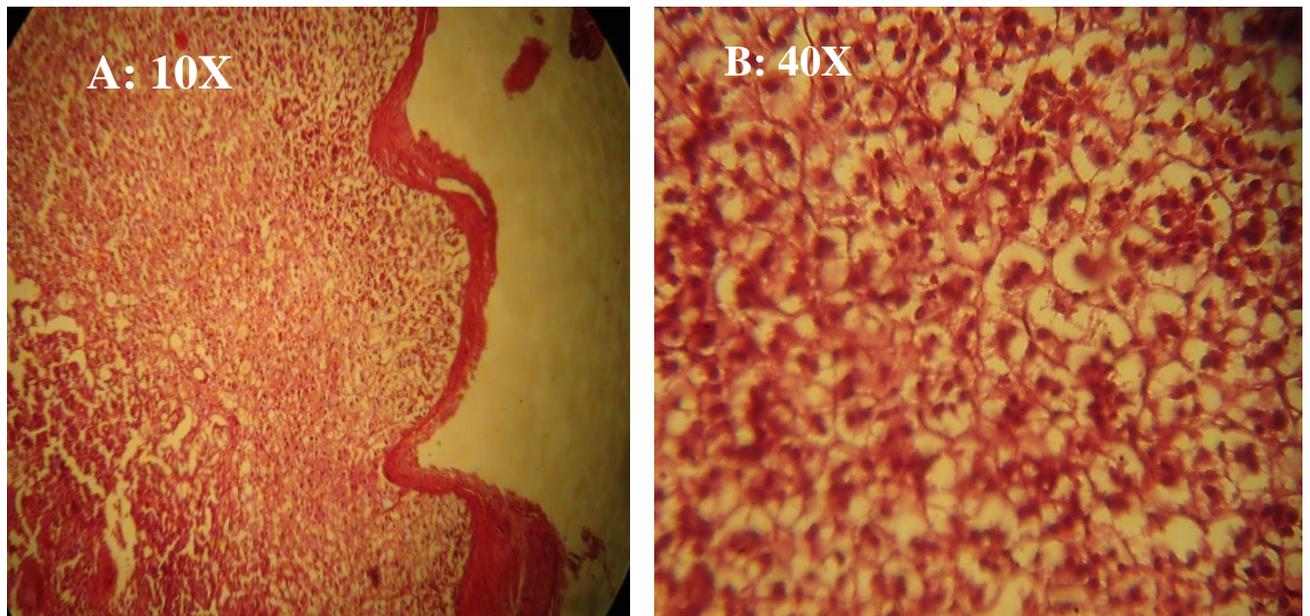


Figure (1): (control) Adrenal gland of female rabbit injection (1gm/kg.bw.) Ivermectin weekly for 8 weeks showed normal architecture. Eosin –hematoxylin stain (A: 10X and B: 40X)

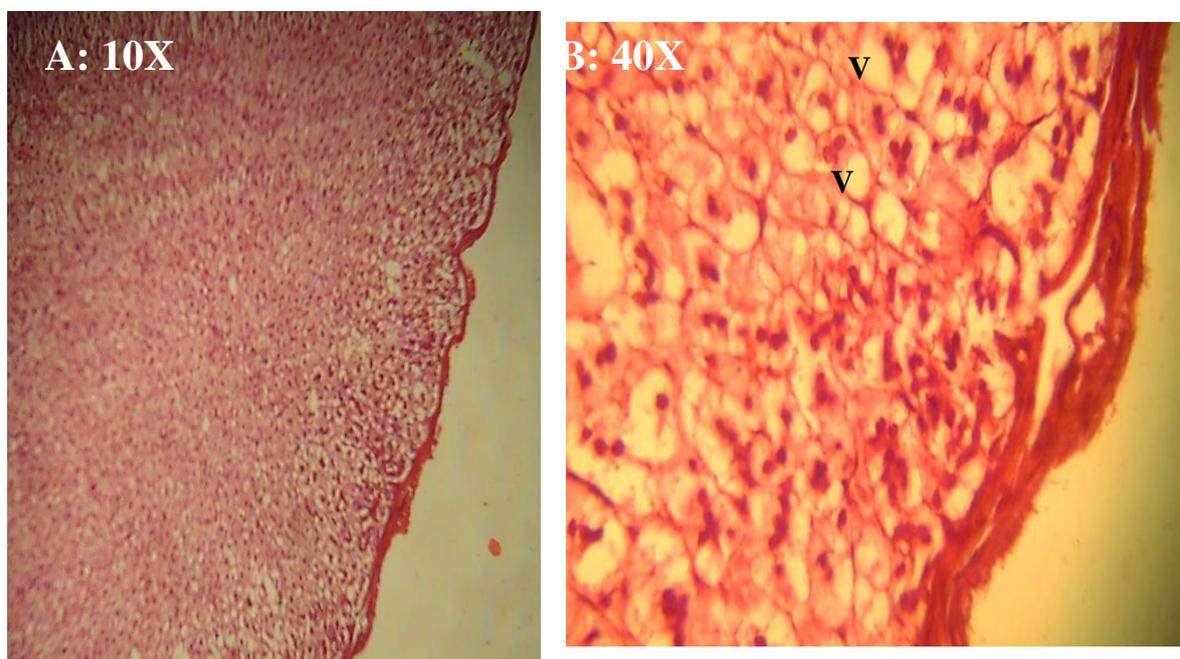


Figure (2): adrenal gland of female rabbit injection (0.5gm/kg.bw)s/c weekly for 8 week. Show vacuolation (V) of zona glomerulase and minimum vacuolation of zona faseculata. Eosin –hematoxylin stain (A: 10X and B: 40X).

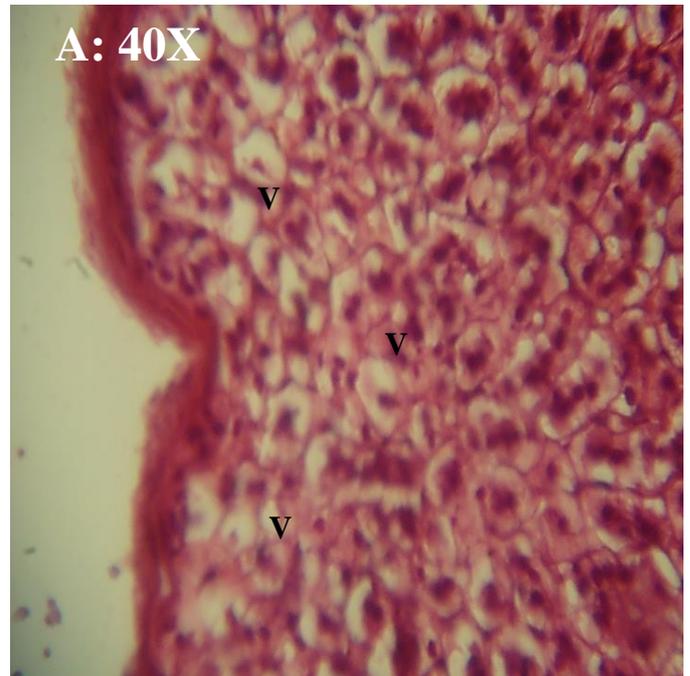
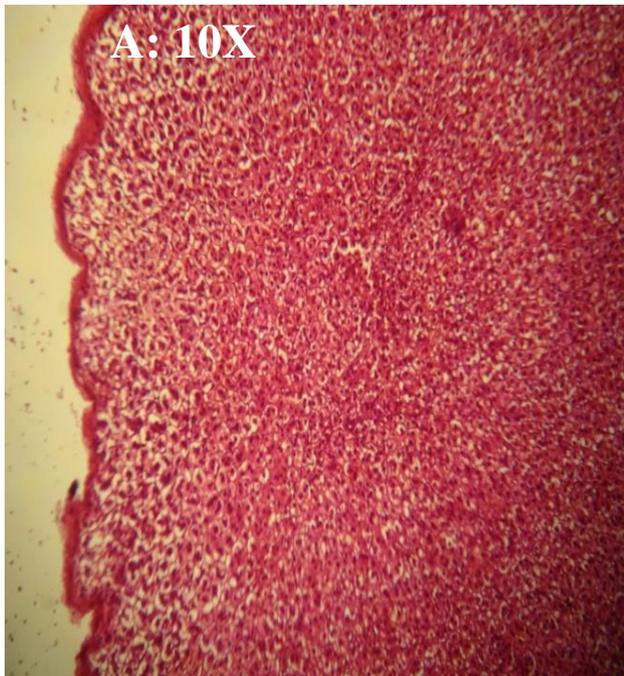


Figure (3): adrenal gland of female rabbit injection(1gm/kg.bw)s/c weekly for 8 week. Show moderated vacuolation of zona glomerulase. Eosin –hematoxylin stain (A: 10X and B: 40X).

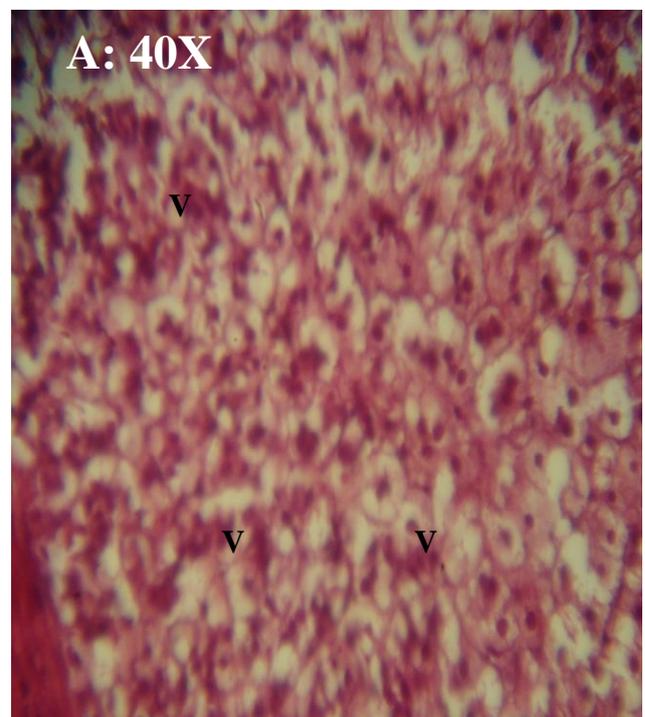
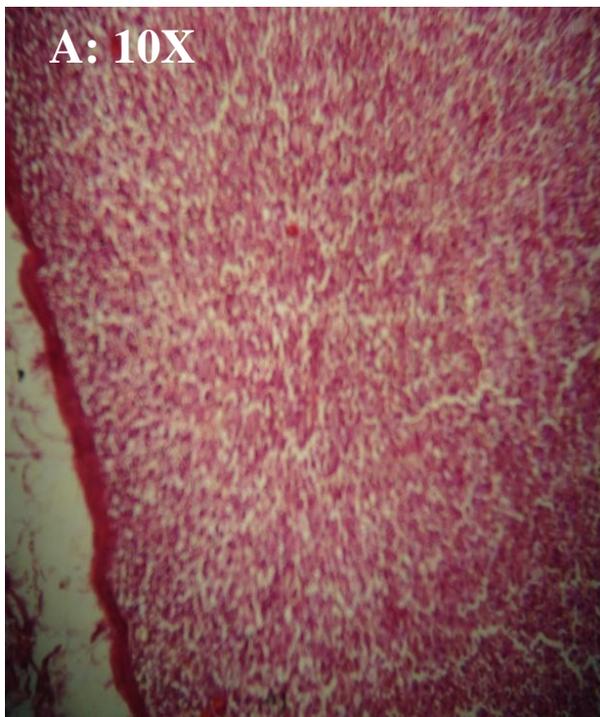


Figure (4): adrenal gland of female rabbit injection (2gm/kg.bw)s/c weekly for 8 week. Show severs vacuolation of zona glomerulose and vacuolation of zona faseculata. Eosin – ematoxlylin stain (A: 10X and B: 40X).

Discussion

The result from table 1 showed a significant increase in plasma cortisol levels when injection ivermectin s/c 2mg/kg.bw compared with other dose of ivermectin 1gm/kg.bw or 0.5 gm/kg.bw and control group. This result is Clearfield the effect of ivermectin on adrenal gland histologically and physiologically through the study the effect of ivermectin on adrenal biomarker (plasma cortisol) and conducted to the increases significantly in plasma cortisol in group injected with ivermectin at dose 2mg /kg.bw. weekly for 8 weeks compared with control and other doses, whereas the concentration 1gm/kg.bw increased but not reached to the significant. So that come to agreement with Muhammed *et al.* (2022) have reported the negative effects of avermectins like ivermectin, abamectin, doramectin, and eprinomectin on the host animals. These harmful effects arise from avermectins targeting GABA and glutamate-gated chloride channels present both in the parasites and the host animals. The neurotoxicity induced by damaging the brain, which is responsible for production of reproductive hormones; therefore, they indirectly affect the reproductive system of animals (El-shafey *et al.*, 2011 and Labue *et al.*, 2020) Also 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 ivermectin at different periods. Similarly, the therapeutic dose of ivermectin in adult rats was found to impair neurochemical and behavioral attitudes injected 1 mg/Kg subcutaneously causes increased serotonergic and dopaminergic in rats activity in association with stress (Parisi *et al.*, 2019). Also GabAllh *et al.* (2017) conducted to injected 0.8 mg/Kg subcutaneously ivermectin for 8 weeks rabbits Meningitis and brain degeneration.

So from earlier study we believe there is a relationship between the effect of ivermectin on neural activity and adrenal gland hormones that resemble with state that is observed a trend towards an increase in serum cortisol levels in chronically infected animals. Serum cortisol is often used in stress and welfare assessments (Orihuela *et al.*, 2009) that is agreement with study In Baladi cows, injected a therapeutic dose (0.2 mg/Kg) of ivermectin one day post-parturition caused 3 months delay in estrous and caused disturbances in the levels of luteinizing hormone, follicle-stimulating hormone, cortisol, estradiol, progesterone, and prolactin (Sadek and Shaheen, 2015), there is a resemble in result with Omshi *et al.* (2018) they found the wide range of use of ivermectin in the treatment of many conditions in animals and humans explains the need of studying its effects on vertebrates especially mammals

Cortisol is a steroid hormone produced by the zona fasciculata of the adrenal cortex. Elevated levels of cortisol in Ivermectin treated rabbit's indication of the stress condition of those animals (El-sawy *et al.*, 2016). Ivermectin is considered a very safe drug when therapeutic doses are respected. However, several toxic effects were reported in sensitive populations or are related to involuntary overdoses. the dose and dosage of ivermectin for animals and humans are changing depending on the clinical case and the type of parasites (Ahmed *et al.*, 2020), while sub-chronic administration of the oral high dose of emamectine benzoate, an avermectin insecticide, was associated only with decreased activity and increasing weakness (Khaldoun-oulabi *et al.*, 2015), ivermectin may produce free radicals and thus results in cytotoxic effect on the parasite. Nitric oxide is involved in various path physiological processes. it acts as free radicals and as host defense mechanisms through cytotoxic effect (Tamarozzi *et al.*, 2011). Since cortisol is a significant hormone that is released as a response to stress (Orihuel *et al.*, 2009; Muehlenbein and Watts, 2010). Cortisol levels in rabbits went up significantly in ivermectin-treated and untreated infested control group.

Histological Changes: In the present study conducted to the modifications in plasma cortisol levels markers were confirmed by the histopathological alterations found in the ivermectin treated group including vacuolization in zona fasciculata cells when treated with dose 2mg/kg bw. The results correspondence with (Elzoghby *et al.*, 2015; El sawy *et al.*, 2016) in therapeutic and double therapeutic doses of ivermectin treated groups in rats and in rabbits (Gaballh *et al.*, 2017)

According to the literature research, no studies were performed on the histopathological effects of ivermectin on the adrenal gland tissues of animals. Makhoul *et al.* (2021) found severe alterations of the brain tissues of rabbits when administered a therapeutic dose of ivermectin (degenerative changes in neurons) for 8 weeks and a double therapeutic dose (vesiculation of brain structure) for the same period. In the aforementioned study, it was conducted that the brain lesions were related to the dose and frequency of use of ivermectin results were reported to ivermectin injections alone weekly for 8 weeks of treatment at a dose of 2 mg/kg s/c, resulted in histopathological effects on the liver tissue, including severe swelling and necrosis of some liver cells, hyperplasia of the epithelial cells lining the bile ducts, vascular congestion, and hydropic degeneration of hepatocytes. These findings are consistent with those of another study, which found pathological alterations in the livers cells of ivermectin treated mice (Nashat *et al.*, 2018).

Conclusion:

we concluded the high dose of ivermectin (2mg/kg.bw.) cause a stress effect on adrenal gland of the host, specially zona fasciculate that is led to released cortisol hormone from it.

References

- 1-Makhlouf, C., Khaldoun, O.H., Bokreta, S., Tarzali, D.B., Asma1, B.M., Daoudi, Z.N., 2020. Beneficial effects of ascorbic acid on Ivermectin repeated high-dose therapy in rabbits: biochemical and histopathological investigations. *European Journal of Biological Research*, ISSN 2449-8955.
- 2- Hotson, I.K. (2020).The avermectins: a new family of antiparasitic agents. *J S Afr. Vet. Assoc.*, 53(2):87–90
- 3- Suarez, G., Alvarez, L., Castells D., Correa, O., and P. Fagiolino *et al.*, 2013. Relative bioavailability and comparative clinical efficacy of different ivermectin oral formulations in lambs. *BMC Vet. Res.*, 9: 27. DOI: 10.1186/1746-6148-9-27
- 4- Yovany, M., Joseph, F.N., Jonathan, S., Charles, D.M. and Timothy, G.G. 2010. Ivermectin disrupts the function of the excretory-secretory apparatus in microfilariae of *Brugia malayi*. *PNAS*, 107: 20120- 20125. DOI: 10.1073/pnas.1011983107
- 5- Epstein, S.E. and Hollingsworth, S.R. 2013. Ivermectin induced blindness treated with intravenous lipid therapy in a dog. *J. Vet. Emerg Crit. Care*, 23:58-62.
- 6- Trailovic, S.M. and Nedeljkovic, J.T. 2011. Central and peripheral neurotoxic effects of ivermectin in rats. *J. Vet. Med. Sci.*, 73: 591-599. DOI: 10.1292/jvms.10- 0424
- 7- Hutchinson, G.W.; Dawson; K., Fitzgibbon, C.C. and Martin, P.J. 2009. Efficacy of an injectable combination anthelmintic (nitroxynil +clorsulon+ivermectin) against early immature *Fasciola hepatica* compared to triclabendazole combination flukicides given orally or topically to cattle. *Vet. Parasitol.* 162: 278- 284. PMID: 19375232.
- 8- *Archives of Toxicology* (2021) 95:1535–1546.
- 9- Diao, H.; Cheng, N.; Zhao, Y.; Xu, H.; Dong, H.; Thamm, D. H.; Zhang, D. and Lin, D. 2019. Ivermectin inhibits canine mammary tumor growth by regulating cell cycle progression and WNT signaling. *BMC veterinary research*, 15(1) 1-10.
- 10-Gonçalves, K.; Vasconcelos, A.; Barbirato, D.; Vasconcelos, C. and Vasconcelos, B Burkhart, C.N. 2000. Ivermectin: an assessment of its pharmacology microbiology and safety. *Vet. Hum. Toxicol.* (2020). Therapeutic potential of ivermectin for COVID-19. Authorea Preprints.
- 11-Plumb, D. C. 2008. *Plumbs veterinary drug handbook*. Blackwell publishing. 6th Edi. Stockholm
- 12-WHO, 2013. Model list of essential medicine (<http://apps.WHO.int/iris/bitstream/10665/93142/1/EUa=1>)WHO.october 2013. Retrieved 22 April (2014)
- 13-Muehlenbein, M.P. and Watts, D.P. 2010. The costs of dominance: testosterone, cortisol and intestinal parasites in wild male chimpanzees. *Biopsychosoc Med.*, 9:4–21
- 14-Orihuela, A., Aguirre, V., Hernandez, C., Flores-Perez, I. and Vazquez, P. 2009. Breaking down the effect of electro-ejaculation on the serum cortisol response, heart and respiratory rates in hair sheep (*Ovis Aries*). *JAVA*, 8(10):1968–1972.
- 15-Luna, L.G. 1968 . *Manual of histology staining methods of the Armed Forces Institute of Pathology*.3rd ed., New York, McgrawHill(*Salvia officinalis*). *Food Chem*; 75:197-202.
- 16- Ming, L., Tian-Zi, Y., Wen-Jun, Z., Jian-Ping, Q., Ci, L., Bing, Z., Shi-Wen, X. and Shu, L., 2013. Antioxidant response and histopathological changes in brain tissue of pigeon exposed to avermectin. *J. Ecotoxicol.* 22, 1241–12.
- 17-Mahmoud, S, GabAllh, AbdEl-baset, E, El-mashad, Aziza, A, Amin, Marwa, M, Darweish. D. 2017. Pathological studies on effects of ivermectin on male and female rabbits benha veterinary medical journal, 32(1): 104- 112.
- 18-Elzoghby, R.R., Amin, A., Hamouda, F.A., Ali, A., 2015. Toxicological and pathological studies of Ivermectin on male albino rats. *Journal of American Science* 11, 73-83.
- 19-Omshi, F. S. H.; Abbasalipourkabir, R.; Abbasalipourkabir, M.; Nabyan, S.; Bashiri, A. and Ghafourikhosroshahi, A. (2018). Effect of vitamin A and vitamin C on attenuation of ivermectin-induced toxicity in male Wistar rats. *Environmental Science and Pollution Research*, 25(29) 29408-29417.

- 20-Ahmed, A. E.; Alshehri, A.; Al-Kahtani, M. A.; Elbehairi, S. E. I.; Alshehri, M. A.; Shati, A. A.; Alfaifi, M. Y.; Al-Doais, A.A.; Taha R., Morsy, K. and El-Mansi, A. A. 2020. Vitamin E and selenium administration synergistically mitigates ivermectin and doramectin-induced testicular dysfunction in male Wistar albino rats. *Biomedicine & Pharmacotherapy*, 124 109841.
- 21-Khaldoun-Oularbi, H.; Allorge, D.; Richeval, C.; Lhermitte, M. and Djenas, N. 2015. Emamectin benzoate (Proclaim®) mediates biochemical changes and histopathological damage in the kidney of male Wistar rats (*Rattus norvegicus*). *Toxicologie Analytique et Clinique*, 27(2) 72-80.
- 22-Tamarozzi, F., A. Halliday, K. Gentil, A. Hoerauf and E. Pearlman *et al.*, 2011. Onchocerciasis: the Role of *Wolbachia* bacterial endosymbionts in parasite biology, disease pathogenesis and treatment. *Clin.Microbiol. Rev.*, 24: 459-468.
- 23-El-Sawy, M.A.; M.E. El-Speiy; M.A., Tony, And Sadakal, T.A., 2016. Comparative studies on reproductive male rabbits as affected by therapeutic of Ivermectin or both of garlic and cinnamon oils treatments. b. biochemical blood, hormones and semen characteristics in male rabbit. *Egyptian Journal of Rabbit Science*, 26(1): 57- 87.
- 24- Muehlenbein, M.P. and Watts, D.P. 2010. The costs of dominance: testosterone, cortisol and intestinal parasites in wild male chimpanzees. *Biopsychosoc Med.* 4, 21, <https://doi.org/10.1186/1751-0759-4-21>
- 25- Orihuela, A., Aguirre, V., Hernandez, C., Flores-Perez, I. and Va´zquez, R. 2009. Breaking down the effect of electro-ejaculation on the serum cortisol response, heart and respiratory rates in hair sheep (*Ovis aries*). *J. Anim. Vet. Adv.* 8, 1968–1972
- 26- GabAllh, M. S.; El-mashad, A. B. E.; Amin, A. A. and Darweish, M. M. 2017. Pathological studies on effects of ivermectin on male and female rabbits. *Benha Veterinary Medical Journal*, 32(1) 104-112.
- 27-Makhlouf, C., Khaldoun, O. H., Bokreta, S., Oularbi, Y., Zerrouke, H. and Daoudi, Z. N. 2021. Protective Effects of Vitamin C on Ivermectin Induced Toxicity on Kidney Functions and Brain Tissue in Rabbits (*Oryctolagus cuniculus*) Egypt. *Acad. J. Biolog. Sci.*, 13(1): 63- 77.
- 28- Parisi, D.P.; Santos, S.A.; Cabral, D.; Queiroz-Hazarbassanov, N.; Florio, J.C.; Bernardi, M.M.; Kirsten, T.B. 2019. Therapeutical doses of ivermectin and its association with stress disrupt motor and social behaviors of juvenile rats and serotonergic and dopaminergic systems. *Res. Vet. Sci.*, 124, 149–157.
- 29- Yas-Natan, E.; Shamir, M.; Kleinbart, S.; Aroch, I. **2003**, Doramectin toxicity in a collie. *Vet. Rec.* 153, 718.
- 30**-Muhammad, S., Rao Z. A., Khalid M., Riaz, H., Sehar, S., Mehwish, F., Tean, Z., Asghar, A., Bernardo, M., Ina, A. and José, L. M. 2022: Assessment of Avermectins-Induced Toxicity in Animals *Pharmaceuticals* 15, 332.
- 31-Guizelini, C.C., Pupin, R.C., Möck, T.B.M. and Morais, D.R. 2020. Arredondo, J.A.C., Robalinho, L.L., Gimelli, A., de Lemos, R.A.A. Approaches for a field diagnosis of abamectin poisoning in calves. *Pesqui. Vet. Bras.*, 40, 155-157.
- 32- Hopper, K.; Aldrich, J.; Haskins, S.C. 2002. Ivermectin toxicity in 17 collies. *J. Vet. Intern. Med.*, 16, 89–94.
- 33- Sadek, K.M. and Shaheen, H.M. 2015. The biochemical effects of ivermectin on reproductive hormones and mineral homeostasis in Baladi cows post parturition. *Vet. Arh.*, 85, 95–103.
- 34-Nicolas, P., Maia, M.F., Bassat, Q., Kobylinski, K.C., Monteiro,W., Rabinovich, N.R., Menéndez, C., Bardají, A. and Chaccour, C. 2020. Safety of oral ivermectin during pregnancy: A systematic review and meta-analysis. *Lancet Glob. Health*, 8, e92–e100.
- 35-Ahmed, A.E.; Alshehri, A.; Al-Kahtani, M.A.; Elbehairi, S.E.I.; Alshehri, M.A.; Shati, A.A.; Alfaifi, M.Y.; Al-Doaiss, A.A.; Taha, R.;Morsy, K.; et al. 2020. Vitamin E and selenium administration synergistically mitigates ivermectin and doramectin-induced testicular dysfunction in maleWistar albino rats. *Biomed. Pharmacother.*, 124, 109841.
- 36- GabAllh, M.S.; El-mashad, A.B.E.; Amin, A.A. and Darweish, M.M. 2017. Pathological studies on effects of ivermectin on male and female

- rabbits. *Benha Vet. Med. J.*, 32, 104–112.
- 37- El-Shafey, A.A.M., Seliem, M.M.E., El-Mahrouky, F., Gabr, W.M. and Kandil, R.A. 2011. Some physiological and biochemical effects of oshar extract and abamectin biocide on male albino rats. *J. Am. Sci.*, 7, 254–261.
- 38- Laube, C., van den Bos, W. and Fandakova, Y. 2020. The relationship between pubertal hormones and brain plasticity: Implications for cognitive training in adolescence. *Dev. Cogn. Neurosci.*, 42, 100753.
- 39- European Food Safety Authority (EFSA). 2016. Peer review of the pesticide risk assessment of the active substance abamectin. *EFSA J.*, 14, e04491.