Egypt. J. Vet. Sci. **Vol. 54,** No. 6, pp. 1111-1120 (2023)

Egyptian Journal of Veterinary Sciences
https://ejvs.journals.ekb.eg/

Detection of Ivermectin Toxicity Using Some Biochemical and Immunological Assays in Mice

Farah F. Almawla* and Banan Kh. Al Baggou

Department of Physiology, Biochemistry, and Pharmacology at the College of Veterinary Medicine, University of Mosul, Iraq.

OUR study aims to detect acute and subacute toxicity caused by ivermectin by using some biochemical and immunological assays in mice. Non-lethal toxic doses of ivermectin (30 and 60) mg/kg.B.Wt. caused a pronounced significant decrease in the concentration of glutathione (GSH) in the brain and liver of mice after 1 and 7 days of treatment as a result of oxidative stress caused by poisoning with this drug, it's also caused a significant increase in the malondialdehyde (MDA) concentration in the brain and liver of mice compared to the control group.

The results of our experiments did not show apparent effects in the immune response of mice to poisoning with non-lethal toxic doses of ivermectin, as its development was very simple in the concentrations of some cytokines as the concentration of interleukin-6 (IL-6) & tumer necrosis alpha (TNF-α) in the blood plasma of mice after 1 and 7 days of treatment. Mice poisoned with ivermectin at doses (75 and 100) mg /kg B. wt. after oral dosing showed a significant decrease in acetylcholinesterase (AchE) activity with inhibition rates of up to (25 and 33 %) in the blood plasma of mice and (75 and 68%) in the brain respectively, high percentages indicated a significant inhibition of enzyme activity compared to the control group after 4 and 24 hr of treatment.

Inspite of the clinical saftey of ivermectin, our results showed that the drug produces toxic biochemical effects at a level of oxidative stress , cholinesterase activity not reported before.

Keywords: Ivermectin, oxidative stress, immunological response, AchE activity, mice.

Introduction

Ivermectin is a semi-synthetic derivative of the family of macrocyclic lactones, and its broad spectrum anti-parasitic effect against various nematodes and ectoparasites [1]. Products of the actinomycete *Streptomyces avermitilis*, administered orally, topically, or by injection, have been used for the treatment of mites and nematodes [2,3,4], and are used as an antinematodes such as intestinal and lungworms [5,6].

Ivermectin was increase the potential of the inhibitory neurotransmitter gamma-aminobutyric acid (GABA) which leads to paralysis of the parasite, In vertebrate muscle and nerve cells of the microflora [7], it binds selectively and with high affinity to glutamate-gated chloride ion channels, acting as an agonist of the GABA and disrupting GABA-mediated CNS neuro synaptic transmission [8,9].

High doses of ivermectin or mutation in p-glycoprotein may allow ivermectin to pass through the blood-brain barrier (BBB) to cause neurotoxicity in animals manifested as incoordination, mydriasis, emesis, diarrhea, salivation, depression, ataxia, tremors, coma and death [10,11,12]. Ivermectin induced adverse

*Corresponding author: Farah F. Almawla, E.mail: farahshihab293@gmail.com, farah.21vmp1@student.uomosul.edu.iq, Tel.: 07703770944
(Received 05/07/2023, accepted 29/07/2023)
DOI: 10.21608/EJVS.2023.221045.1537

reactions have high lighted its oxidative nature that increasing toxic oxygen intermediates ,then it induced biochemical changes in the host animals as GSH and MDA concentration in rats blood plasma [13],or in carp fish tissues [14].

When reactive oxygen species (ROS) are produced more quickly than they can be removed by antioxidant systems, oxidative stress is created, which damages biological macromolecules and disturbs normal physiology and metabolism [15]. Organisms engage several antioxidant mechanisms as well as antioxidant defense systems necessary to diminish the antioxidant activity of ROS in order to prevent or minimize ROS formation from oxidative metabolism [16], Numerous studies have demonstrated that numerous xenobiotics, including insecticides, can cause neurotoxicity in many animals, including rats and mice, due to oxidative stress [17].

Ivermectin inhibit production of inflammatory cytokines in mice [1,18]. Cytokines Interleukin 6 (IL-6) and tumor necrosis factor alpha(TNF-α) are small proteins [19,20], produced by almost every cell to regulate and influence the immune response, however, recent research indicates that the simultaneous release of pro-and anti-inflammatory cytokines is mandatory in any immune response [21,22].

The AchE is present in the body of mammals and birds in the blood, muscles, as well as nervous tissues such as the autonomic and CNS. AchE was responsible for the decomposition of acetylcholine (Ach) into choline and acetic acid [23]. Measuring the AchE activity is very important in detecting cases of poisoning with organophosphates, pyrethroids, and other pharmacological preparations in mammals and birds [24]. Ivermectin is a very safe drug, however, there are many studies on its toxic effects in different types of animals due to misuse, high doses, accidental administration, or due to allergy to the drug [25].

Due to the widespread use of ivermectin for being a safe drug, our study shed light on the detection of some acute and sub-acute toxic effects on some biochemical parameters in mice.

Material and Methods

Ethics

The use of experimental animals and the trials were approved according to the ethical code number UM. VET. 2022.041.from the Scientific Council of the Department of Physiology, Biochemistry, and Pharmacology at the College of Veterinary Medicine, University of Mosul, Iraq. The study is part of Master's thesis.

Animals

Fourty-five white Swiss-origin mice (male and female), 2-3 months, its body weights between 22-36 g, were raised in the animal house of the College of Veterinary Medicine at the University of Mosul. and we provided standard conditions as a light cycle (10 hr /light and 14 hr/ dark), the temperature was 22 ± 2°C. The mice were distributed to special cages made of plastic designed for breeding mice and were provided with sufficient water and animal feed available throughout the day, and we prepared the feed from the local markets of Mosul.

Preparation of drugs

Ivermectin **(** Pioneer Company, Sulaymaniyah, Iraq) was dissolved in 10 ml of propylene glycol (99%)(Sigma chemicals, USA [26] for oral dosing by a gavage needl, volume of administration 10 ml/kg B.Wt., they given in different doses to evaluate the acute and sub-acute toxic effects of the drug.The doses were chosen according to a pilot study in mice in which ivermectin did not produce over toxicity.

Blood samples collection

After dosing the mice and at the end of the observation period, they were anesthetized the mice with ether by inhalation, and blood was collected from the venous plexus of the eye by using the capillary tubes [27], it was collected in sterile tubes containing heparin(B/Braun Melsungen AG Germany) diluted in a ratio (1: 10) with normal saline, then separate the plasma by using a centrifuge at a speed of 3000 cycles/ min for 15 min, and the plasma was kept in the freezer at - 20 º C until measurements were made on it.

Extraction and preservation of the brain and liver

After collecting the blood, we sacrificed the mice and extracted the brain and liver there preserving them in aluminum foil and freezing at a temperature of - 20º C until they were homogenized to make their measurements.

The process of homogenization

Both brain and liver were homogenized by using a homogenizer (OMNI BEAD RUPTOR , 24 USA), at a speed of 400 cycles / 10 sec, then the samples were transferred to a centrifuge to obtain the leachate, which was separated and kept in freezing at a degree of -20 º C until it was used for measurements of each organ.

Experimental design

Effects of non-lethal doses of ivermectin on GSH and MDA in the brain and liver and on IL-6 and TNF-α in the blood plasma of mice after 1 and 7 days

Thirty mice (male and female), divided into 3 groups, the mice were treated for 1 and 7 days (the mice were given 3 treatments during 7 days) as follows**:**

1st group: (control group): mice were dosed with propylene glycol 10 ml /kg of B. wt., orally.

2nd group: The mice were dosed with ivermectin at a dose of 30 mg/kg of B. wt., orally.

3rd group: The mice were dosed with ivermectin at a dose of 60 mg/ B. wt., orally.

After 1 and 7 days of treatment. Brain and liver glutathione (GSH) and malondialdehyde (MDA) concentrations were measured by the methods of some researchers[28,29], After 1 and 7 days of treatment, blood plasma was collected to measure the (IL-6) and (TNF-α) concentrations by using kits (Sunlong Biotech Co., Ltd) measured by the ELISA technique.

Acute toxic effects of ivermectin on AchE activity in blood plasma and brain of mice after 4 and 24 hr.

Fifteen mice (male and female), randomly divided into 3 groups as follows;

1st group: (control group) mice were dosed with propylene glycol 10 ml/kg of B. wt. orally.

2nd group: mice were given ivermectin at a dose of 75 mg/kg of B. wt. orally.

3rd group: mice were given ivermectin at a dose of 100 mg/kg of B. wt. orally.

After 4 and 24 hr of treatment blood was collected from the mice were killed, and the brains were extracted and frozen at 20- °C until they measured the activity of the AchE by an electrometric method [30], for the determination of brain and blood plasma cholinesterase activity within 4 and 24 hr.

Electrometric technique was used to assess the ChE activity in the plasma and brain samples[31,32]. 0.2 milliliters of plasma or whole brain homogenate, 3 milliliters of pH 8.1 buffer, and 3 milliliters of distilled water made up the reaction mixture in a beaker. A pH meter (HANNA, Romania) was used to determine the combination's initial pH (pH1). After that, 0.10 mL of the substrate, 99% acetylcholine iodide (Avouchem, UK), was added to the mixture, which was then incubated at 37° C for 30 min. The pH of the reaction mixture (pH2) was measured at the end of the incubation period. The enzyme activity was calculated as follows:

ChE activity (ΔpH/30 min.) = (pH1 – pH2) - Δ pH of blank

The blank was without the plasma or brain homogenate sample, The unit of ChE activity was expressed as ΔpH/30 min.

The % of ChE inhibition was calculated as follows: % ChE inhibition= [ChE activity (without ivermectin)-ChE activity (with ivermectin)/ ChE activity (without ivermectin)] X 100.

Statistical analysis

We statistically analyzed the results of our study based on the version 20 SPSS program by analyzing the data using the Two-way analysis of variance test and then we subjected these results to the least significant difference test (LSD) the significant difference in all our tests was at the probability level ($p \leq 0.05$).

Results

Effects of non-lethal doses of ivermectin on the GSH and MDA concentration in brain and liver of mice after 1 and 7 day.

The effects of ivermectin on GSH concentration in the brain and liver of mice after 1 and 7 days.

Oral administration of ivermectin (30 mg/kg B.wt.) after 1 and 7 days caused a slight decrease in the GSH concentration in the mice brain (0.29 ±0.003 &0.21±0.01) µmol/g, respectively, compared to the control groups (0.34±0.01 & 0.37±0.01) µmol/g, respectively, with a decrease in the GSH concentration about (14% & 43%), respectively.

Whereas ivermectin (60 mg/kg B.wt.)1 and 7 days after oral dosing caused a significant decrease in the GSH concentration in the brain (0.20 ±0.01 & 0.20±0.005) µmol/g, respectively, compared to the control groups (0.34±0.01 & 0.37±0.01) µmol/g, respectively, GSH concentration reduced by a value of (41% and 45%) respectively. Fig (1-a).

Oral administration of ivermectin (30 mg/kg B. wt). after 1 day resulted in a slight decrease in the GSH concentration in the liver (7.01 ±0.18) µmol/g and a significant decrease in the concentration after 7 days (5.86±0.19) µmol/g compared to the control groups (7.65±0.10&7.42±0.02) µmol/g, respectively, GSH concentration reduced by a value of (8% & 21%), respectively. Whereas

the ivermectin (60 mg/kg B. wt.), after 1 and 7 days caused a significant decrease in the GSH concentration in the liver (4.99 ±0.05 & 3.22±0.19) µmol/g, respectively, compared to the control groups (7.65±0.10&7.42±0.02) µmol/g, respectively, GSH concentration reduced by a value of (34% &56%) respectively.

High doses of ivermectin were significantly influential and caused a decreased liver GSH concentration significantly compared to the control group at both times. Fig (1-b).

Effects of ivermectin on MDA concentration in the brain and liver of mice after 1 and 7 days.

Ivermectin (30 mg/kg B. wt.) after 1 day resulted in a slight increase in the MDA concentration of the brain (783 ±11.10) µmol/g and a significant increase in the concentration (988±23.53) µmol/g after 7 days of treatment of mice compared to the control groups (746±10.02 & 777±7.57) µmol/g, respectively, with an increased in the value (4% & 27%) respectively, whereas ivermectin (60 mg/kg B.wt.)1 and 7 days after oral dosing caused a significant increase in the MDA concentration of the brain (863 ±8.34 &1064±9.39) µmol/g, respectively, compared to the control groups (746±10.02) and (777±7.57) µmol/g, respectively, with an increased in the value (13% & 37%) respectively. Fig (2-a).

Oral administration of ivermectin (30 mg/kg B. wt.) after 1 and 7 days resulted in a significant increase in the MDA concentration in the liver (744±11.51&871±10.69) µmol/g, respectively, compared to the control groups (696±6.05&753±6.30) µmol/g, respectively, with an increased in the value (6% & 15%) respectively. Ivermectin (60 mg/kg B. wt.) 1 and 7 days after oral dosing caused a significant increase in the MDA concentration in the liver (886±4.84 & 1053±8.07) µmol/g, respectively, compared to the control groups (696±6.05&753±6.30) µmol/g, respectively, with an increased in the value (27% & 39%) respectively. Fig (2-b).

The two doses of ivermectin caused a significant increase in the MDA concentration in the liver of mice compared to the control group at both times.

Effects of non-lethal toxic doses of ivermectin on the concentration of interleukin-6 (IL-6) and tumor necrosis factor-alpha (TNF-α) in the blood plasma of mice after 1 and 7 days.

The oral administration of ivermectin (30 mg/kg B. wt.) caused a slight decrease in the plasma concentration of both cytokines (IL-6 & TNF-α) (11.91±0.13 & 10.83±0.02 ng / L), respectively, compared with the control groups (11.92± 0.13&10.83±0.09 ng/L), respectively. Ivermectin (60 mg/kg B.wt.) caused a significant decrease in the IL-6 concentration (10.65±0.29 ng /L) after 1 day of treatment compared with the control group (11.92± 0.13 ng/L), while it caused a slight decrease in the TNF-α concentration (10.78± 0.05) ng /L compared with the control group (10.83±0.09 ng /L).Fig(3-a).

Ivermectin (30 mg/kg B. wt) slightly affected the concentration of both cytokines (IL-6 & TNF-α), while ivermectin (60 mg /kg B. wt.) caused a significant decrease in the IL-6 concentration (9.71± 0.17 ng/L) and TNF-α concentration (8.46±0.17 ng/L), compared with the control groups(12.01± 0.16&10.89±0.02 ng/L), respectively, 7 days after the treatment of mice. Fig (3- a and b).

Effects of non-lethal toxic doses of ivermectin on the AchE activity in the blood plasma and brain of mice 4 and 24 hr

Oral administration of ivermectin (75 and 100 mg/kg B.wt.) 4 hr after the treatment caused a significant decrease in plasma AchE activity (1.77±0.01&1.54±0.1), respectively, compared to the control group (2.08±0.05), an inhibition activity by a value of (14%& 25%), respectively. also when use measuring the AchE activity in the blood plasma of mice 24 hr after treatment, ivermectin (75mg/kg B. wt), caused a slight decrease in AchE activity compared to the control group with an inhibition ratio of about (10.34%), while ivermectin as (100 mg/kg B. wt.) caused a significant decrease in the enzyme activity (0.77 ± 0.18) compared to the control group with an inhibition ratio of (33 %). Fig.(4-a).

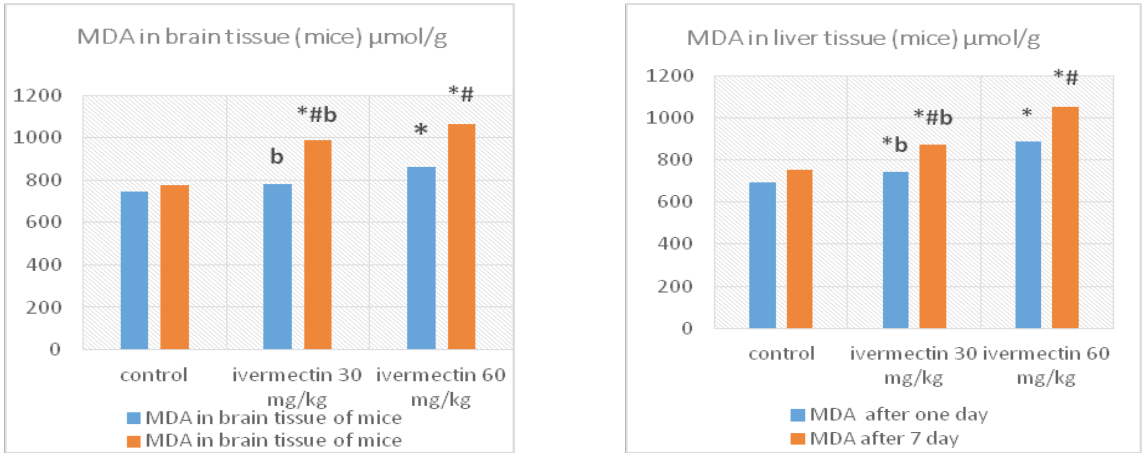
When we compared the enzyme activities at 4 and 24 hr of treatment, we observed a significant decrease in the AchE activity after 24 hr of treatment in all ivermectin-treated mice compared to its activity in the control group (Fig. 4-a).

Oral administration of ivermectin with its two doses (75 and 100 mg/kg B. wt.) 4 hr after the treatment caused a significant decrease in the AchE activity (0.26±0.01 & 0.13±0.01), respectively, compared to the control group (0.54±0.03) with a percentage of inhibition in the activity by a value of (51% & 75%) respectively, also but when measuring the AchE activity in the mice brain 24 hr after oral administration,

ivermectin (75 & 100 mg/kg of B.wt), caused a significant decrease in enzyme activity (0.18±0.03 & 0.13±0.02), respectively compared to the control group (0.41±0.07), inhibition rate of (56% & 68%), respectively, hence the two doses of ivermectin the AchE activity significantly inhibit in the brain of mice compared to the control group at both times (Fig. 4-b) .

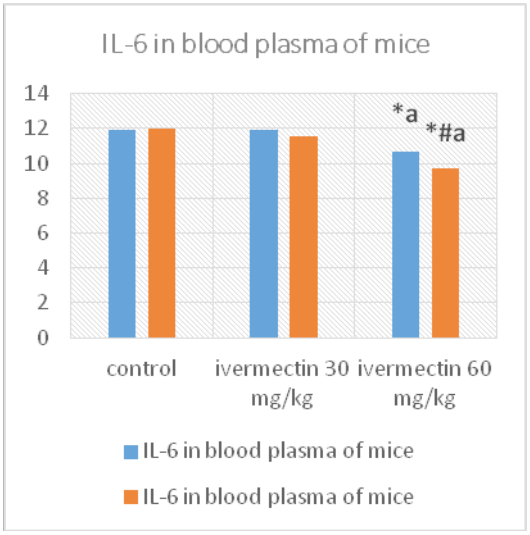
(a) (b)

Fig. 1 (a and b). Effects of ivermectin (30 and 60 mg/kg) in brain and liver GSH conc. after 1 and 7 days.
(*) Significantly different in comparison with the control group at P≤0.05
(#) Significantly different in comparison between the two times (after 1 and 7 days) of treatment at P≤0.05
(a) Significantly different with the group treated with ivermectin at a dose of 30 mg/kg of B. wt. at P≤0.05.

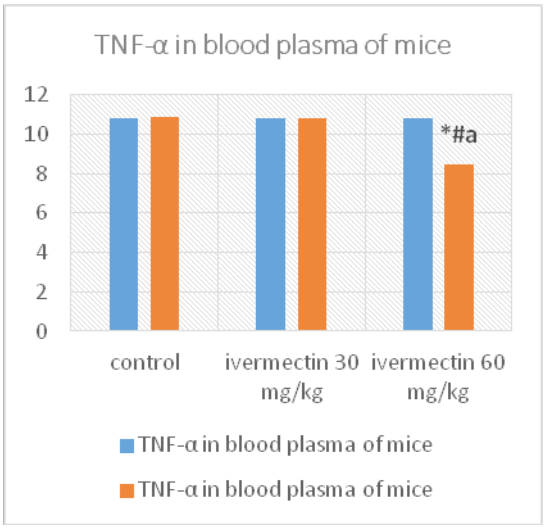


(a) (b)

Fig. 2(a and b). Effects of ivermectin (30 and 60 mg/kg) in the brain and liver MDA conc. after 1 and 7 days.
(*) Significantly different in comparison with the control group at P≤0.05
(#) Significantly different in comparison between the two times (after 1 and 7 days) of treatment at P≤0.05
(a) Significantly different with the group treated with ivermectin at a dose of 30 mg/kg of B. wt. at P≤0.05.
(b) Significantly different with the group treated with ivermectin at a dose of 60 mg/kg of B. wt. at P≤0.05.



(a)



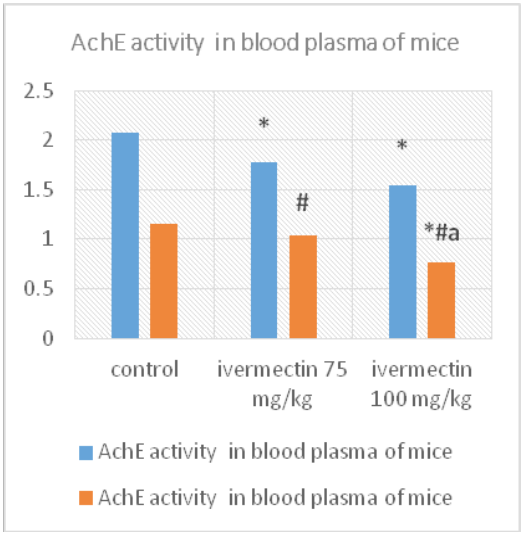
(b)

Fig. 3(a and b). Effects of ivermectin (30 and 60 mg/kg) in the (IL-6) conc. in the blood plasma of mice after 1 and 7 days.

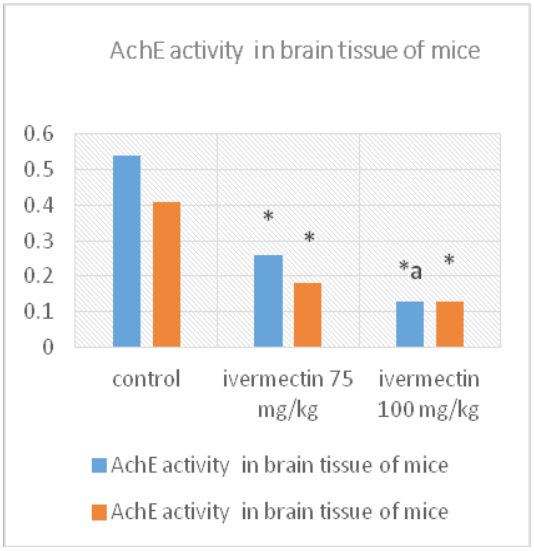
(*) Significantly different in comparison with the control group at P≤0.05

(#) Significantly different in comparison between the two times (after 1 and 7 days) of treatment at P≤0.05

(a) Significantly different with the group treated with ivermectin at a dose of 30 mg/kg of B. wt. at P≤0.05.



(a)



(b)

Fig.4(a and b). AchE activity in the blood plasma and brain of mice treated with ivermectin (75 and 100 mg/kg) after 4 and 24 hr.

(*) Significantly different in comparison with the control group at P≤0.05

(#) Significantly different in comparison between the two times (after 1 and 7 days) of treatment at P≤0.05

(a) Significantly different with the group treated with ivermectin at a dose of 75 mg/kg of B. wt. at P≤0.05.

Discussion

Ivermectin is an antiparasitic medicine that belongs to the avermectins family of drugs. It is a mixture of at least 80% of an avermectin B1a analog and at least 20% of an avermectin B1b analog. Ivermectin is widely used in veterinary medicine [33]. Ivermectin is a very safe drug, however, there are many studies on its toxic effects in different types of animals, which reported in dogs , cats, horses , pigs, cattle and frogs [25], Its toxicity was more for young animals than for adults due to increase in the sensitivity to ivermectin as a result to lack of BBB development [34].

The oral ivermectin LD_{50} in mice was 115.25 mg/kg of B.wt and approximate lethal dose of ivermectin was 121 mg/kg of B.wt. [35]. In our experiments we use doses were not lethal or even overtly toxic.

Oxidative stress and reactive oxygen species ROS are strong indicators of diseases produced as a natural product of cellular metabolism [36,37]. Lipid oxidation, a complex chain reaction that creates numerous compounds such as lipid hydroperoxides and malondialdehyde as a result of free radicals' impact on lipids [**38].** Oral administration of ivermectin (30 and 60) mg/kg B.wt. after 1 and 7 days caused a significant decrease in the GSH concentration in the mice brain and liver compared to the control groups with a high values of decrease in the GSH concentration after 7 days reach to (41% & 72%) in the brain and (21% and 56%) in the liver , respectively, this indicates the ability of ivermectin to induce an oxidative imbalance which leads to increased oxidative stress and lipid peroxidation [14], When ivermectin induced adverse reactions have highlighted its oxidative nature that increasing toxic oxygen intermediates then it induced biochemical changes in the host animals as GSH and MDA concentration in rats blood plasma [13], or in carp fish tissues [14].

The main functions of GSH include detoxification of free radicals and ROS in the cells and acts on the synthesis of DNA and proteins and has an important role defensively as an antioxidant and regulates the growth and death of cells in processes such as regulation of the oxidation-reduction state within the cell [39-41]. Ivermectin (30 and 60)mg/kg cause an increase in the MDA concentration as a result to the formation of more free radicals which cause damage to the lipid bio membrane, mainly polyunsaturated fatty acids, which cause hyper oxidation of fats, leading to an increase in the concentration. The increase in MDA levels shows that there is a reduced generation of OH free radicals and less damage to the biomembrane [42,43].

When we studied the toxic effects of ivermectin on the immune response in mice, by measuring the concentration of some cytokines(IL-6) and the (TNF-α) concentration in the blood plasma of mice, the results showed minor effects of ivermectin of these cytokines compared to the control group after 1 and 7 days of treatment, which indicates ivermectin did not produce any immunological stimulation in monocytes, macrophages, T and B lymphocytes, and mast cells [44], therefore, the immune response did not appear when we measured the concentration of IL-6 and TNF- α, perhaps because these doses are in low toxicity and the number of exposure to these doses is low that they did not stimulate the release of these cytokines [1].

On the other hand, non-lethal toxic doses of ivermectin (75 and 100) mg/kg B.wt., caused a significant decrease in the AchE activity in the blood plasma (25% and 33 %) and in the brain (75% & 68%), respectively, after 4 and 24 hr of treatment, which are a high percentage compared to the decrease in the activity of the enzyme in the blood plasma of mice, and this disparity in the inhibition rates between brain and blood plasma may be due to the nonspecific inhibition, and its possible that GABA-mimetic effects of ivermectin has overcome any other excitatory toxic manifestations in mice as a results of cholinesterase inhibition. The clinical significance of the present finding is to avoid the combination of anticholinesterase drugs or insecticides with ivermectin intended for animal therapy [45].

Conclusion

Non-lethal toxic doses of ivermectin affected the oxidative stress, Did not affect the immune response of treated mice, High doses of ivermectin affected the activity of AchE.

Acknowledgment

The work gets support from the University of Mosul, College of Veterinary Medicine. Farah Almawla & Banan kh. AlBaggou planned, analyzed, evaluated, wrote, and approved this article.

Conflicts of Interest

The authors declare that no prospective conflicts of interest exist.

Funding statement

This work was not support.

References

1. Zhang, X., Song, Y., Ci, X., An, N., Ju, Y., Li, H., Wang, X., Han, C., Cui, J. and Deng, X., 2008
2. . Ivermectin inhibits LPS-induced production of inflammatory cytokines and improves LPS-induced survival in mice. *Inflamm. Res*, **57**, 524–529(2008).
3. Lierz, M. Evaluation of the dosage of ivermectin in falcons. *Vet. Rec.;* **148**(19), 596–600 (2001).
4. Wolstenholme AJ, Rogers AT. Glutamate-gated chloride channels and the mode of action of the avermectin/milbemycin anthelmintics. *Parasitology*., **131**(S1), S85-95(2005).
5. Chandler, R.E. Serious neurological adverse events after ivermectin—do they occur beyond the indication of onchocerciasis?. *The American Journal of Tropical Medicine and Hygiene*, **98**(2),382(2018).
6. Novelli, A., Vieira, B.H., Cordeiro, D., Cappelini, L.T.D., Vieira, E.M. and Espíndola, E.L.G. Lethal effects of abamectin on the aquatic organisms Daphnia similis, Chironomus xanthus and Danio rerio. *Chemosphere*, **86**(1),36–40(2012).
7. Laing, R., Gillan, V. and Devaney, E. Ivermectin–old drug, new tricks?.*Trends in Parasitology*, **1**, 33 (6), 463-72(2017).
8. Aktaruzzaman, M., Islam, M.S., Hasan, M.M., Bhuiyan, M.J., Hossain, M.M., Hossain, M.K., Lucky, N. and Howlader, M. Evaluation of anthelmintic efficacy of ivermectin, levamisole HCL and albendazole administered through different routes against naturally occuring gastrointestinal nematodiasis in Black Bengal Goat inducing hematological parameters and live weight indices. *International Journal of Natural Sciences*, **5**(1), 26-349(2015).
9. Dong, Z., Xing, S., Zhang, J. and Zhou, X. 14-Day Repeated Intraperitoneal Toxicity Test of Ivermectin Microemulsion Injection in Wistar Rats. *Front. Vet. Sci.,* **7**, 598313, eCollection (2020). doi: 10.3389/fvets.2020.598313.
10. Othman, H.M.A., Othman, F.M.A. and Aljali, A.A. The effect of different dosages on hematological and some biochemical parameters of ivermectin after administration in goats. *Libyan J. Basic Sci*., **17**(1),35–43 (2022).
11. Yang, C-C. Acute human toxicity of macrocyclic lactones. Curr. Pharm. *Biotechnol*., **13**(6),999–1003 (2012).
12. Al-Rekabi, F.M.K., Alsadawi, A. and Al-ameedi, A.I. A new approach in treatment acute ivermectin toxicity in male balb-c mice. *Iraqi J. Agric. Sci*., **52**(2),301–308(2021).
13. Donfo-Azafack, C., Nana-Djeunga, H.C., Wafeu-Sadeu, G., Dongmo-Yemele, R. and Kamgno, J. Successful management of poisoning with ivermectin (Mectizan) in the Obala health district (Centre Region, Cameroon): a case report. *Journal of Medical Case Reports*, **17**(1), 1-4(2023).
14. Qureshi, S. Biochemical toxicity of ivermectin in Wistar albino rats. *Am-Eur J. Toxicol. Sci*., **5**(1),15–19 (2013).
15. Kolarova, J., Stara, A., Zuskova, E. and Velisek, J. Safety of the anthelminthic drugs levamisole, fenbendazole, and ivermectin administered in therapeutic baths for the common carp Cyprinus carpio. *Vet. Med.(Praha).,* **67**(7),371–378 (2022).
16. Abd Ellah, M.R. Involvement of free radicals in animal diseases. *Comp. Clin. Path*., **19**, 615–619(2010).
17. Pamplona, R. and Costantini, D. Molecular and structural antioxidant defenses against oxidative stress in animals. *Am. J. Physiol. Integr. Comp. Physiol*., **301**(4),R843–863(2011).
18. Xing, H., Li, S., Wang, Z., Gao, X., Xu, S. and Wang, X. Histopathological changes and antioxidant response in brain and kidney of common carp exposed to atrazine and chlorpyrifos. *Chemosphere,* **88**(4),377–383(2012).
19. Ma, Y., Xu, X., Wu, H., Li, C., Zhong, P., Liu, Z., Ma, C., Liu, W., Wang, C., Zhang, Y. and Wang, J. Ivermectin contributes to attenuating the severity of acute lung injury in mice. *Biomedicine & Pharmacotherapy*, **1**(155),113706(2022).
20. Hos, J. and Zelova, H. TNF- a signalling and inflammation : Interactions Between old acquaintances. *Inflammation Research*, 62(7);641–651(2013). DOI:10.1007/s00011-013-0633-0

21. Rose-John, S. Interleukin-6 family cytokines. Cold Spring Harb Perspect *Biol*., **10**(2), a028415(2018).

22. Geginat, J., Larghi, P., Paroni, M., Nizzoli, G., Penatti, A. and Pagani, M. The light and the dark sides of Interleukin-10 in immune-mediated diseases and cancer. *Cytokine Growth Factor Rev.,* **30**, 87–93(2016).

23. Kany, S., Vollrath, J.T. and Relja, B. Cytokines in inflammatory disease. *Int J Mol Sci*.;**20**(23):6008(2019).

24. Lakshmanan, M. Cholinoceptor Agonists and Anticholinesterase Agents. Introd to Basics Pharmacol Toxicol Vol. 2 Essentials Syst. Pharmacol. From Princ. to *Pract*., **3**–24(2021).

25. Masson, P., Nachon, F., Broomfield, C.A., Lenz, D.E., Verdier, L., Schopfer, L.M. and Lockridge, O. A collaborative endeavor to design cholinesterase-based catalytic scavengers against toxic organophosphorus esters. *Chemico-biological Interactions*, **175**(1-3), 273-280(2008).

26. Hayes, Jr. W.J. and Laws, Jr. E.R. Handbook of Pesticide Toxicology. Volume 2. Classes of Pesticides. New York, NY: Academic Press, *Inc.* (1991).

27. Al-Najmawi, T. K. and Al-Zubaidy, M. H.Acute toxicity events of ivermectin in chicks' model. *Iraqi Journal of Veterinary Sciences,* **36**(4), 1119–1124(2022).

28. Teilmann, A.C., Nygaard Madsen, A., Holst, B., Hau, J., Rozell, B. and Abelson, K.S.P. Physiological and pathological impact of bloodsampling by retro-bulbar sinus puncture and facial vein phlebotomy in laboratory mice. *PLoS* One., **9**(11),e113225(2014). 28. James, R.C., Goodman, D.R. and Harbison, R.D. Hepatic glutathione and hepatotoxicity: changes induced by selected narcotics. *J. Pharmacol.Exp. Ther*., **221**(3),708–714(1982).

29. Ohkawa, H., Ohishi, W. and Yagi, K. Colorimetric method for determination of MDA activity. *Biochemistry*, **95**,351(1979).

30. Michel, H.O. An electrometric method for the determination of red blood cell and plasma cholinesterase activity. *J. Lab. Clin. Med*., **34**,1564–1568(1949).

31. Mohammad, F.K., Bhattacharyya, H.K., Fazili, M.R., Nasreen, S., Jeelani, S.G. and Sheikh, N.A Review of a Practical Electrometric method for determination-of Blood and Tissue Cholinesterase activities in Animals. *Feedback*, **2**, 16(2007).

32. Al-Baggou, B.K., Naser, A.S. and Mohammad, F.K. Hydrogen peroxide potentiates organophosphate toxicosis in chicks. *Hum. Vet. Med*., **3**(2),142–149(2011).

33. Hopper, K., Aldrich, J. and Haskins, S.C. Ivermectin toxicity in 17 collies. *J. Vet. Intern. Med.*, **16**(1), 89–94(2002).

34. Lankas, G. R. and Gordon, L. R. Toxicology .In". Ivermectin and abamectin ". WC Campbell,(Ed.) Springer-Verlag. New York, *USA*(1989).

35. Almawla, F. F. and Baggou, A. Acute Toxic Effects of levamisole and Ivermectin in Mice. *Journal of Applied Veterinary Sciences,* **8** (3),75-81(2023) .

36. Ince, S., Kozan, E., Kucukkurt, I. and Bacak, E. The effect of levamisole and levamisole+ vitamin C on oxidative damage in rats naturally infected with Syphacia muris. *Exp. Parasitol*., **124**(4),448–452(2010).

37. Lightbody, J.H., Stevenson, L.M., Jackson, F., Donaldson, K. and Jones, D.G. Comparative aspects of plasma antioxidant status in sheep and goats, and the influence of experimental abomasal nematode infection. *J. Comp. Pathol*., **124**(2–3),192–199(2001).

38. Kucukkurt, I., Ince, S., Fidan, A.F. and Ozdemir, A. The effects of dietary supplementation of different amount of Yucca schidigera powder (Sarsaponin 30®) on blood and tissue antioxidant defense systems and lipid peroxidation in rats. *J. Anim. Vet. Adv*., **7**(11),1413–1479(2008) .

39. Rana, S.V.S., Allen, T. and Singh, R. Inevitable glutathione, *then and now*. *Indian Journal of Experimental Biology,* **40**, 706-716 (2002).

40. Lobo, V., Patil, A., Phatak, A. and Chandra, N. Free radicals, antioxidants and functional foods: Impact on human health. *Pharmacogn. Rev*., **4**(8),118(2010).

41. Deponte, M. Glutathione catalysis and the reaction mechanisms of glutathione-dependent enzymes. *Biochimica et Biophysica Acta (BBA)-General Subjects*, 1830(5), 3217-3266(2013).

42. Sarkar, B., Kulharia, M. and Mantha, A.K. Understanding human thiol dioxygenase enzymes: structure to function, and biology to pathology. *Int. J. Exp. Pathol*., **98**(2),52–66(2017).

43. Grune, T., Sommerburg, O., Petras, T. and Siems, W.G. Postanoxic formation of aldehydic lipid peroxidation products in human renal tubular cells. *Free Radic. Biol. Med*., **18**(1),21–27(1995).

44. Jha, R.K., Ambad, R., Kamble, A. and Lamture, Y. Comparative Study of Malondialdehyde, Superoxide Dismutase and Glutathione Peroxidase in Urolithiasis Patients: A Case Control Study. *J. Pharm. Res*., **15**,208–213(2021).

45. Sadiq, S.A. Monitoring exposure of sheep treated with insecticide ivermectin. Diploma report. College of Veterinary Medicine, University of Mosul, Mosul, Iraq. (2011).

الكشف عن سمية الإيفرمكتين باستخدام بعض القياسات الكيمو حيوية والمناعية في الفئران

فرح فتح الله شهاب المولى و بنان خالد عبد الرحمن البكوع
قسم الفسلجة والكيمياء الحياتية والأدوية - كلية الطب البيطري - جامعة الموصل - الموصل - العراق.

هدفت دراستنا الكشف عن السمية الحادة وتحت الحادة المحدثة بالايفرمكتين باستخدام بعض المعايير الكيمياحيوية والمناعية في الفئران. سببت الجرع السامة غير المميتة من الإيفرمكتين انخفاضا معنويا ملحوظا في تركيز قلوتاثايون في مخ وكبد الفئران بعد 1 و 7 أيام من المعاملة نتيجة الإجهاد التأكسدي الناجم عن التسمم بهذا الدواء، كما سبب ارتفاعا معنويا في تركيز المالونداياالديهايد في مخ وكبد الفئران مقارنة بمجموعة السيطرة.

لم تظهر النتائج تأثيرات واضحة في الاستجابة المناعية للفئران عند تسممها بجرع سامة غير مميتة من الإيفرمكتين ، حيث كان تأثيره بسيطا جدا على بعض السايتوكينات المتمثلة بتركيز الانترلوكين 6 (IL-6) وعامل تنخر الورم الفا (TNF-α) في بلازما دم الفئران بعد 1 و 7 أيام من المعاملة، وأظهرت الفئران المتسممة فمويا بالإيفرمكتين (75 و 100) ملغم /كغم من وزن الجسم انخفاضا معنويا في نشاط انزيم الاسيتيل كولين استراز بمعدلات تثبيط وصلت إلى (25 و 33٪) في بلازما دم الفئران و (75 و 68٪) في مخ الفئران على التوالي ، واظهرت نسب تثبيط عالية في نشاط الانزيم بعد 4 و 24 ساعة من المعاملة ، و بالرغم من الاستخدام الامن للايفرمكتين أثبتت نتائجنا ان هذا العقار يمتلك تأثيرات سمية وكيميا حيوية على مستوى الاجهاد التاكسدي ونشاط وفعالية انزيم الاسيتيل كولين استريز لم تسجل من قبل.

الكلمات المفتاحية: الايفرمكتين ، الاجهاد التاكسدي ، الاستجابة المناعية ، نشاط انزيم الكولين استراز ، الفئران.