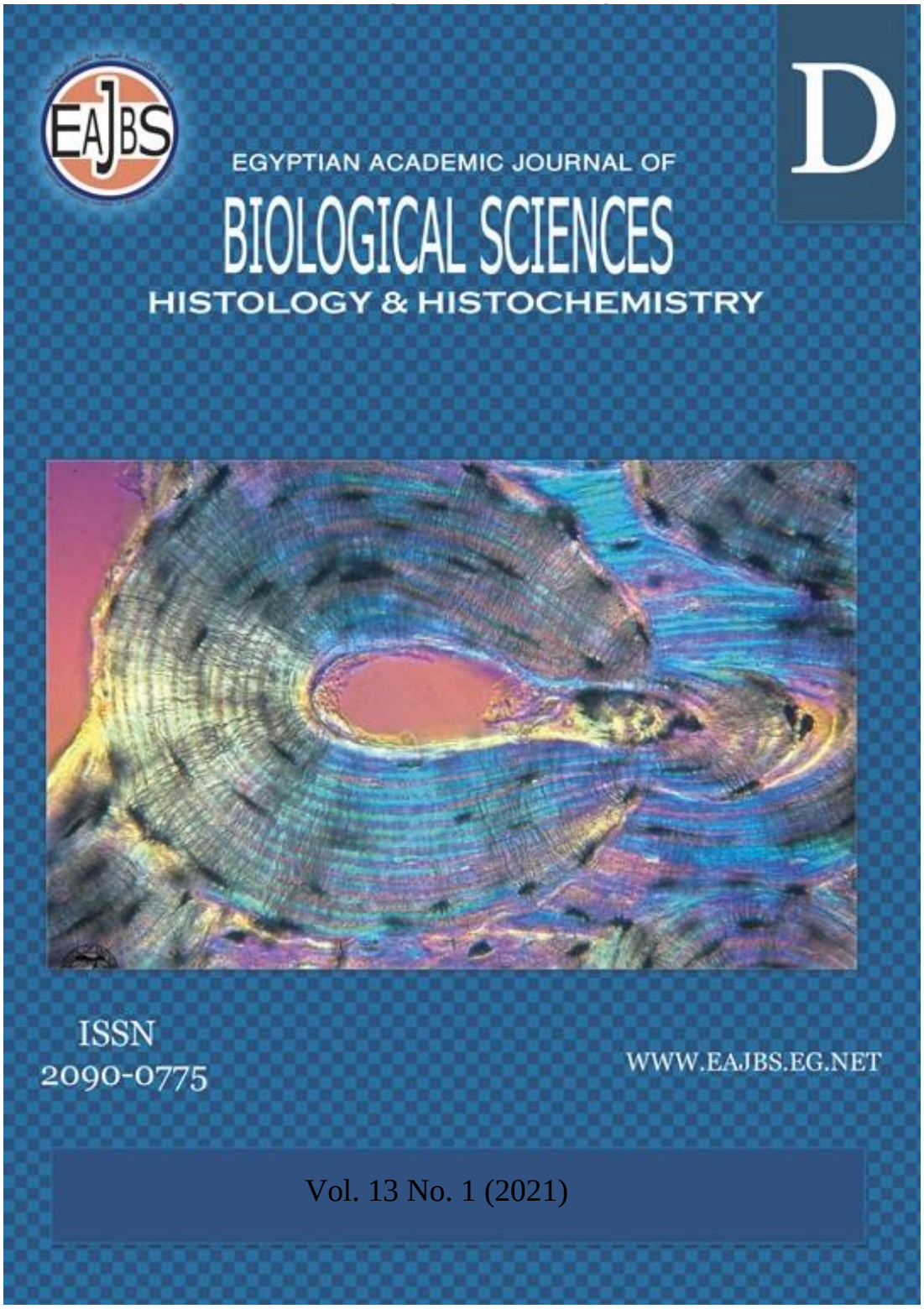

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Protective Effects of Vitamin C on Ivermectin Induced Toxicity on Kidney Functions and Brain Tissue in Rabbits (*Oryctolagus cuniculus*)

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ABSTRACT

The aim of this study was to evaluate the effects of repeated intake of a high dose of Ivermectin (IVM) alone or with co-administration of Vitamin C on kidney biochemical parameters and histological structure of kidney and brain of male rabbits (*Oryctolagus cuniculus*). Male rabbits were distributed into four groups (5 rabbits/group) and treated for three consecutive weeks: receiving distilled water; IVM 2 mg/kg b.w. subcutaneously, 3 times a week; IVM + Vitamin C 20 mg/1mL by gavage and IVM + Vitamin C 200mg /kg of diet in food. IVM significantly ($p < 0.05$) increased the plasma level of creatinine compared to the control group. Co-treatment with vitamin C orally by gavage ameliorated this level compared to IVM group. The histopathological examination of kidney sections in IVM alone treated group showed dilated Bowmans' spaces, dilated renal tubules, vascular congestion and cell infiltration. The brain tissues demonstrated vacuolated neuropil and degenerative changes in nerve cells in both the cerebral cortex and hippocampus of IVM-treated rabbits. However, these histological changes were moderate in both vitamin C co-treated groups. In conclusion, vitamin C has a protective effect against nephrotoxicity and neurotoxicity induced by subacute administration of a high dose of IVM.

INTRODUCTION

Macrocyclic lactones (MLs) represent a wide family of antiparasitic drugs of a broad spectrum (McKellar and Benchaoui 1996). MLs are divided into two groups of molecules: avermectins (AVMs) and milbimycins. AVMs were firstly discovered in the fermentation bouillon of *Streptomyces avermitilis*, a soil microorganism (GabAllh *et al*., 2017). After performing many bioassays, eight natural components, specifically A1a, A1b, A2a, A2b, B1a, B1b, B2a and B2b, were found. AVMs molecules of B series were extremely effective over helminthes and arthropods. Ivermectin (IVM; 22, 23-dihydro-avermectin B1) was commercialized for animal and clinical use in 1981 (Shoop *et al*., 1995).

Based on its high efficacy and tolerability, IVM is a miraculous endectocide drug widely used as an antiparasitic agent in veterinary and human therapy (Trailović and Nedeljković 2010).

IVM is utilized in a large range of animals for internal and external parasites, it is used with different doses as heartworm prophylaxis, ectoparasite treatment and a microfilaricide (Papich 2015). In human medicine, IVM was very effective in treating onchocerciasis (Crump and Omura 2011), strongyloidiasis, scabies, gnathostomiasis, pediculosis, myiasis, leishmaniasis (Dos Santos *et al*., 2009; Geary, 2005), children head lice and scabies (Bockarie *et al*., 2000), it was also found efficient against malaria parasite and vector (Azevedo *et al*., 2019; Mekuriaw *et al*., 2019) and *Papulopustular rosacea* (De Ménonville *et al*., 2017). As well, the use of IVM in a mice model was associated with a decrease in alcohol intake (Yardley *et al*., 2012). The anticancer and the antiviral activity of IVM are well known. Thus recently, IVM 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.

IVM is a lipophilic drug that acts by preserving the transmission of electrical impulses in nerves and muscles by intensifying the glutamate-gated chloride ions channels (GluCls) in parasites. This leads to cell penetration of more chloride ions resulting in hyperpolarization and paralysis of the neuromuscular systems of invertebrates (Bloomquist 2003).

The GluCls are entirely absent in the nervous system of vertebrates. However, MLs are well known to link to other transmembrane receptors that are existing in Mammals as well as γ-aminobutyric acid type A ($GABA_A$), glycine, neuronal α_7-nicotinic and purinergic P_{2x4} receptors. AVMs are well tolerated in vertebrates and characterized with few to no side effects. This can be explained by their low affinity to the aforementioned receptors compared to GLuCls (Spampanato *et al*., 2018). On the other hand, the most common adverse effects of AVMs are related to $GABA_A$ receptor activation (Moreira *et al*., 2017). According to (Kiki-Mvouaka *et al*., 2010), this lack of side effects can be due to the presence of a P-glycoprotein pump in the blood-brain barrier that limits the AVMs penetration in the central nervous system (CNS). Even the wide safety of MLs in both animals and human, they can be toxic and even mortal in mammals with P-glycoprotein deficiencies (Menez *et al*., 2012). In another way, whereas the proper mechanism is unclear, MLs administered at high doses may penetrate through the blood-brain barrier and cause GABA-mimetic toxic effects in mammals. This toxicity starts with hyper-excitability, incoordination, tremors and hypotension and later expands into ataxia, coma and respiratory failure and may lead to death (Yang 2012).

IVM when taken orally or in other routes is well diffused to all tissue compartments via blood circulation, the widest quantity is found in the liver, while the smallest one is found in the brain tissue because of the blood-brain barrier limitation. IVM is almost metabolized in the liver by the cytochrome P450; it is mainly eliminated in the feces while only < 5% is excreted via the kidneys. This unequal distribution of IVM in different organs can explain the diverse toxicity of IVM between invertebrates and their vertebrate hosts (Kudzi *et al*, 2010).

In addition to the clinical signs of IVM neurotoxicity, actually many studies have detected the effect of Therapeutic and double therapeutic doses of IVM on kidney function in rats (Arise and Malomo 2009; El Sawy *et al*., 2015; Elzoghby *et al*., 2015; Omshi *et al*., 2018) and brain tissue especially the cerebral cortex in rabbits (GabAllh *et al*., 2017). Conversely, few studies were conducted on the functional and histopathological effects of IVM on the hippocampus. The hippocampus which is fully developed only in mammals is located under the cerebral cortex and form a key brain zone for many forms of learning and memory (Amin *et al*., 2013). While there are various studies on the toxic effect of IVM, there is a need to study this effect at repeated high-dose therapy.

The toxic effects of AVMs are mainly attributed to oxidative stress (Zhu *et al*., 2017), which explains the need to use antioxidant products to alleviate their harmful effects in mammals. The utilization of natural antioxidants to ameliorate different xenobiotic-induced alterations in vertebrates becomes a field of interest for many recent researchers. Amongst natural antioxidants, vitamin C (ascorbic acid) is a water-soluble vitamin that protects cellular compartments in the face of reactive oxygen species (Jurczuk *et al*., 2007). It is one of the main important elements in the biological system (Magdy *et al*., 2016). This vitamin must be incorporated into the diet for the reason that it cannot be produced in mammals' organisms (Radi *et al*., 2020). It has been demonstrated that vitamin C ameliorates AVMs induced biochemical; histopathological and/or oxidative stress parameters alterations in kidneys (Omshi *et al*., 2018; Magdy *et al*., 2016; Al-Jassim *et al*., 2016**)**, brain tissues (Radi *et al*., 2020) and other organs (Khaldoun Oularbi *et al*., 2017).

The objectives of this study were to examine the impact of sub-acute exposure to a high dose of IVM (Avimec®) on the renal biochemical parameters and histopathological modifications in kidneys and brain tissues of male rabbits (*Oryctolagus cuniculus*). Moreover, the ameliorative effect of vitamin C against IVM toxicity was also tested in this experiment.

MATERIALS AND METHODS

Chemicals:

The injectable solution of IVM (AVIMEC®, 10 mg/ml) and Vitamin C (the content of which was more than 99%) were purchased from the Arab Veterinary Industrial Company, Jordan and Sigma–Aldrich Chemicals Co. (St. Louis, Missouri, USA), respectively.

Animals:

To perform this study, young male rabbits of the local breed (*Oryctolagus cuniculus*) with an initial weight of 0.85 to 0.95 kg were used in this work. Rabbits were supplied from a breeding barn of Djebla (Tizi-Ouzou) and maintained for experimentation in the Saidal CRD, Algiers. All animals were lodged in temperature and hygrometry-controlled rooms with a 12 h light/dark cycle. Animal experimentation was consistent with the Guiding Principles in the Use of Animals in Toxicology (Derelanko and Auletta 2014). Before any treatment, the rabbits were subjected to a 14-day acclimation period and were fed with a commercial pellet diet and water *ad libitum*.

Experimental Design:

The experiment was carried out on 20 male rabbits divided into four groups (n=5) according to their homogeneous mean weight in each group. A control group was given distilled water by gavage; IVM group, treated subcutaneously with a high dose of IVM (2 mg/kg of body weight, three times/week) for 21 days (Lu *et al*., 2017); IVM + Vit C_g group; treated with the same dose of IVM and Vitamin C by gavage (20 mg / 1ml, three times/week) and IVM + Vit C_f group, treated with the same dose of IVM and Vitamin C supplemented in food (200

mg/kg of food, three times/week) (Fetoui *et al*., 2010). Vitamin C was administered 24 h after IVM injection. During the acclimation (2 weeks) and experimentation (3 weeks) periods, all animals were weighed daily. While the blood samples were collected at 14 and 21 days of the experiment in heparin tubes from the lateral marginal vein of the ear for renal biomarkers analysis. After 21 days of treatment, all rabbits were sacrificed by cervical decapitation, and kidneys and brain were carefully removed and weighed.

Biochemical Analysis:

To evaluate the effect of ascorbic acid on IVM toxicity, Blood samples were gathered after a fasting period of 12 hours and plasma was separated by centrifugation at 3000 rpm for 20 min. The following renal parameters; creatinine, uric acid and urea were evaluated in plasma using a commercially available spectrophotometric enzymatic kit (Biolabo, France) and analyzed by an auto-analyzer (Hitachi 912) instrument (Roche Diagnostics, Mannheim, Germany).

Histopathological Examinations:

For the histopathological study, the kidneys and the brain were dissected out from all rabbits and fixed in 10% neutral formalin buffer, processed through graded alcohols and xylene and embedded in paraffin blocks. Organ sections were cut and stained with haematoxylin and eosin (H&E) for histopathological examinations.

Statistical Analysis:

Statistical analysis was carried out using Statistica version 10.0 (Stat Soft Inc., Tulsa, USA). Data were analyzed using one-way analysis of variance (ANOVA) followed by Duncan's post hoc tests. Data were expressed as the mean ± SD. A p-value < 0.05 was considered as a level of significance.

RESULTS

No deaths occurred in any group during the experiment. However, severe physical signs (tremor and commotion), hair loss, diarrhea and reduced activity were observed in IVM group rabbits compared to the control group.

Effect on Absolute and Relative Organ Weights:

As mentioned in **Table 1,** the absolute and relative weight of both right and left kidneys showed a significant ($p<0.05$) increase in the IVM group compared to control group. On the other hand, these weights were not significantly changed in the IVM + Vit C-treated groups compared to control group. However, the absolute and relative weights of the brain were significantly decreased in the IVM group compared to control group without a significant modification of the brain weight in both IVM + Vit C-treated groups compared to control.

Table 1: Effect of IVM and/or Vitamin C on absolute and relative kidneys' and brain weights, 21 days after treatment.

Organ weight (g) / groups			Control	IVM	IVM + $VitC_g$	IVM + $VitC_f$
Kidneys	RK	Absolute	5.7 ± 0.9	6.8 ± 1.3*	5.9 ± 0.7	6.0 ± 0.8
		Relative	0.3 ± 0.01	0.4 ± 0.1*	0.3 ± 0.02	0.3 ± 0.1
	LK	Absolute	6.0 ± 0.7	6.7 ± 1.4*	6.1 ± 0.7	6.1 ± 1.8
		Relative	0.3 ± 0.01	0.4 ± 0.1*	0.3 ± 0.03	0.3 ± 0.1
Brain	Absolute		8.4 ± 0.1	7.6 ± 0.3*	8.3 ± 0.3	8.3 ± 2.4
	Relative		0.4 ± 0.01	0.35±0.03*	0.5 ± 0.01	0.5 ± 0.1

IVM: Ivermectin; VitC: Vitamin C; $VitC_g$: Vitamin C by gavage; $VitC_f$: Vitamin C supplemented in food; RK: right kidney; LK: left kidney. Results are given as a mean ± SD for five rabbits in each group.

*: $p < 0.05$ (significant difference between IVM group and control).

Effect on Serum Biochemical Parameters:

The results of renal biochemical parameters are shown in Table 2. IVM induced nephrotoxicity as proved by the elevation of kidneys' biomarkers in plasma. On day 14 of experimentation, only the creatinine level increased significantly ($p<0.05$) in the IVM group compared to the control group. This level was significantly decreased in IVM + $VitC_g$ group compared to IVM group. On day 21 of experimentation, the plasma level of creatinine continues to be increased significantly in the IVM group compared to control without any significant change in the same level in IVM + VitC-treated groups. However, the levels of uric acid and urea didn't change significantly between the four studied groups on days 14 and 21 of the experimental period.

Table 2: Effect of IVM and/or Vitamin C on renal biomarkers at 14 and 21 days of experimentation.

Period	Biochemical parameters	Experimental groups			
		Control	IVM	IVM + $VitC_g$	IVM + $VitC_f$
14 day	Creatinine (mg/dl)	7.16 ± 0.01	10.8 ± 0.01[a]	8.85± 0.13[b]	7.4 ± 0.36
	Uric acid (mg/dl)	4.4 ± 0.02	5.1 ± 0.01	4.9 ± 0.6	4.8 ± 0.4
	Urea (mg/dl)	0.27 ± 0.2	0.32 ± 0.02	0.24 ± 0.03	0.29 ± 0.01
21 day	Creatinine (mg/dl)	7.24 ± 1.69	12.24 ± 0.88 [a]	7.38 ± 0.51	7.72 ± 1.3
	Uric acid (mg/dl)	4.4 ± 0.03	4.4 ± 0.01	4.91 ± 1.4	4.5 ± 0.3
	Urea (mg/dl)	0.27 ± 0.02	0.34 ± 0.01	0.25 ± 0.02	0.30 ± 0.03

IVM: Ivermectin; VitC: Vitamin C; $VitC_g$: Vitamin C by gavage; $VitC_f$: Vitamin C supplemented in food. Results are given as a mean ± SD for five rabbits in each group.

A and b: $p < 0.05$ (a: significant difference between all treated groups and control, b: significant difference between IVM and IVM + $VitC_g$ groups).

Histopathological Results:

In the control group, the kidney showed normal morphology with a normal appearance of renal corpuscles, glomeruli, surrounded by Bowman's spaces and cortical tubules with proximal and distal convoluted tubules (Fig. 1: A). In IVM alone treated group, the kidney cortex showed severe pathological alterations, including vascular congestion, mononuclear infiltrating cells, dilation of the Bowmans' spaces, vacuolization in tubular cells and dilated proximal and distal convoluted tubules (Fig. 1: B & C). These pathological changes were ameliorated in IVM + Vitamin C groups (Fig. 1: D).

The cerebral cortex of control (Fig. 2: A & B) rabbits exhibited normal cellular architecture. However, IVM group (Fig. 2: C, D & E) showed abnormal cellular morphology of granule cells and pyramidal cells in granular and pyramidal layers, respectively accompanied by severe vacuolation of neuropil and necrosis of neurons in the brain cortex. In Vitamin C-treated group's (Fig. 2: F & G) modulation of the abnormalities in brain cortex histopathology as compared to IVM group are shown.

The histological structure of the hippocampus of IVM alone treated group (Fig. 3: C & D) showed degenerative changes mainly neuropil vacuolation and intracellular vacuoles, which result in foam structure cells as compared to control (Fig. 3: A & B) and Vitamin C exposed rabbits (Fig. 3: E & F).

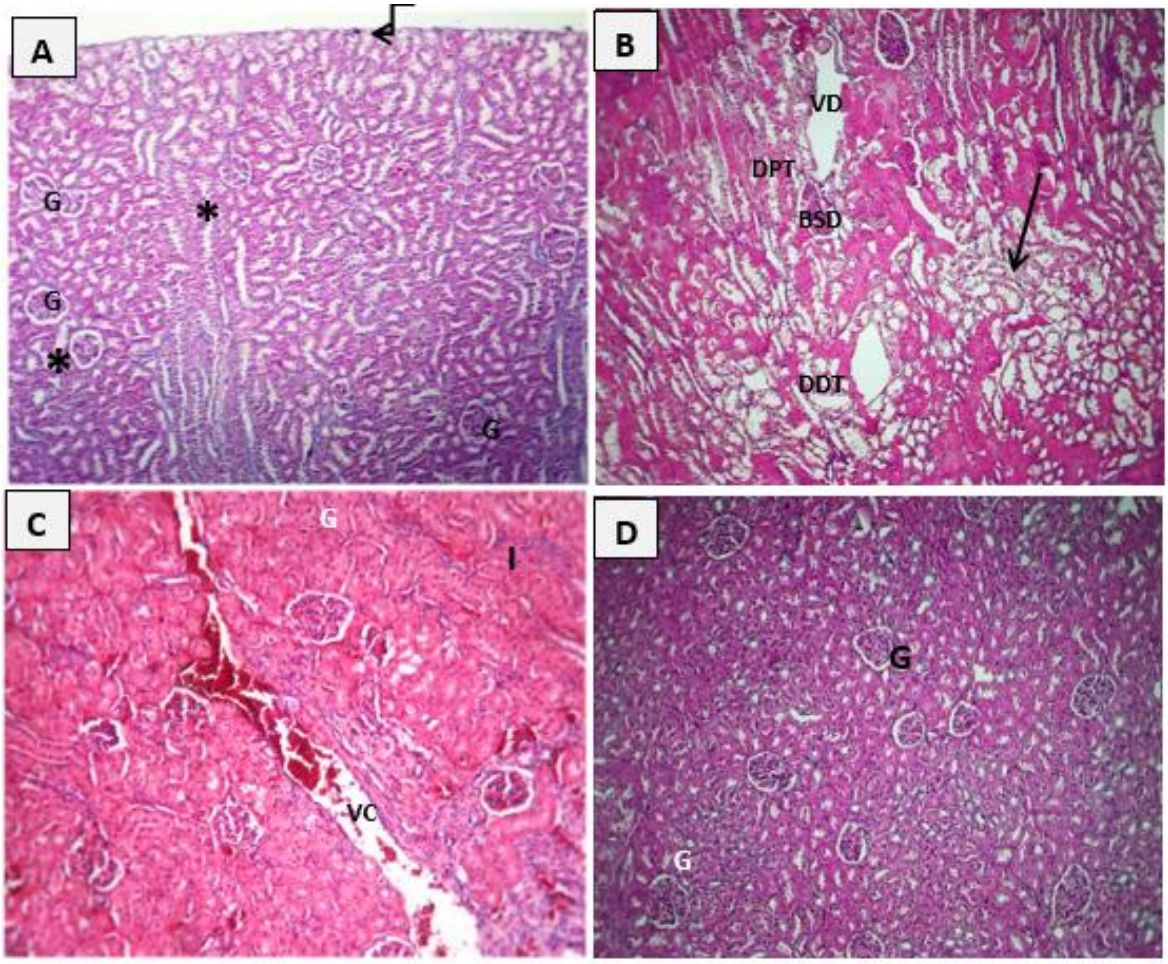

Fig. 1: Photomicrographs of rabbit kidney sections (hematoxylin-eosin) showing (**A**): the normal appearance of renal cortex limited with renal capsule (arrow) and normal histology of glomeruli (G); surrounded with Bowmans' spaces and distal and proximal convoluted tubules (astirisks) in the control group. (**B** and **C**): vacuolation in tubular cells (arrow), vascular dilation (VD), glomeruli with Bowmans' spaces dilation (BSD), dilated distal and proximal convoluted tubules (DDT and DPT, respectively), vascular congestion (VC) and mononuclear cells' infiltrations in IVM group (I). (**D**): ameliorated architecture of renal cortex in IVM + $VitC_g$ and IVM + $VitC_f$ groups, respectively. (Magnification A, B, C and D: Gr × 100).

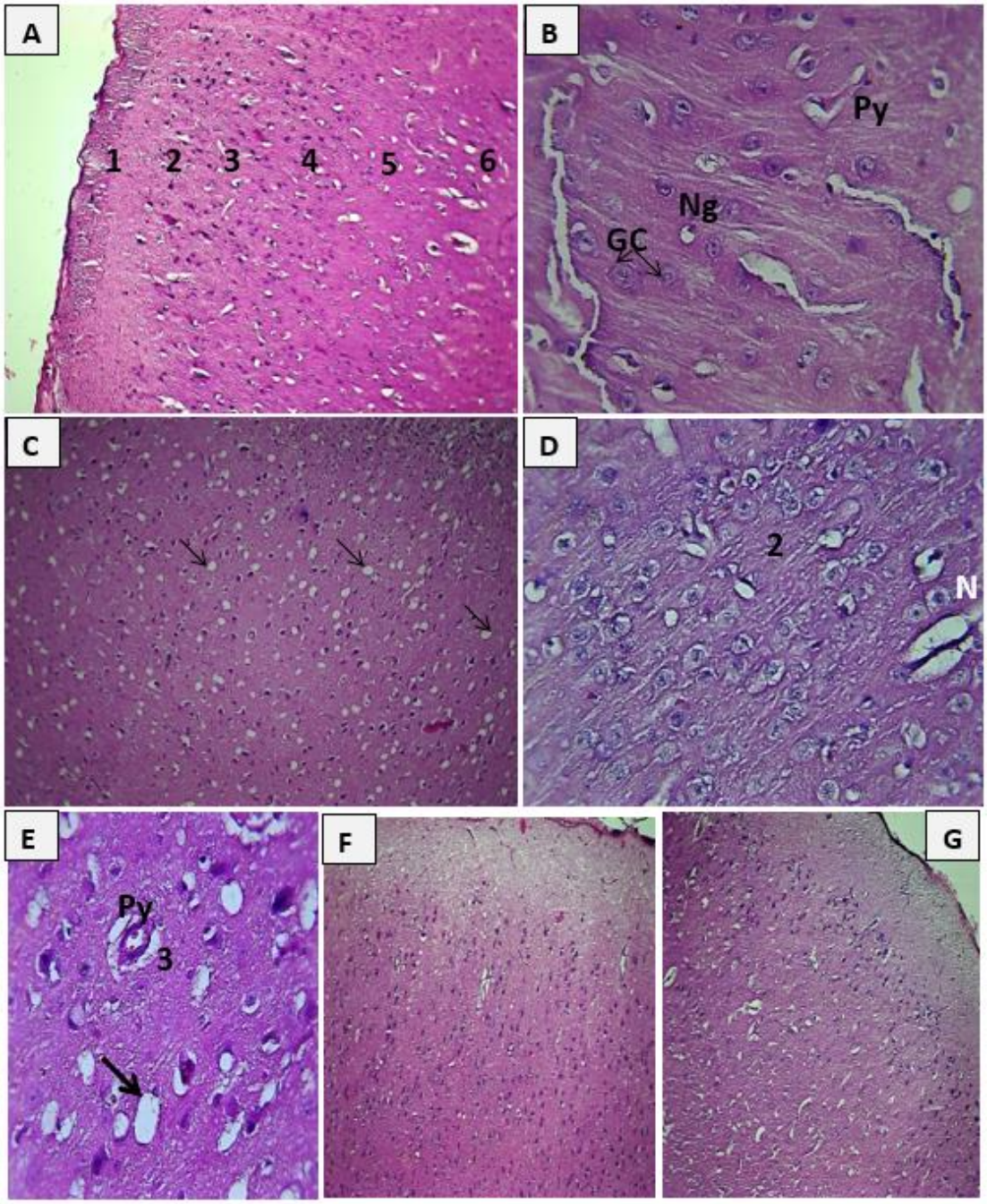

Fig. 2: Photomicrographs of rabbit cerebral cortex sections (hematoxylin-eosin) showing (**A** and **B**): the normal architecture of cells (GC: granue cells; Py: pyramidal cell; Ng: neuroglial cell) in cerebral cortex layers (**1**: molecular layer; **2**: outer granular layer; **3**: outer pyramidal layer; **4**: inner granular layer; **5**: inner pyramidal layer; **6**: multiform layer). (**C, D** and **E**): vacuaolated neuropil of the cerebral cortex (arrows), the outer granular layer becomes more cellular with abnormal granule cells (**2**), irregular shaped pyramidal cells (Py) surrounded by pericellular halos in the outer pyramidal layer and neurons necrosis (N) in IVM group. (**F** and **G**): Apparently normal architecture of cerebral cortex layers and cells with less vacuolated neuropil in IVM + $VitC_g$ and IVM + $VitC_f$ groups. Magnification: A, C, F and G × 100; B, D and E × 400.

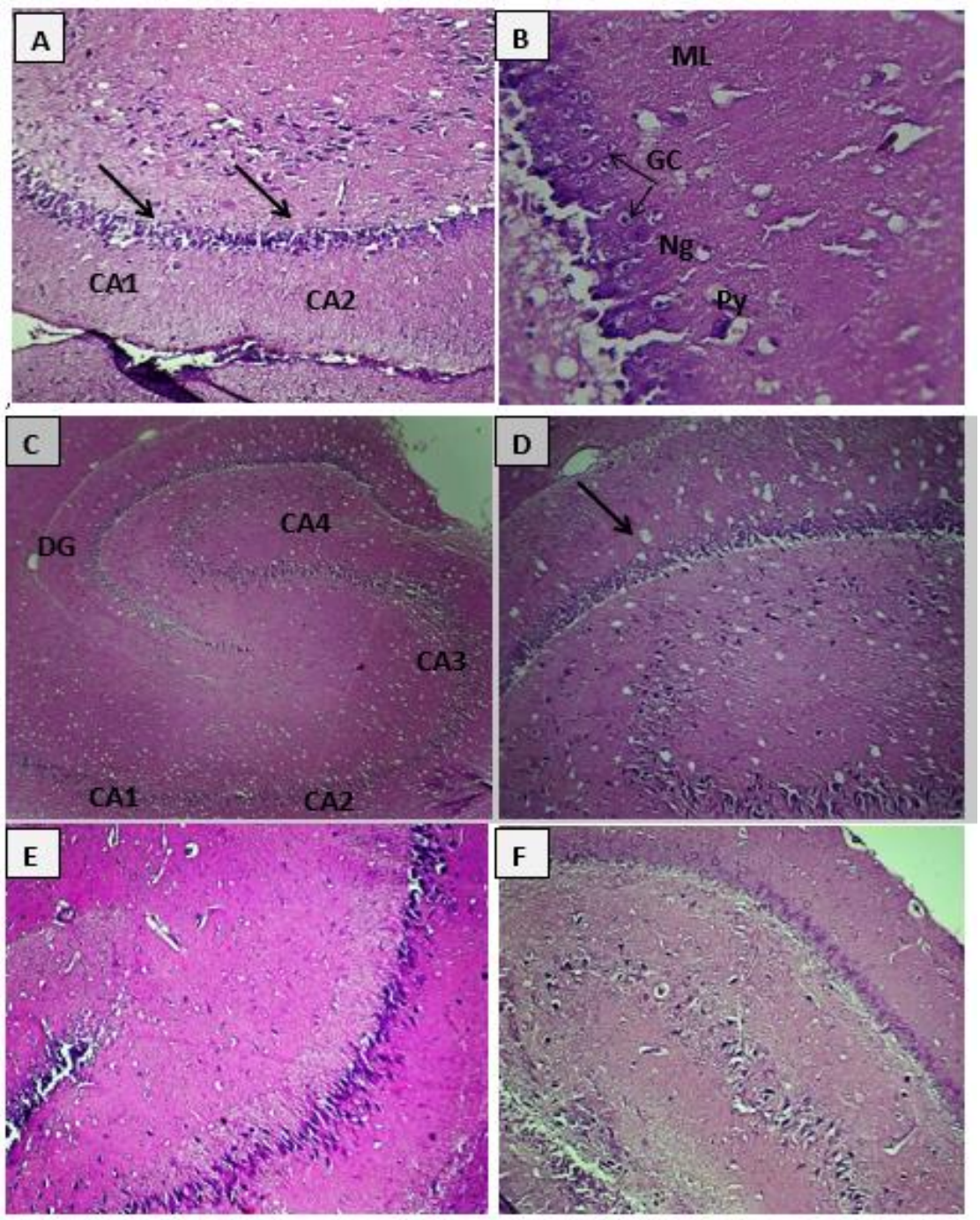

Fig. 3: Photomicrographs of rabbit hippocampus sections (hematoxylin-eosin) showing (**A**): the normal appearance of pyramidal cells of Cornu Ammonis (CA1 and CA2) region, (**B**): granular layer with normal granule cells (CG) and normal neuroglial cells (Ng) and pyramidal cells (Py) of the molecular layer in Dentate Gyrus region in the control group. (**C** and **D**): vacuolated neuropil of all layers of both Cornu Ammonis (CA1, CA2, CA3, CA4) and Dentate Gyrus (DG) regions and intracellular vacuoles with the formation of foam cells (arrow) in IVM group. (**E** and **F**): corrected pathological changes of hippocampus in IVM + $VitC_g$ and IVM + $VitC_f$ groups. Magnification: A, C, D, E and F × 100; B × 400.

DISCUSSION

AVMs are the largest marketed anthelmintic drugs in the world. AVMs are potent antiparasitic used for the treatment of both internal and external parasites in animals, they are lipophilic compounds known by a broad spectrum of activity with noticed long-lasting efficacy (GabAllh *et al*., 2017). The wide range of use of IVM in the treatment of many conditions in animals and humans explains the need of studying its effects on vertebrates especially mammals (Omshi *et al*., 2018). IVM 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 IVM for animals and humans are changing depending on the clinical case and the type of parasites (Ahmed *et al*., 2020). According to (Barrett *et al*., 2016), parenteral IVM is better tolerated compared to oral administration which is associated with embarrassing gastrointestinal side effects.

The present work evaluated the ameliorative and protective effect of vitamin C administered either orally by gavage or supplemented in food on the toxic effects associated with the administration of a high dose of an antiparasitic drug, AVIMEC® (2 mg/kg b.w) given at a dose up to 10 times the highest therapeutic dose of 0.2 mg/kg (0.2 mg/kg b.w) (Barrett *et al*., 2016) subcutaneously for 21 days in a male rabbit. To achieve this goal, the animals' behaviors, as well as the evolution of the organs' weights, the analysis of renal biochemical parameters and the histopathological changes of the kidney and brain were evaluated.

In the present study, no death was marked in all rabbit groups throughout the acclimation or experimental period. At therapeutic doses, IVM is well tolerated with fewer adverse effects (Grayson *et al*., 2017). During experimentation, some behavioral changes were reported in rabbits treated with IVM alone: tremor, commotion, decreased animal activity, hair loss and diarrhea. The observed tremors and commotion are probably related to the IVM neurotoxicity. Despite the fact that IVM does not normally traverse the blood-brain barrier invertebrates due to the expression of P-glycoprotein which restrict the passage of many drugs in the CNS, high-dose animal studies have noticed signs and symptoms of central nervous system toxicity, including emesis, mydriasis, and ataxia (Edwards 2003). Severe signs of neurotoxicosis (depression, ataxia, somnolence, mydriasis, salivation, and tremor) are also provoked by the use of IVM therapeutic dose of 0.2 mg⁄ kg in P-glycoprotein lacking dogs (Hopper *et al*., 2002). From (Trailovic and Nedejkovic 2010), IVM at 2.5, 5, 7 and 10 mg/kg b.w didn't cause visible CNS depression but at 15 mg/kg b.w produced CNS depression similar to general anesthesia in rats. In the opposite of our results, 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).

The results revealed a significant ($p<0.05$) increase in the absolute and relative weight of both right and left kidneys in the IVM group compared to control group. Our results are in disagreement with those of (Khaldoun-Oularbi *et al*., 2015) who found a non-significant change in the kidney weight in the emamectin-benzoate treated rat. In contrast, the absolute and relative weights of the brain were significantly decreased in the IVM group compared to control group. This can be explained by the possible penetration and accumulation of IVM in the brain when administered at high doses (Menez *et al*., 2012). The kidneys' and brain weights were not significantly changed

in the IVM + Vit C-treated groups compared to the control group. These results are in line with other studies that proved the protective effects of vitamin C co-administration on body weight in rats (Omshi *et al*., 2018; Khaldoun-Oularbi *et al*., 2017).

Renal dysfunction can be assessed by plasma levels of creatinine, urea and uric acid (Omshi *et al*., 2018). In our study, the plasma analysis of renal biomarkers at day 14 of experimentation showed a significant increase in creatinine level in IVM group compared to the control. This level continues to be significantly increased at day 21 of the experiment. However, both uric acid and urea concentrations showed a non-significant increase in the IVM group compared to the control group on both days 14 and 21 of the experimental period. Depending on (Arise and Malomo 2009), these high levels of renal plasma parameters may be attributed to the impairment of the glomerular filtration that produces the retention of creatinine and urea. Our results were matched with the findings of several studies that determine the effects of therapeutic and/or double therapeutic doses of IVM on the variation of renal biomarkers (Arise and Malomo 2009 ; El Sawy *et al*., 2015 ; Elzoghby *et al*., 2015 ; Al-Jassim *et al*., 2016; Ahmed *et al*., 2020). In the other hand, our results are in disagreement with those of (Omshi *et al*., 2018) who determined a non-significant change of creatinine level in IVM treated group compared to the control. For the levels of renal biochemical parameters in the IVM + Vitamin C co-treated groups, only the plasma creatinine level was significantly decreased in the IVM + $VitC_g$ group compared to IVM group. These findings are in agreement with those found by a previous study (Omshi *et al*., 2018). Conversely, (Aljassim *et al*., 2016) detected a significant increase of creatinine level even in the vitamin C co-treated groups and a significant decrease of urea in IVM + vitamin C groups compared to IVM group in rabbits. (Magdy *et al*., 2016) concluded that vitamin C was able to preserve the kidney tissues against the abamectin-induced oxidative stress that resulted in the abnormal levels of renal biomarkers.

In the current study, all these modifications in plasma renal markers were confirmed by the histopathological alterations found in the IVM treated group including vascular congestion, hemorrhages, mononuclear cell infiltration, dilation of Bowmans' spaces, vacuolization in tubular cells and dilated renal tubules. The same results are found in therapeutic and double therapeutic doses of IVM treated groups in rats (El Sawy *et al*., 2015; Elzoghby *et al*., 2015) and rabbits (GabAllh *et al*., 2017). Vitamin C co-administration orally by gavage or supplemented in food showed less histopathological changes compared to IVM group. According to (Omshi *et al*., 2018), vitamin C was effective in reducing the level of serum oxidative stress parameters when co-administered to IVM. Similarly, vitamin C was found to have protective effects against renal histology damage when taken orally with IVM (Al-Jassim *et al*., 2016) and abamectin (Magdy *et al*., 2016) and intraperitoneally with emamectin benzoate in rats ((Khaldoun-Oulabi *et al*., 2017).

As mentioned in (Menez *et al*., 2012), IVM is very safe in mammals. In fact, the P-glycoprotein limits their passage in the brain, thereby preserving their binding to $GABA_A$ receptors. In contrast, IVM neurotoxicity has been stated in mammals in cases of P-glycoprotein failure (Geyer *et al*., 2009) or overdoses (Trailovic and Nedejkovic 2010). As another finding of the present study, the cerebral cortex histological examination showed severe vacuolated neuropil, abnormal structure of granule cells, the irregular shape of pyramidal cells surrounded with pericellular halos and neurons necrosis in the IVM group compared to the control group. In the same manner, the hippocampal sections

demonstrated vacuolated neuropil and intracellular neuropil with the appearance of foam cells in IVM group compared to control. According to the literature research, few studies were performed on the histopathological effects of IVM on the brain tissues of mammals. Compared to our results, severe alterations of the brain tissues were found in rabbits when administered a therapeutic dose of IVM (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 IVM. Identical results were observed in the brain tissue of pigeon when exposed to avermectin (Li *et al*., 2013). Accordingly, this neurotoxicity was induced by oxidative damage of P-glycoprotein. Recently, the effects of abamectin, an AVM insecticide, on brain oxidative stress parameters and histopathological changes were evaluated in rats (Radi *et al*., 2020). In this study, the cerebral cortex of the abamectin-treated group showed vacuolated neuropil and hemorrhages, the hippocampus of the same group displayed pyknosis and degenerative changes in pyramidal layers. These results are in line with our findings. In the present work, all treated groups with IVM + VitC orally or in food showed an ameliorated architecture of both cerebral cortex and hippocampus with less vacuolated neuropil. According to (Radi *et al*., 2020), the histopathological alterations of the cerebral cortex and hippocampus were accompanied by a significant increase in MDA concentration and a significant decrease in GSH and CAT activity in brain tissues. All these oxidant/antioxidant markers were significantly corrected in abamectin + vitamin C treated groups; this finding confirms the antioxidant activity of vitamin C against AVMs neurotoxicity. The neuroprotective effect of vitamin C was confirmed in previous studies in vivo (Shokouhi et al., 2005; Han et al., 2007).

CONCLUSION

The repeated administration of a high dose of IVM for three consecutive weeks in male rabbits may disturb the renal function and alter the histological structure of kidneys and brain tissue. Vitamin C co-treatment could ameliorate the IVM produced nephrotoxic and neurotoxic effects.

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Ethical approval

This study was approved by the Scientific Council of Biotechnology Laboratory of Animal Reproduction, Institute of Veterinary Sciences, University of Saad Dahlab Blida 1 (Algeria).

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