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Beneficial effects of ascorbic acid on ivermectin repeated high-dose therapy in rabbits: biochemical and histopathological investigations

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ABSTRACT: Ivermectin (IVM) is a lipophilic anthelmintic drug widely used for the control of internal and external parasites in both human and veterinary medicine. Conversely, overdoses of IVM are associated with resistance and efficacy problems. The present study aimed to evaluate the effects of repeated administration of a high dose of IVM alone or with combination of ascorbic acid (AA) in male young rabbits (*Oryctolagus cuniculus*) via biochemical and histological investigations. Twenty rabbits were divided into four groups (n=5) and treated for three consecutive weeks: Control group; IVM group (2 mg/kg of body weight subcutaneously, 3 times a week); IVM + AA_g (20 mg/mL) group and IVM + AA_f (200 mg/kg of diet) group. IVM induced a disruption of hepatic biochemical parameters and lipid profile with a statistically significant ($p < 0.05$) increase in glucose, ALT, AST, GGT, HDL-C and a significant decrease of TC, TG, LDL-C, VLDL-C in IVM group compared to control group. Co-administration of AA moderately improved those biochemical parameters. Histopathological changes following IVM treatment in liver comprised loss of normal hepatocytes structure, central vein dilation and portal vein congestion. The lung showed abnormal structure of intrapulmonary bronchus, dilated bronchioles and alveoli and congested pulmonary artery. Nevertheless, the AA treatment groups revealed significant improvement when co-administered orally with IVM. This study suggested that AA has a beneficial ameliorative role against toxic effects induced by repeated high-dose of IVM.

Keywords: Ivermectin; Ascorbic acid; Biochemical parameters; Histopathology; Rabbit.

1. INTRODUCTION

In veterinary medicine, macrocyclic lactones (MLs) has a position of prominence in the control of parasites and are probably the most widely used anti-parasitic agents in the treatment of food producing animals, poultry, aquaculture and crops. Ivermectin (IVM) was the first commercially available endectocide macrocyclic lactone (ML), discovered in the mid-1970s and used in veterinary and humans clinical medicine [1, 2]. IVM is a chemical derivative of avermectin. Since its discovery, a number of alternative products such

abamectin, doramectin, emamectin, eprinomectin, moxidectin, milbemycin and selamectin, have been marketed [3, 4]. The antiparasitic effect of IVM is said to be due to its interaction with glutamate and GABA-gated chloride channels, which cause efflux of chloride ions across the cell membranes and lead to paralysis in many types of parasites [5].

In addition to its efficacy in the control of endo and ectoparasites [6], IVM a '*Splendid gift from microorganisms*' has been demonstrated to have anticancer activities on various types of cancer, including glioblastoma [7], chronic myelogenous leukemia [8], breast cancer [9], ovarian cancer [10] and renal cancer [11]. Recently, it was reported that IVM is also an inhibitor of the replication of SARS-CoV-2 (COVID-19) *in vitro* [12].

The use of endectocide drugs for the treatment of parasitic diseases in rabbits has been increasing recently. Lu et al. [13] revealed that treatment of female rabbits naturally infected with *Psoroptes cuniculi* with a commercial IVM injection (Ivomec® at 0.2 mg/kg) showed rapid and high efficacy against ear-mite. Gokbulut et al. [14] indicated that subcutaneous administration of two doses of IVM (0.3 mg/kg) with a 15- 20 day interval in-between could be necessary for treatment of ectoparasites.

Guzzo et al. [15] reported that IVM at doses up to 10 times the highest FDA (Food and Drug Administration), approved dose of 200 µg/kg is generally well tolerated, with no indication of associated central nervous system toxicity. Nonetheless, at higher concentrations IVM has a broad range of effects in many different organisms [16, 17] and repeated administration of different doses of IVM induced histopathological alterations in liver [18], kidneys and lungs [19] of rabbit; testis [20] of rat and brain tissues [21] of pigeon.

Recently, the protective effects of natural antioxidants against the toxicity of various xenobiotic are the focus of interest. As have been approved by the study of Abdeldaim and Abdellatief [22], Caffeic Acid Phenethyl ester and Betaine have a protective effect against Abamectin induced toxicity. Also, some antioxidant can be administered with IVM to improve its side effects such as vitamin A [23], vitamin K [24], Selenium and vitamin E [25] and some phenols and flavonoids [26].

Ascorbic acid (AA) or vitamin C is a water-soluble antioxidant which efficiently scavenges free radicals, protecting cell membranes from oxidative damage [27, 28]. Previous animal studies suggested that vitamin C treatment may have potential protective effects on oxidative stress and environmental toxicities [29, 18]. Recently we demonstrated the beneficial effects of Vitamin C in attenuating emamectin benzoate toxicity, an avermectin insecticide formulation [30].

Accordingly, this study has two stages of demonstration, first to describe the toxic effect of repeated subcutaneous injections of IVM on the hepatic biochemical parameters, lipid profile and the liver and lung histology in male young rabbits of (*Oryctolagus cuniculus*) strain. Secondly, to investigate the beneficial effects of AA on IVM repeated high-dose therapy in rabbits.

2. MATERIALS AND METHODS

2.1. Chemicals

Ivermectin (AVIMEC®, 10 mg/ml) was purchased from the Arab Veterinary Industrial Company (AVICO, Jordan). This formula is used by subcutaneous injection for rabbits: 0.15 ml per 1 kg of body weight (1.5 mg/kg). Vitamin C of purity 99%, was purchased from Sigma–Aldrich Chemicals Co. (St. Louis, Missouri, USA). All other reagents used were obtained from commercial sources: Biosolve Chimie (Valkenswaard, Netherlands) and BIOLABO SA. (France).

2.2. Animals

Healthy male young Algerian rabbits, *Oryctolagus cuniculus* (aged 4–5 weeks; 850 to 950 g) were used in this study. Rabbits were obtained from a state breeding unit of Djebbla (Tizi-Ouzou) and kept for experimentation in the CRD of Saidal, Algeria. All animals were housed in temperature-controlled rooms with 12-h light/dark cycle. Animal experimentation was consistent with the Guiding Principles in the Use of Animals in Toxicology [31]. Rabbits were acclimated to the laboratory conditions for 2 weeks before treatment and had free access to a commercial pellet diet and water *ad libitum*.

2.2.1. Experimental design

Twenty rabbits were divided into four groups (n=5). A control group, received distilled water by gavage and three groups treated with a high dose of IVM (2 mg/kg of body weight subcutaneously, 3 times/week) [13] for three consecutive weeks including; a group was treated only with IVM (IVM group); a group was co-treated orally with ascorbic acid by gavage (IVM + AA_g group; 20 mg/ml, 3 times/week) and a group was co-treated with ascorbic acid enriched diet (IVM + AA_f group; 200 mg/kg of food, 3 times/week) [32]. AA is administered with a mean interval of 12 h after IVM injection. Animals were weighed daily throughout the acclimation (2 weeks) and experimentation (3 weeks) periods in order to follow their weight evolution. At 14 and 21 days of the experiment, blood samples were collected with heparinized syringes from the ear vein for biochemical analysis.

2.2.2. Samples collection

At the end of the experiments, the rabbits were euthanized by cervical decapitation, and liver and lung were carefully dissected out and weighed. Blood samples were collected after a fasting period of 12 hours and plasma was separated by centrifugation at 4000 r/min for 15 min.

2.3. Biochemical analysis

To assess the effect of vitamin C on IVM toxicity, the following hepatic parameters; alanine aminotransferase (ALT), aspartate aminotransferase (AST), gamma-glutamyltransferase (GGT) and glucose and lipid profile tests; triglycerides (TG), total cholesterol (TC), high density lipoprotein cholesterol (HDL-C) and low density lipoprotein cholesterol (LDL-C) were assessed in plasma using a commercially available spectrophotometric enzymatic kit (Biolabo, France) and analyzed by an auto-analyzer (Hitachi 912) instrument (Roche Diagnostics, Mannheim, Germany). The plasma level of very low density lipoprotein cholesterol (VLDL-C) was evaluated using the following formula: $VLDL-C = TC - (HDL-C + LDL-C)$. In addition, for better expression of the rapport between triglycerides and HDL-C, atherogenic index of plasma (AIP) factor based on the ratio of the values of triglycerides to high-density lipoprotein (HDL-C) levels was calculated according to the following formula: $AIP = \text{Log} [TGs] / [HDL-C]$, where both of them are measured in the plasma [33]. Otherwise, other atherogenic factors (AF) were calculated as the ratio between; total Cholesterol and HDL-C, LDL-C and HDL-C [34].

2.4. Histopathological examinations

For histopathological examination, the liver and lung were excised from all rabbits and fixed in 10% neutral formalin buffer, processed through graded alcohols and xylene and embedded in paraffin blocks. Organ sections (2–3 μm thick) were cut and stained with haematoxylin and eosin (H&E) for histopathological studies.

2.5. Statistical analysis

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

3. RESULTS

3.1. Effect on general body health condition, body weight and weight gain

No deaths occurred in any group throughout the experiment. Some signs of general toxicity (hair loss and diarrhea) and decreased food intake were apparent in male rabbits treated with IVM alone. As shown in the Table 1, the body weight as well as the percentage of body weight gain (% BWG) and the food intake of the rabbits in the control, IVM, IVM + AAg and IVM + AAf groups during the acclimation period (2 weeks) are normal. However, during the experimental period (3 weeks), in the IVM treated group, the food intake (21.01 ± 0.9) was significantly ($p < 0.05$) decreased compared to control group (33.03 ± 0.1), also the final body weight and the % BWG (1587.0 ± 71.6 and 19.3 ± 0.8) was significantly decreased compared to control (1806.4 ± 63.2 and 34.1 ± 0.5), respectively. Conversely, the food intake (27.5 ± 0.2 ; 35.2 ± 0.5), the final body weight (1693.0 ± 47.1 ; 1678.1 ± 48.0) and the % BWG (25.3 ± 0.7 ; 26.4 ± 0.8) in the IVM + AAg and IVM + AAf groups, respectively were significantly increased compared to the food intake, the final body weight and % BWG of rabbits treated with IVM alone.

Table 1. Effect of IVM and/or AA on body weight and weight gain for the acclimation and experimental periods.

Body weight / groups		Control	IVM	IVM + AAg	IVM + AAf
Acclimation	Initial BW (g)	862.1 \pm 9.8	914.8 \pm 22.7	946.7 \pm 25.6	932.2 \pm 27.8
	Final BW (g)	1085.7 \pm 18.9	1147.7 \pm 21.9	1156.8 \pm 26.2	1149.1 \pm 30.8
	% BWG	25.9 \pm 0.9	25.5 \pm 0.9	22.23 \pm 0.7	23.31 \pm 0.8
	Food intake %	28.1 \pm 0.1	30.3 \pm 0.2	26.01 \pm 0.1	23 \pm 0.4
Experimentation	Initial BW (g)	1347.1 \pm 21.1	1330.6 \pm 19.8	1351.0 \pm 26.6	1328.3 \pm 36.4
	Final BW (g)	1806.4 \pm 63.2	1587.0 \pm 71.6 ^a	1693.0 \pm 47.1 ^b	1678.1 \pm 48.0 ^c
	% BWG	34.1 \pm 0.5	19.3 \pm 0.8 ^a	25.3 \pm 0.7 ^b	26.4 \pm 0.8 ^c
	Food intake %	33.03 \pm 0.1	21.01 \pm 0.9 ^a	27.5 \pm 0.2 ^b	35.2 \pm 0.5 ^c

IVM: Ivermectin; AA: Ascorbic acid; AAg: Ascorbic acid by gavage; AAf: Ascorbic acid supplemented in food; BW: body weight; BWG: body weight gain. Results are given as a mean \pm SD for five rabbits in each group. a, b and c: $p < 0.05$ (a: significant difference between all treated groups and control, b: significant difference between IVM and IVM + AAg groups, c: significant difference between IVM and IVM + AAf groups).

3.2. Effect on absolute and relative organ weights

According to table 2, the absolute and relative weight of lung showed no significant change in the IVM group compared to control group, but these weights were significantly ($p < 0.05$) increased in the IVM + AA-treated groups (9.8 ± 0.8 g and 11.7 ± 0.7 g) compared to IVM group (8.0 ± 1.0 g) and the control group (8.8 ± 0.9 g) and with no significant difference between IVM + AAg and IVM + AAf groups. However, the absolute and relative weight of liver showed no significant change in the IVM group when compared to control and IVM + AA-treated groups.

Table 2. Effect of IVM and/or AA on absolute and relative liver and lung weights, 21 days after treatment.

Organ weight (g) / groups		Control	IVM	IVM + AAg	IVM + AAF
Liver	Absolute	76.5 ± 9.2	75.8 ± 6.5	77.7 ± 8.7	77.3 ± 9.5
	Relative	4.1 ± 1.0	4.6 ± 0.4	4.4 ± 0.5	4.5 ± 1.2
Lung	Absolute	8.8 ± 0.9	8.0 ± 1.0	9.8 ± 0.8 ^{a, b}	11.7 ± 0.7 ^{a, c}
	Relative	0.5 ± 0.01	0.5 ± 0.1	0.6 ± 0.03 ^{a, b}	0.7 ± 0.2 ^{a, c}

IVM: Ivermectin; AA: Ascorbic acid; AAg: Ascorbic acid by gavage; AAF: Ascorbic acid supplemented in food. Results are given as a mean ± SD for five rabbits in each group. a, b and c: $p < 0.05$ (a: significant difference between all treated groups and control, b: significant difference between IVM and IVM + AAg groups, c: significant difference between IVM and IVM + AAF groups).

3.3. Effect on serum biochemical parameters

As shown in Table 3, IVM induced hepatic disorders as demonstrated by the elevation of liver biomarkers in plasma. The glucose plasma level didn't change significantly in different treated groups at day 14 of experimentation but at day 21, the glucose level was significantly ($p < 0.05$) increased in the IVM group compared to control group. However, this level was significantly decreased in the IVM + AAg group compared to IVM and IVM + AAF groups. At day 14 of experimentation, the plasma levels of GGT, ALT and AST were significantly increased in the IVM group compared to control group. These levels were significantly decreased in both IVM + AA-cotreated groups compared to IVM group. In the same manner, the levels of GGT, ALT and AST continue to be significantly increased in IVM group compared to control group at day 21 of the experimentation. These levels didn't change significantly in both IVM + AA-cotreated groups.

Table 3. Effect of IVM and/or AA on liver biomarkers at 14 and 21 day of experimentation.

Period	Biochemical parameters	Experimental groups			
		Control	IVM	IVM + AAg	IVM + AAF
14 day	Glucose (g/L)	0.89 ± 0.01	1.19 ± 0.11	1.18 ± 0.02	1.17 ± 0.01
	Gamma GT (U/L)	10.0 ± 0.02	13.0 ± 0.01 ^a	12.5 ± 2.1 ^b	12.0 ± 6.4 ^c
	ALT (U/L)	27.0 ± 0.2	43.5 ± 7.9 ^a	32.0 ± 0.1 ^b	38.0 ± 4.8 ^c
	AST (U/L)	33.0 ± 0.05	57.0 ± 0.02 ^a	50.2 ± 7.2 ^b	52.5 ± 6.4 ^c
21 day	Glucose (g/L)	1.02 ± 0.19	1.64 ± 0.13 ^a	1.20 ± 0.09 ^{b, d}	1.34 ± 0.17
	Gamma GT (U/L)	11.0 ± 0.03	18.0 ± 4.6 ^a	11.2 ± 0.6	12.1 ± 0.8
	ALT (U/L)	32.0 ± 2.7	44.5 ± 3.7 ^a	31.5 ± 7.9	33.2 ± 3.8
	AST (U/L)	36.5 ± 8.7	42.0 ± 6.7 ^a	34.2 ± 3.2	38.2 ± 1.9

IVM: Ivermectin; AA: Ascorbic acid; AAg: Ascorbic acid by gavage; AAF: Ascorbic acid supplemented in food. Results are given as a mean ± SD for five rabbits in each group. a, b, c and d: $p < 0.05$ (a: significant difference between all treated groups and control, b: significant difference between IVM and IVM + AAg groups, c: significant difference between IVM and IVM + AAF groups and d: significant difference between IVM + AAg and IVM + AAF groups).

As indicated in Table 4, all lipid parameters were significantly decreased in the IVM group compared to control group at day 14 of experimentation, except the HDL-C level which was significantly increased in the same group. The co-administration of vitamin C to the IVM treated rabbits, especially in the IVM + AAg group, resulted in a significant recovery of all lipid parameters compared to IVM group except the level of HDL-C. At day 21, only LDL-C and TG levels and the LDL-C/HDL-C ratio were still significantly

decreased in the IVM group compared to control group. In addition to the total cholesterol/ HDL-C ratio, the above mentioned parameters were significantly better corrected in the IVM + AA_g group compared to IVM group. The AIP was increased in the IVM group compared to the control and decreased in the IVM + AA-cotreated groups compared to IVM group at 14 day of experimental period. However, at 21 day of experimentation, the AIP was still increased in the IVM group and both IVM + AA-cotreated groups compared to control group.

Table 4: Effect of IVM and/or AA on lipid profile at 14 and 21 day of experimentation.

Period	Biochemical parameters	Experimental groups			
		Control	IVM	IVM + AA _g	IVM + AA _f
14 day	TC (g/l)	1.05 ± 0.001	0.78 ± 0.001 ^a	0.86 ± 0.24 ^b	0.66 ± 0.10
	TG (g/l)	1.75 ± 0.004	0.11 ± 0.002 ^a	1.17 ± 0.16 ^b	0.94 ± 0.24 ^c
	HDL-C (g/l)	0.29 ± 0.003	0.39 ± 0.001 ^a	0.34 ± 0.07	0.27 ± 0.03
	LDL-C (g/l)	0.41 ± 0.002	0.21 ± 0.003 ^a	0.30 ± 0.12 ^b	0.19 ± 0.10 ^c
	VLDL-C (g/l)	0.35 ± 0.01	0.23 ± 0.04 ^a	0.24 ± 0.03 ^b	0.17 ± 0.08 ^c
	TC/HDL-C	3.62 ± 0.001	2.22 ± 0.003 ^a	2.49 ± 0.23 ^b	2.32 ± 0.27 ^c
	LDL-C/HDL-C	1.41 ± 0.002	0.58 ± 0.001 ^a	0.82 ± 0.33 ^b	0.70 ± 0.29 ^c
	AIP	0.83 ± 0.001	-2.66 ± 0.002	-0.15 ± 0.17	0.15 ± 0.09
21 day	TC (g/l)	0.93 ± 0.05	0.83 ± 0.10	0.85 ± 0.17	0.73 ± 0.08
	TG (g/l)	1.10 ± 0.17	0.87 ± 0.12 ^a	0.90 ± 0.24 ^b	0.84 ± 0.18
	HDL-C (g/l)	0.37 ± 0.01	0.36 ± 0.05	0.37 ± 0.05	0.40 ± 0.06
	LDL-C (g/l)	0.37 ± 0.03	0.28 ± 0.07 ^a	0.27 ± 0.08 ^b	0.21 ± 0.07 ^c
	VLDL-C (g/l)	0.21 ± 0.07	0.19 ± 0.03	0.18 ± 0.02	0.17 ± 0.01
	TC/HDL-C	2.53 ± 0.19	2.34 ± 0.20	2.19 ± 0.37 ^b	1.82 ± 0.12 ^c
	LDL-C/HDL-C	1.00 ± 0.11	0.79 ± 0.15 ^a	0.71 ± 0.23 ^b	0.47 ± 0.11 ^c
	AIP	0.11 ± 0.08	-0.17 ± 0.06	-0.20 ± 0.11	-0.17 ± 0.15

IVM: Ivermectin; AA: Ascorbic acid; AA_g: Ascorbic acid by gavage; AA_f: Ascorbic acid supplemented in food. Results are given as a mean ± SD for five rabbits in each group. a, b and c: p < 0.05 (a: significant difference between all treated groups and control, b: significant difference between IVM and IVM + AA_g groups, c: significant difference between IVM and IVM + AA_f groups).

3.4. Histopathological findings

Histological examination of the control liver sections showed a normal histological architecture with normal hepatocytes arranged in cords that are separated from each other by sinusoids (Figure 1 a, b). H&E-stained sections of the liver of rabbits treated with IVM revealed severe hepatic damage, including, loss of normal hepatocytes architecture, sinusoidal dilation, central vein dilation, congested branches of portal vein (Figure 1 c, d). These changes were reduced in the liver of (IVM + AA_g) (Figure 1 e, f) and (IVM + AA_f) (Figure 1 g, h) groups of rabbit.

In the control group, the lung sections showed normal limits of intrapulmonary bronchus, bronchiole and alveoli (Figure 2 a, b). However, in the IVM group, the rabbit lung sections showed an irregular intrapulmonary bronchus limit with altered proliferation of mucosal epithelium, dilated bronchiole with a thick epithelium, dilated alveoli with thickening of their septa, pulmonary hemorrhage and pulmonary artery congestion (Figure 2 c, d). These histopathological modifications were less apparent in the lung of (IVM + AA_g) (Figure 2 e, f) and (IVM + AA_f) (Figure 2 g, h) groups of rabbit.

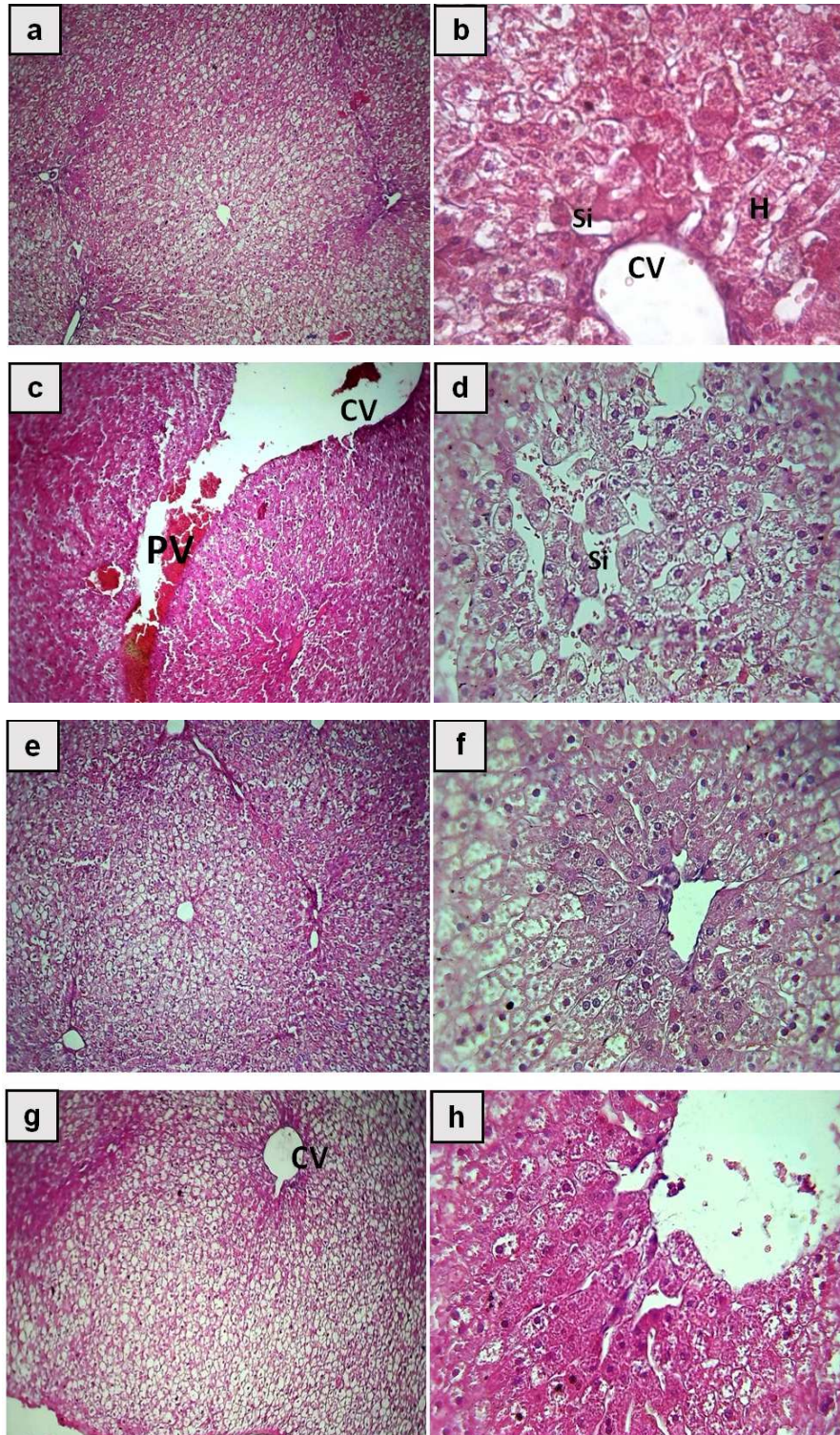


Figure 1. Photographs of liver sections from control rabbits (a and b) showing normal lobular architecture with a central vein (CV) and normal structure of hepatocytes (H) and hepatic sinusoids (Si). From IVM group (c and d), showing a loss of normal hepatocytes architecture, dilated and congested central vein (CV), dilated and congested portal vein (PV), dilated hepatic sinusoids (Si). From IVM + AA (e and f) and IVM + AAF (g and h) showing normal central vein (CV) and portal vein (PV) with restoration of hepatocytes (H) structure. H&E: (a, c, e and g) $\times 100$ and (b, d, f and h) $\times 400$.

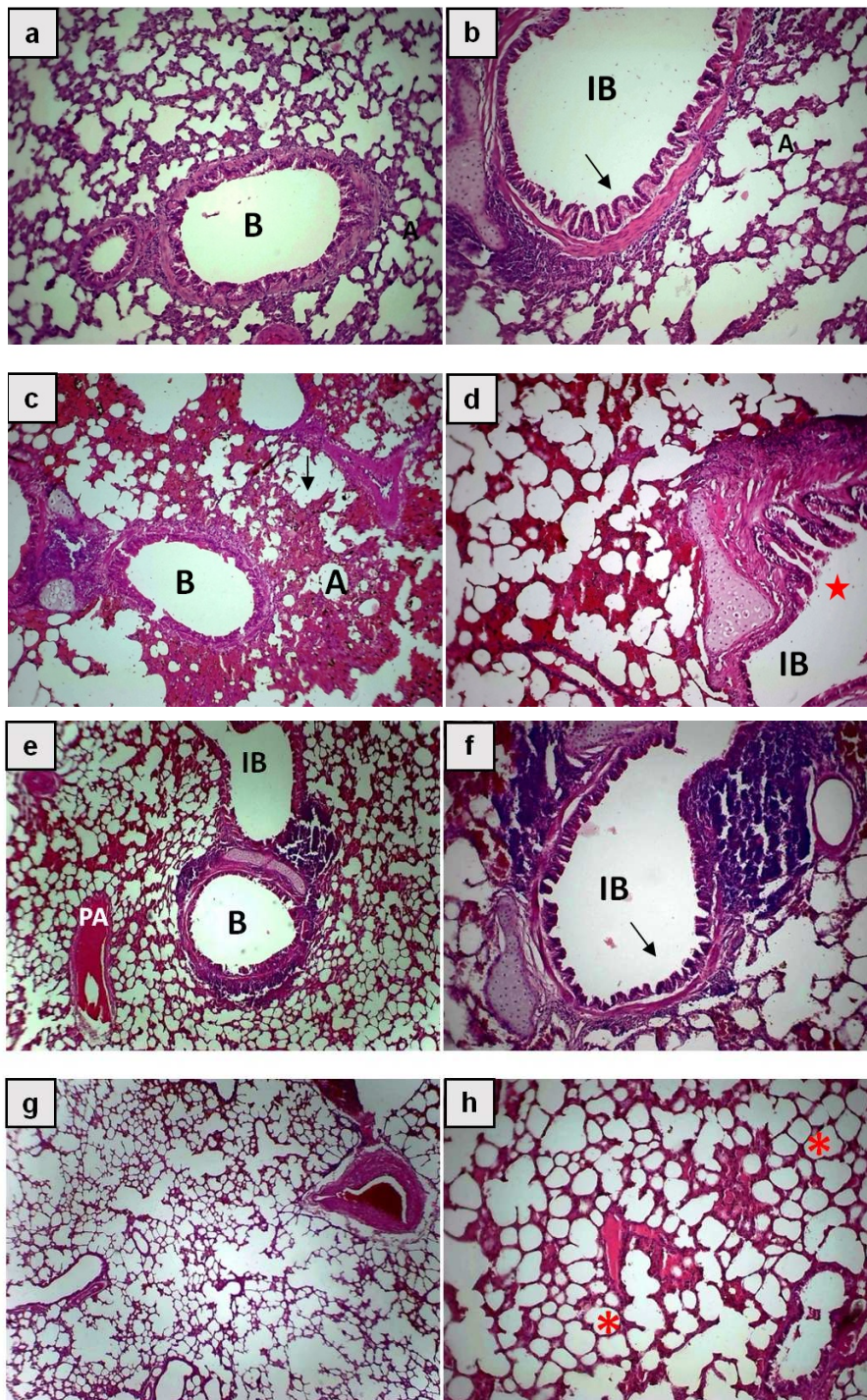


Figure 2. Photographs of lung sections from control rabbits (a and b) showing normal limit of intrapulmonary bronchus (IB) with folded mucosa (arrow), bronchiole (B) and alveoli (A). From IVM group (c and d) showing dilated alveoli (A) with thickening of their septa and pulmonary hemorrhage (arrow), irregular intrapulmonary bronchus (IB) limit with altered proliferation of mucosal epithelium (star), dilated bronchiole (B) with a thick epithelium and congested pulmonary artery. From IVM + AA group (e and f) and IVM + AAf (g and h) showing intrapulmonary bronchus (IB) with normal limit and folded mucosal epithelium (arrow), restoration of pulmonary artery congestion (PA) and mild dilation of bronchiole (B) and alveoli (asterisk). H&E: (a, c, e and g) $\times 100$ and (b, d, f and h) $\times 400$.

4. DISCUSSION

MLs resistance has become a problem in human and veterinary medicine thus threatening the sustainable efficacy of antiparasitic drugs [35]. IVM is one of the most important drugs for the control of parasitic infection and was the joint focus of the 2015 Nobel Prize in Physiology or Medicine, after 35 years of its remarkable discovery [16]. In Algeria, Many generic preparations of IVM drugs are used for the treatment and prevention of major parasitic diseases in animal, caused by both endo and ectoparasites [36]. However, increases in the use of these compounds are associated with resistance and efficacy problems [37].

Avermectin confers its cytotoxic effects by inducing DNA damage and mitochondria-associated apoptosis [38]. Omshi et al. [23] revealed that treatment with IVM at a concentration of 0.4 mg/kg of body weight orally once a week for three consecutive weeks resulted in insignificant oxidative degradation in the drug-treated group.

In the present study, IVM was given at a dose up to 10 times the highest FDA-approved dose of 200 µg/kg (0.2 mg/kg) [13, 15] for which no death was observed during the experiment. However, a decrease in the body weight and the % BWG of rabbits were observed after IVM treatment. This decrease might be due to the reduced food intake as a result of loss of appetite of the young male rabbits. Our results are similar to those of Khaldoun et al. [30]. The results showed a significant increase in absolute and relative weight of lung in ascorbic acid co-treated groups compared to control group. These findings may be explained by oxidative stress generation due to repeat administration of IVM and its accumulation in lung tissue [19]. For the liver weight, it was found no change in the absolute and relative weight of the liver in all treated groups. These results are not in agreement with previous studies showing a significant increase in liver weight after avermectin administration [20, 30, 39]. Interestingly, the co-administration of AA orally by gavage or in food to IVM rabbits was capable of protecting against the lowered body and organ weight and overall health of rabbits.

Liver damage is caused by excessive exposure to drugs and toxins, which overwhelm the detoxifying power of the liver leading to enzyme leakage, lipid peroxidation, and oxidative stress [40, 41]. The current study showed significant increase in glucose level after 21 day of experimentation and significant increase of GGT, ALT and AST at 14 and 21 of experimental period in rabbits treated with IVM alone which correlated with liver histopathological results. Many reports have stated that avermectins exposure has a significant potential to induce damages in rat liver by increasing plasma transaminases and/or glycemia [30, 39, 41, 42].

In contrast, these parameters were reduced when IVM treated-rabbits were co-administered AA compared to rabbits administered with IVM alone. The IVM + AA_g treatment was found to better decrease the level of plasma glucose compared to IVM + AA_f. The effects of IVM and its combination with vitamin C on hepatic biomarkers in rabbits indicate that vitamin C provides better protection against IVM-induced hepatic damage. Our results are in agreement with those found by Khaldoun Oularbi et al. [30] that confirmed the ameliorative effect of vitamin C on glucose, AST and ALT plasma level when co-administered to emamectin benzoate during 28 days.

Miyajima et al. [43] demonstrated a significant correlation between the increases of total cholesterol (TC) and IVM concentrations in rabbit's plasma. The authors proposed that the pharmacokinetic profile of IVM is influenced not only by the promotion of IVM dissolution in gastrointestinal tract but also the change of plasma cholesterol concentration. Conversely, our study demonstrated that IVM administration provoked a decrease in all lipid parameters except the HDL-C level which was increased at 14 day. However, the co-administration of vitamin C to the IVM-treated rabbits resulted in a partial recovery of TC, HDL-C and

VLDL-C, as well as TG levels. Moreover, the LDL-C and TG levels were still significantly inferior to those of the control rabbits at 21 day.

Our results are in line with those of Jin et al. [44] who demonstrated that ivermectin decreased serum cholesterol, including high-density lipoprotein and low-density lipoprotein (LDL)/very LDL levels in mice. They established that the antiparasitic drug, IVM, is a Farnesoid X receptor (FXR) ligand that maintain bile acid and cholesterol homeostasis and can effectively improve hyperglycemia and hyperlipidemia in diabetic mice model by regulating genes expression. According to Al-Jassim et al. [18], the level of TC did not change significantly in IVM and IVM + vitamin C treated groups of rabbits compared to control group while this study showed a significant decrease in plasma level of TG in two groups exposed to IVM + vitamin C orally (0.5 mg/kg + 50 mg/kg and 2 mg/kg + 50 mg/kg; respectively).

Atherogenic indexes are considered as a better indicator of coronary heart disease risk than individual lipoprotein concentration [33]. The most important finding of our study is that the atherogenic index of plasma (AIP) was found to be augmented in IVM group compared to that of the control group. Thus, the co-administration of AA into a diet or by gavage to rabbits had statistically significant effects on the lipid profile compared to IVM group, and corrected the atherogenic index of plasma. In our study, the two atherogenic factors (total cholesterol/HDL-C and LDL-C/HDL-C) were found to be significantly decreased in IVM group compared to the control rabbits. Thus, the co-administration of AA into a diet or by gavage to rabbits had statistically significant effects on the lipid profile compared to IVM group, and corrected the atherogenic indexes.

The aforementioned results revealed that IVM administration 3 times/week for three consecutive weeks in young rabbits displayed histological alterations in liver and lung of male rabbits. Liver histological damage's consist essentially in loss of normal hepatocytes architecture, sinusoidal dilation, central vein dilation and congested branches of portal vein. These results explained the significant high level of liver biochemical parameters and are in accordance with many work's which demonstrated that the repeated administration of either therapeutic or double therapeutic doses of IVM induced severe degenerative changes and necrosis in some parenchymatous organs [20].

The histological testing of lung in IVM group revealed irregular intrapulmonary bronchus limit with altered proliferation of mucosal epithelium, dilated bronchiole with a thick epithelium, dilated alveoli with thickening of their septa, pulmonary hemorrhage and pulmonary artery congestion. These results are in line with those obtained by AL-Jassim et al. [19] and Abd-Elhady and Abou-Elghar [45] who observed interstitial pneumonia with congestion and edema in the lung section of rat exposed to abamectin for 30 days, while local hemorrhages associated with atelectasis was observed in the lung of animals exposed to abamectin for 210 days.

Vitamin C prevents the subsequent histological damage induced in liver and lung tissues. From our results we can assume that liver and pulmonary histological damages of ivermectin are mainly attributed to oxidative stress increase since the effects were largely prevented by ascorbic acid supplementation especially when this later was administered by gavage. Previously, studies have also shown the curative and antioxidative efficiency of orally administered AA and other vitamins against avermectin toxicity [19, 23, 25, 30].

5. CONCLUSION

The findings presented from this study have revealed that IVM repeat high-dose therapy in rabbits may cause toxic effect on liver function, lipid profile and several changes of the liver and lung histological structure. AA co-treatment could reduce the level of IVM toxicity. In the light of these results, vitamin C by

gavage is recommended over that supplemented in the food. Careful application should be considered when using IVM on a wide scale in rabbits and others farm animals.

Abbreviations

IVM: ivermectin; AA: ascorbic acid; AAg: ascorbic acid by gavage; AAF: ascorbic acid supplemented in food; AST: alanine aminotransferase; ALT: aspartate amino transferase; GGT: gamma-glutamyltransferase; MLs: macrocyclic lactones; ML: macrocyclic lactones; BW: body weight; BWG: body weight gain; H&E: haematoxylin and eosin; TC: total cholesterol; TG: triglycerides; HDL-C: high density lipoprotein cholesterol; LDL-C: low density lipoprotein cholesterol; VLDL-C: very low density lipoprotein cholesterol; AF: atherogenic factor; AIP: atherogenic index of plasma; FDA: Food and Drug Administration; SD: standard deviation; DNA: deoxyribonucleic acid; GABA: Gama-aminobutyric acid.

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Conflict of Interest: The author has no conflict of interest to declare.

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).

Availability of data and materials: The datasets used and/or analyzed during this study are available from the corresponding author on reasonable request.

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