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Co-administration of vitamin E and selenium *in vivo* and *in vitro* ameliorates the toxic effects caused by ivermection and doramectin

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Abstract: Avermectins are used in animals and humans for their broad-spectrum effects against parasites causing cytotoxicity and damage to the cellular DNA. In this study, we examined the toxicological changes of ivermectin (IVM) and doramectin (DME) with or without the co-administration of vitamin E (Vit. E) and selenium (Se). The drugs used were for animal use. Twenty-five adult male rats were divided into five groups. Group 1 (control) was given saline, Group 2 was given IVM (0.2 mg/kg b.w.), Group 3 was given IVM and Vit. E/Se (80/1.6 mg/kg b.w., respectively), Group 4 received DME (0.2 mg/kg b.w.), and Group 5 received DME and Vitamin E/Se. Both IVM and DME were given by subcutaneous injections whereas Vit. E and Se were given orally. All the treatments were given once per week throughout the eight weeks. Although the doses were off-label use, they were given in a long-term course to unveil their toxicity effects in a clear manner and the response of the amelioration. By 24-h after the 8th week, the rats were sacrificed. Their blood was sampled for the haematological and sero-biochemical examinations. Histopathological changes and caspase-3 were determined in the hepatic and renal tissues. The histopathological findings showed that Vit. E and Se reduced the cellular changes induced by IVM or DME, indicating that Vit. E and Se protect against both types of avermectins, and that DME was safer than IVM. The cytotoxicity was assessed on a human embryo kidney (HEK) and skin cells by the SRB/IC_{50} method and AO/EB (acridine orange-ethidium bromide) staining. Both IVM and DME caused apoptosis in the cultured HEK more than in the skin cells (80% vs. 30%, respectively). The cellular apoptosis in response to the IVM was more than that of DME, and the use of Vit. E and Se reduced the cytotoxicity as observed by caspase-3, *in vivo,* and IC_{50}, *in vitro*.

Keywords: avermectins; HEK; apoptosis; caspase-3; antioxidants; α-Tocopherol; HEK skin cells

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Avermectins are macro-cyclic lactone derivatives used as potent anthelminthic drugs (Omura and Shiomi 2007; Pitterna et al. 2009). They generate by *Streptomyces avermitilis* after fermentation. Eight avermectins contain a-major and b-minor components for the prevention of cellular hyperpolarisation (Merola and Eubig 2012). Ivermectin (IVM), doramectin (DME) are the two main types of avermectins (Pitterna et al. 2009). Both drugs are fermentation products of avermectins. IVM is formed from a B1-a, and B1-b (80 : 20) mixture of natural avermectin, used in animals and human antiparasitic medication (Ali et al. 2017). It is oxidised in the liver primarily and excreted in the faeces and urine causing significant alterations in the liver and kidney tissues (Utu-Baku 2009). The persistence of avermectins' residues in the tissues and fluids is dose-dependent and producing variable side effects from mild to extremely severe (Crooks et al. 2000; Epstein and Hollingsworth 2013), up to CNS (central nervous system) depression as a sign of poisoning (Trailovic and Nedeljkovic 2011). Vitamin E (Vit. E) and selenium (Se) are antioxidant sweepers for the reactive oxygen species (ROS) inhibiting the lipid peroxidation in the tissues (El-Demerdash 2004; Biller-Takahashi et al. 2015). In the present study, we aimed at examining the toxicological effects of repeated administrations of IVM or DME, with or without the co-administration of Vit. E and Se on the haematological and biochemical profiles in rats. Furthermore, the characteristics of the cellular apoptosis in response to those drugs were studied with regard to the responsive effects to the Vit. E/Se cotreatments. The ability of IVM or DME, co-administered with Vit. E/Se, to induce apoptosis will be studied both *in vivo* and *in vitro*, as well.

MATERIAL AND METHODS

Drugs and chemicals

The drugs used were for animal use. Ivermectin® (IVM) (liquid form); 22, 23-dihydroavermectin A1a: C48H74O14 and 22, 23-dihydroavermectin B1a: C47H72O14 (United Company for Chemicals and Medical preparations, UCCMA, Cairo, Egypt), and Dectomax®; doramectin (DME): $C_{50}H_{74}O_{14}$ (Pfizer Inc., Pfizer Animal Health, New York, USA) were used, as 1% injectable solutions, and each 1 ml of Dectomax® contains 10 mg of DME. Both the vitamin E and selenium were obtained from UVEDCO Industrial Co. Ltd. (Amman, Jordan). The blood indicators were determined by an automatic cell counter (Exigo Veterinary Haematology System; Boule Medical AB, Stockholm, Sweden).

Transferase kits, alanine amino transferase (ALT) and aspartate amino transferase (AST), were used (RANDOX laboratories, Ltd., Crumlin, Antrim, United Kingdom). An alkaline phosphatase (ALP) kit (Spectrum; Egyptian company for Biotechnology, Cairo, Egypt), total protein and albumin kits (Biomerieux® SA, Marcy l' Etoil, France), uric acid and blood urea nitrogen kits (Biomerieux® sa69280, Marcy l' Etoil, France), creatinine kits (SAE) were also used. The neutral buffered formalin for fixation: sodium phosphate mono- and dibasic (Loba Chemie Pvt, Ltd, Mumbai, India), formalin (38–40%) (Sigma-Aldrich, Steinheim, Germany), xylene (Pharmaceutical Chemicals, ADWIC, Cairo, Egypt), paraffin wax (El-Nasr Pharmaceuticals Chemicals Co., Abu Zaabal, Egypt), and Canada balsam (Oxford Company for Chemicals, Mumbai, India) were also used.

Cells, chemicals and drugs for *in vitro* cell culture and apoptosis study

All the chemicals used in this study (Sulpho-Rhodamine-B (SRB, Tris-HCl, Trichloroacetic acid (TCA)) were obtained from Sigma Chemical Co. (St. Louis, MO, USA). The media and related products were supplemented by Gibco/Life Technologies Co. (Carlsbad, CA, USA). The cells of a human embryo kidney (HEK-293) and skin were obtained from Vacsera (Giza, Egypt). The medium for the cell culture is supplemented with 100 µg/ml streptomycin, and 10% heat-inactivated foetal bovine serum (FBS).

Animals and experimental design

All the experimental procedures and animal handling were performed according to the guidelines of the Research Ethics Committee of King Khalid University which follows the guidelines established by the US National Institutes of Health (NIH publication No. 85–23, revised 1996). Twenty-five adult male Sprague-Dawley rats were randomly

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divided into five equal groups and each received all the treatments once per week for eight successive weeks. Group 1, the control (CTL); in which the rats were subcutaneously (*s.c.*) injected with saline. The animals in Group 2 were *s.c.* injected with IVM (0.2 mg/kg body weight; b.w.) (Oksanen et al. 1992). The animals in Group 3 treated with IVM, but orally received Vit. E and Se (80 mg/kg and 1.6 mg/kg b.w. respectively) (Weiss et al. 1987; Rey et al. 2015). The animals in Group 4 were *s.c.* injected with DME (0.2 mg/kg b.w.) (Tinar et al. 1997; Yazwinski et al. 1999; Ayaz and Sahin 2003). The animals in Group 5 were treated with DME and Vit. E and Se, as undertaken in Group 3. The doses were chosen according to previous studies, and according to the company recommended doses. Twenty-four hours after the last treatment, the rats were sacrificed. Their blood samples were collected for a blood profile and biochemical analysis. The hepatic and renal tissue samples were used for the histopathology or flow cytometry.

Haematological and serobiochemical analysis

The blood samples were divided in two portions. The whole blood was used for the blood picture; the RBC (red blood cells), WBC (white blood cells), Hb (haemoglobin) and platelet counts. The serum was collected after centrifugation of the coagulated blood and kept frozen at −20 °C until used for the biochemical analyses. The serobiochemical analysis was colorimetrically performed for testing the markers of the liver and kidney functions, including; the ALT (alanine transaminase), AST (aspartate transaminase) (Reitman and Frankel 1987), globulin, albumin (Doumas et al. 1997), total proteins (Fenk et al. 2007), urea, creatinine and uric acid (Lumeij 1987). Furthermore, the alkaline phosphatase (ALP) was measured as a marker for the cellular activity and intact plasma membrane (Kind and King 1954).

Assessment of antioxidants

The glutathione S-transferase (GST) activity was measured in the liver and kidney tissues according to Habig and Jakoby (1981), in the light of the commercial kit instructions. The activity of the glutathione peroxidase (GSH-Px) was measured as explained by Paglia and Valentine (1967) at 340 nm.

Histopathological examination

Hepatic and renal changes. Small fresh specimens from the liver and kidney were dissected and rapidly fixed in formalin (10%) for a minimum period of 24 hours. The specimens were processed throughout the paraffin embedding procedures till obtaining 5 µ tissue sections stained by H&E (Haematoxylin and eosin) (Slaoui and Fiette 2011).

Caspase-3 detection by flow-cytometry. According to the method previously described (Ribble et al. 2005), 100 µl of the cell suspension (1×10^6) cell/ml of the liver samples obtained from the sacrificed rats were prepared for the homogenous suspension by using a Tris-EDTA buffer. The cells were thoroughly washed with a phosphate buffer saline (PBS) and centrifuged at 448 *g* for 5 minutes. The supernatant was removed and then the pellet was resuspended in 100 µl of PBS to be mixed well with 10 µl of caspase-3 FITC (Rabbit anti-active caspase-3, cat. No. 559341), and then incubated for 30 min at room temperature in darkness. The cells were re-washed with PBS/BSA (bovine serum albumin) again; and centrifuged for 5 min at 448 *g* and the supernatant was removed. Finally, the cells were resuspended in 200 µl of 4% paraformaldehyde in PBS and fixed until assayed by the flow cytometer (Bicton Dickinson, Bedford, USA).

IC_{50} detection by sulforhodamine B (SRB) assay. The SRB assay, as previously described (Houghton et al. 2007), was used to assess the cytotoxicity of the human embryo kidney (HEK-293) and skin cells after treatment with IVM or DME, supplemented with Vit. E/Se. The drugs' concentrations were used in a range of 0.01 to 1 000 µg/ml. The tested drugs showed a variable cytotoxicity profile. The cytotoxicity of the drugs on the cultured HEK and skin cell lines was expressed as IC_{50} (concentrations exhibited 50% cytotoxicity). The acridine orange-ethidium bromide staining (AO/EB) method was used to detect the living, early apoptotic, necrotic and late apoptotic cells. The normal HEK and skin cells were seeded in 6-well tissue culture plates containing a cover slide (1×10^6 cells/ well). After incubation for 24 h, the cells were treated with an IC_{50} dose of the drugs and incubated for 48 h, then incubated

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again. After incubation, the cells were washed once with PBS; 100 µl of the mixed AO/EB (50 µl mixture; 100 µg/ml of AO and 100 µg/ml of EB in PBS).

The cells were examined by using a fluorescence microscope (Nikon Eclipse E400; Nikon, Missouri City, TX, USA) (Baskic et al. 2006). Acridine orange emits a green fluorescence immediately after penetration of the dead and living cells and intermixed with the double strand nucleic acid or DNA, but gives off a red fluorescence when it binds to the single stranded RNA fragments. Ethidium bromide emits a red fluorescence when binding to the DNA (Ciniglia et al. 2010). Four kinds of cells are distinguished; a) even-shaped living cells with bright green nuclei, b) early apoptotic cells with irregular green nuclei, and bright green spots of apoptotic chromatins, c) swollen organised structures with red nuclei referred to as necrotic cells, and d) late apoptotic uneven cells with orange to red nuclei and a highly fragmented chromatin (Ciniglia et al. 2010; Zhang et al. 2016). The cells were blindly counted by three observers.

Statistical analysis. All the data were analysed by a one-way analysis of variance (ANOVA). The differences between the groups were considered significant at $P < 0.05$. The IC_{50} was calculated by using the Sigma Plot software (v12; San Jose, USA) by using the fitting curve. The significant difference of the drugs' IC_{50} against the same cell line was also compared by a one-way ANOVA, and the Newman Keuls test was used post-hoc. The data are shown as the mean ± standard error of the mean (SEM). The statistics were correlated using the GraphPad Prism software (v5; San Diego, USA).

RESULTS

Haematological findings

The blood indicators including the blood profile and the serum concentrations of the urea, creatinine, uric acid, total proteins, albumin and globulin in response to IVM, DME with or without the co-administration of Vit. E/Se are shown in Table 1. The data revealed a non-significant increment in the red blood cells (RBCs), the platelet count and the Hb concentration as compared to the controls. On the other hand, treatment with IVM or DME tended to significantly increase the white blood cell (WBC) count compared to the control one. In contrast, the concomitant administration of Vit. E/Se significantly improved the WBC count; they significantly reduced the WBCs than IVM or DME only.

Both IVM and DME exhibited a significant increase in the urea, creatinine and uric acid, but exhibited a significant decrease in the protein when compared to each respective control value ($P < 0.05$). However, treatment with Vit. E/Se induced a significant decrease in the urea, creatinine and uric acid versus an increase in the serum proteins when compared to IVM or DME only ($P < 0.05$). The effect of IVM or DME on the serum concentrations of the transaminases; aspartate aminotransferase (AST), alanine aminotransferase (ALT), and alkaline phosphatase (ALP) are also shown in Table 1. Both IVM and DME significantly increased the AST, ALT and ALP activities when compared to the control ($P < 0.05$). However, those enzymatic changes induced by both drugs were non-significantly different from each other, and the use of Vit. E/Se induced non-significant changes in those enzymes compared to the control.

The effect of IVM or DME and Vit. E/Se on the antioxidant enzymes; glutathione S-transferase (GST) and glutathione peroxidase (GSH-Px) in the liver and kidney tissues are also presented in Table 1. IVM, but not DME, significantly suppressed the release of GST and GSH-Px ($P < 0.05$). The effect was slightly improved in response to the co-treatment with Vit. E/Se that showed variable effects in both types of the tissues. Administration of Vit. E/Se with both types of avermectins slightly increased the activities of those antioxidants rather than the use of IVM and DME only in either the liver or kidney tissues. It was clearly obvious that the effects of both IVM and DME on the serobiochemical indicators were non-significantly different from each other and the concomitant treatment with Vit. E/Se ameliorated those effects showing non-significant differences from each respective control.

Histopathology and apoptosis assessment

The necrotic changes in the hepatocytes and renal tissue cells in response to the IVM and DME and Vit. E/Se administration are shown in Figure 1. The effects of the IVM administration on the hepatic tissue are shown in Figure 1C. It exhibited necrosis in the hepatocytes associated with

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Table 1. The blood picture and serobiochemical parameters in response to the *s.c.* injections of saline (vehicle) as the control (CTL), ivermectin (IVM; 0.2 mg/kg b.w./week) and doramectin (DME; 0.2 mg/kg b.w./week) with or without the oral administration of Vit. E and Se (80 mg/1.6 mg/kg b.w./week) in 5 male albino rats (n = 5) for 8 successive weeks

Parameters		G1	G2	G3	G4	G5
RBCs ($\times 10^{12}$/l)		8.50 ± 0.2	8.70 ± 0.1	8.30 ± 0.2	9.20 ± 0.4	9.40 ± 0.2
WBCs ($\times 10^{9}$/l)		11.90 ± 1.6^{a}	14.40 ± 1.0^{a}	8.60 ± 0.7^{b}	14.70 ± 1.4^{a}	9.60 ± 0.9^{b}
Hb (g/l)		140.8 ± 6.0^{b}	149 ± 3.0^{ab}	143 ± 5.0^{ab}	147 ± 3.0^{ab}	158 ± 2.0^{a}
Platelets ($\times 10^{9}$/l)		660.8 ± 46.3	650.8 ± 34.7	660.8 ± 34.2	694.0 ± 35.1	734.4 ± 54.4
AST (µkat/l)		1.23 ± 0.06^{b}	1.96 ± 0.13^{a}	1.30 ± 0.08^{b}	1.90 ± 0.11^{a}	1.20 ± 0.09^{b}
ALT (µkat/l)		0.92 ± 0.10^{b}	1.48 ± 0.15^{a}	0.90 ± 0.09^{b}	1.39 ± 0.08^{a}	0.86 ± 0.09^{b}
ALP (µkat/l)		2.01 ± 0.13^{b}	2.91 ± 0.29^{a}	1.92 ± 0.17^{b}	2.70 ± 0.22^{ab}	2.10 ± 0.19^{b}
Urea (mmol/l)		12.49 ± 0.86^{b}	19.56 ± 1.54^{a}	12.78 ± 1.07^{b}	18.78 ± 1.39^{a}	13.49 ± 1.46^{b}
Creatinine (µmol/l)		45.97 ± 8.84^{b}	120.22 ± 14.14^{a}	53.04 ± 8.84^{b}	113.15 ± 11.49^{a}	56.58 ± 7.96^{b}
Uric acid (µmol/l)		243.9 ± 11.9^{b}	327.17 ± 17.9^{a}	226.04 ± 14.9^{b}	339.1 ± 17.90^{a}	249.8 ± 29.7^{b}
T. Protein (g/l)		79.0 ± 3.0^{a}	62.0 ± 3.0^{b}	83.0 ± 3.6^{a}	63.0 ± 3.6^{b}	78.0 ± 6.0^{a}
Albumin (g/l)		44.0 ± 2.5^{ab}	33.0 ± 4.3^{b}	46.0 ± 2.5^{a}	35.0 ± 5.1^{ab}	45.0 ± 3.8^{ab}
Globulin (g/l)		35.0 ± 4.1^{ab}	28.0 ± 3.6^{b}	35.0 ± 1.3^{a}	27.0 ± 2.5^{b}	32.0 ± 2.6^{ab}
GST (mM)	liver	0.22 ± 0.01^{a}	0.15 ± 0.01^{b}	0.18 ± 0.01^{b}	0.19 ± 0.01^{ab}	0.21 ± 0.004^{a}
	kidney	0.27 ± 0.02^{a}	0.20 ± 0.01^{b}	0.26 ± 0.01^{a}	0.23 ± 0.01^{ab}	0.26 ± 0.01^{a}
GSH-Px (mM)	liver	0.15 ± 0.01^{a}	0.11 ± 0.004^{b}	0.12 ± 0.01^{ab}	0.13 ± 0.004^{ab}	0.14 ± 0.002^{a}
	kidney	0.13 ± 0.004^{a}	0.099 ± 0.004^{b}	0.12 ± 0.01^{ab}	0.11 ± 0.004^{ab}	0.12 ± 0.003^{ab}

ALP = alkaline phosphatase; ALT = alanine amino transferase; AST = aspartate amino transferase; GSH-Px = glutathione peroxidase; GST = glutathione S-transferase; Hb = haemoglobin; RBCs = red blood cells; WBCs = white blood cell
^{a,b,c}The letters denote the significant difference between the groups; G1; CTL, G2; IVM only, G3; IVM+Vit. E/Se, G4; DME only, G5; DME+Vit. E/Se. All the data were presented as the mean ± SE. ^{a,b,c}The letters on the bars denote the significant differences at $P < 0.05$

a fatty infiltration compared to the control (CTL) (Figure 1A). The effects of the IVM administration on the renal tissues are shown in Figure 1D. It showed necrosis in the renal tubular epithelium with vasodilatation, haemorrhaging between the tubules and tissue depression in the tubular lumens compared to the CTL (Figure 1B).

The effects of the DME administration on the hepatic tissues are clearly shown in Figure 1I. It exhibited a very mild degree of hepatic necro-

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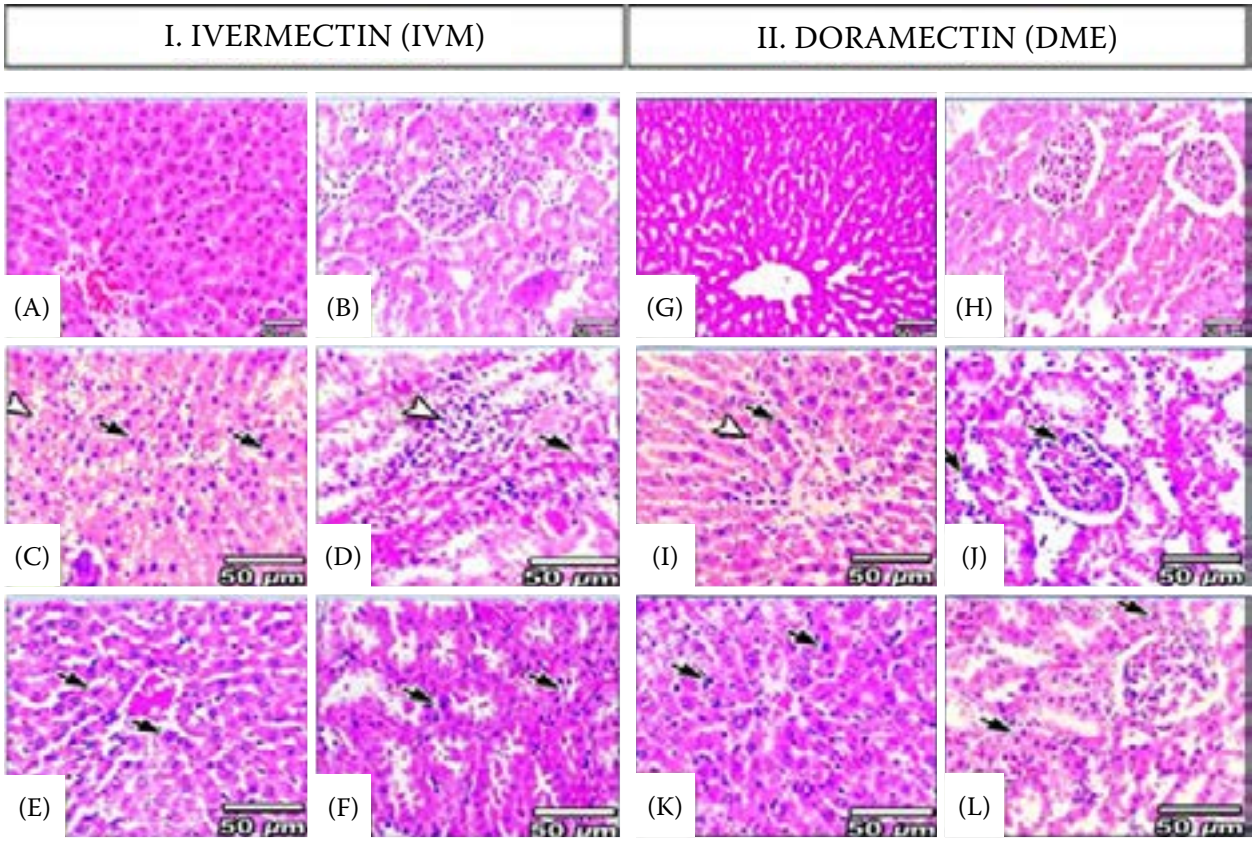

Figure 1. The histological sections of the liver (A, C, E, G, I, K) and kidney (B, D, F, H, J, L) of a male albino rat stained with haematoxylin and eosin (H&E, × 400). Panel I: The normal hepatic or renal tissues in the saline-treated control (CTL) are shown in (A) and (B), respectively. The hepatic tissues in response to IVM show necrosis (white arrow) and fatty changes (black arrow) (C), or IVM plus Vit. E/Se show the intact hepatic cells (E). The renal tissues in response to IVM show tubular necrosis with haemorrhage and necrosis (arrows) (D), or IVM plus Vit. E/Se showing recovery (F). Panel II: The normal hepatic or renal tissues in the CTL are shown in (G) and (H), respectively. The hepatic tissues in response to DME show mild necrosis (white arrow) and mild fatty changes (black arrow) (I), or DME plus Vit. E/Se show intact hepatic cells (K). The renal tissues in response to DME show mild tubular necrosis (arrows) (J), or DME plus Vit. E/Se showing the recovery (L)

sis with dilatation of the central veins compared to the CTL (Figure 1G). The effects of the DME injections on the renal tissues are shown in Figure 1J. It showed necrosis in the tubular epithelium compared to the CTL (Figure 1H). The co-administration of the Vit. E/Se with IVM or DME induced dilatation in the central vein with a recovery of the hepatocytes (Figure 1E and 1K), and a recovery of the renal tubular epithelium with a widening of the tubular lumens (Figure 1F and 1L).

The apoptotic liver cells in the rats treated with IVM or DME with or without the supplementation with Vit. E/Se were marked by the active caspase-3 as shown in Figure 2. The hepatic cellular viability showed variable degrees of apoptosis beyond treatment with IVM, DME and Vit. E/Se. The hepatic cells showed higher numbers of the caspase-3 positive cells as a result of avermectin injections in the rats. The apoptotic cells, marked positive to caspase-3, varied significantly among the groups ($P < 0.05$); CTL, 20.5 ± 1.8; IVM, 54.7 ± 0.6; DME, 36.3 ± 1.4; IVM+Vit. E/Se, 32.8 ± 0.8, and DME+Vit. E/Se, 27.9 ± 1.1%. Supplementation with Vit. E/Se decreased the extent of the apoptosis compared to the use of avermectins only.

The toxic effects of the avermectins and Vit.E/Se were evaluated *in vitro* by testing the inhibition concentration-50 (IC_{50}) of those drugs on the normal human embryo kidney (HEK-293) and human skin cell lines, as non-target cells, and is presented in Figure 3. The avermectins evoked a larger extent in the cellular deaths in the HEK rather than the skin

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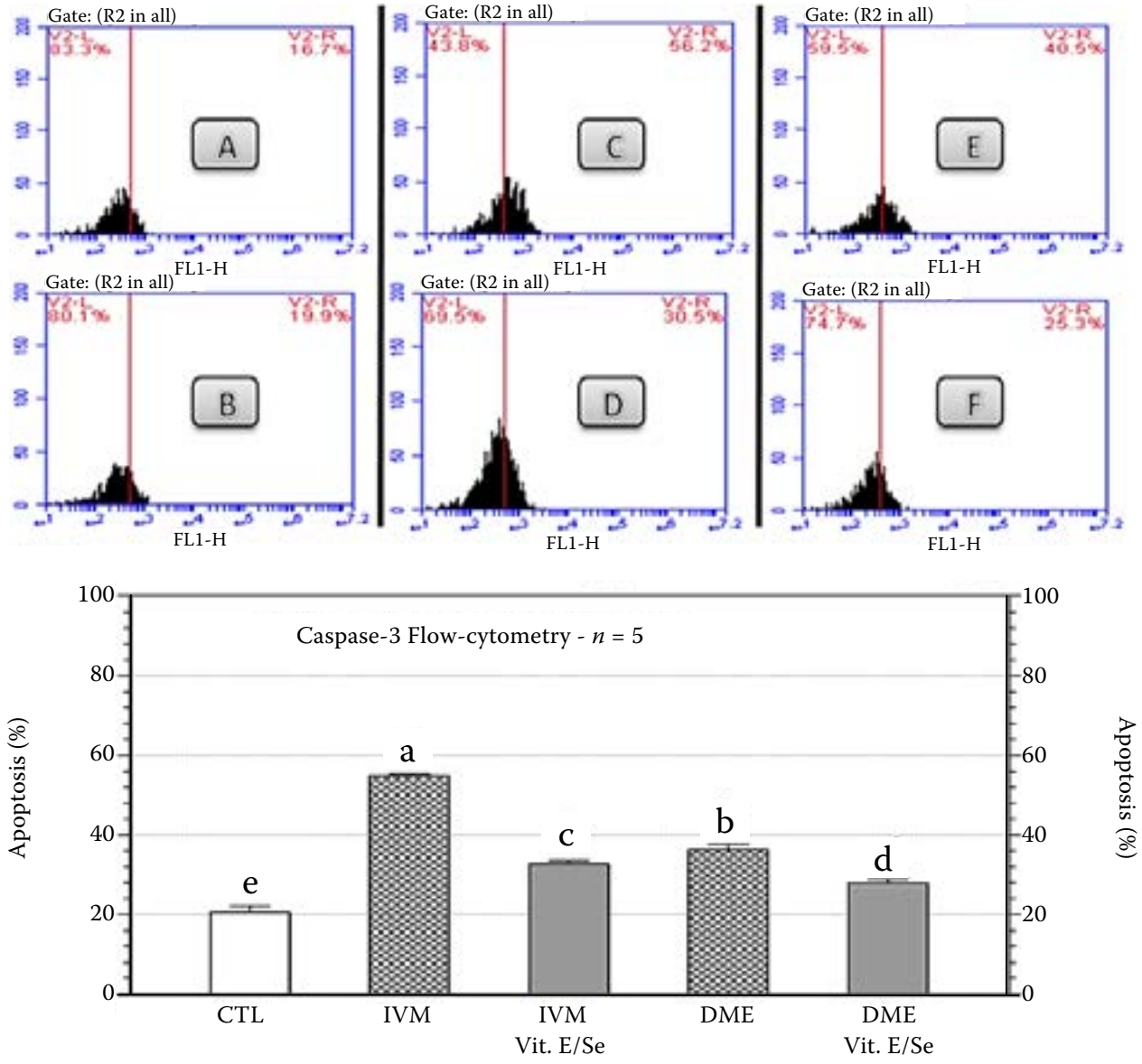

Figure 2. The flow cytometric analysis for caspase-3 in the different rat-experimental groups showing the hepatic cellular apoptosis; V2-L= Non-apoptotic (–ve stain), V2-R= Apoptotic (+ve stain) in response to the treatment; A, B) saline (CTL), C) IVM, D) IVM + Vit. E/Se, E) DME, and F) DME + Vit. E/Se. All the data were presented as the mean ± SE for the five animals (n = 5). The letters on the bars (a, b, c, d) denote a significant difference between the groups at $P < 0.05$

cells (80 vs. 30%, respectively), and that IVM induced the killing of the cells more than DME in both types of cells. DME was safe on both types of human cells ($IC_{50} \geq 100$ µg/ml); 298.11 ± 1.61 *vs.* 217.0 ± 93.5 µg/ml for the HEK and skin, respectively. However, IVM was cytotoxic giving lower values in the IC_{50}; 0.51 ± 0.01 *vs.* 2.21 ± 0.05 µg/ml against the HEK and skin cells, respectively. On the other hand, the effect of Vit. E and Se in a united-form drug was safe on both types of cells showing the highest IC_{50} for the HEK and skin cells; 15 660.0 ± 9.1 and 14 380.71 ± 1.50 µg/ml, respectively.

Supplementation of IVM or DME with Vit. E/Se reduced the IC_{50} on both the cell lines rather than with the treatment of either drug only. The IC_{50} of the IVM treated animals with Vit. E and Se for the HEK and skin cells were increased up to 58.54 ± 0.84 *vs.* 0.51 ± 0.01 and 43.60 ± 1.43 *vs.* 2.21 ± 0.05 µg/ml. Furthermore, the IC_{50} of the DME treated animals with Vit E/Se for the HEK and skin cells were also in-

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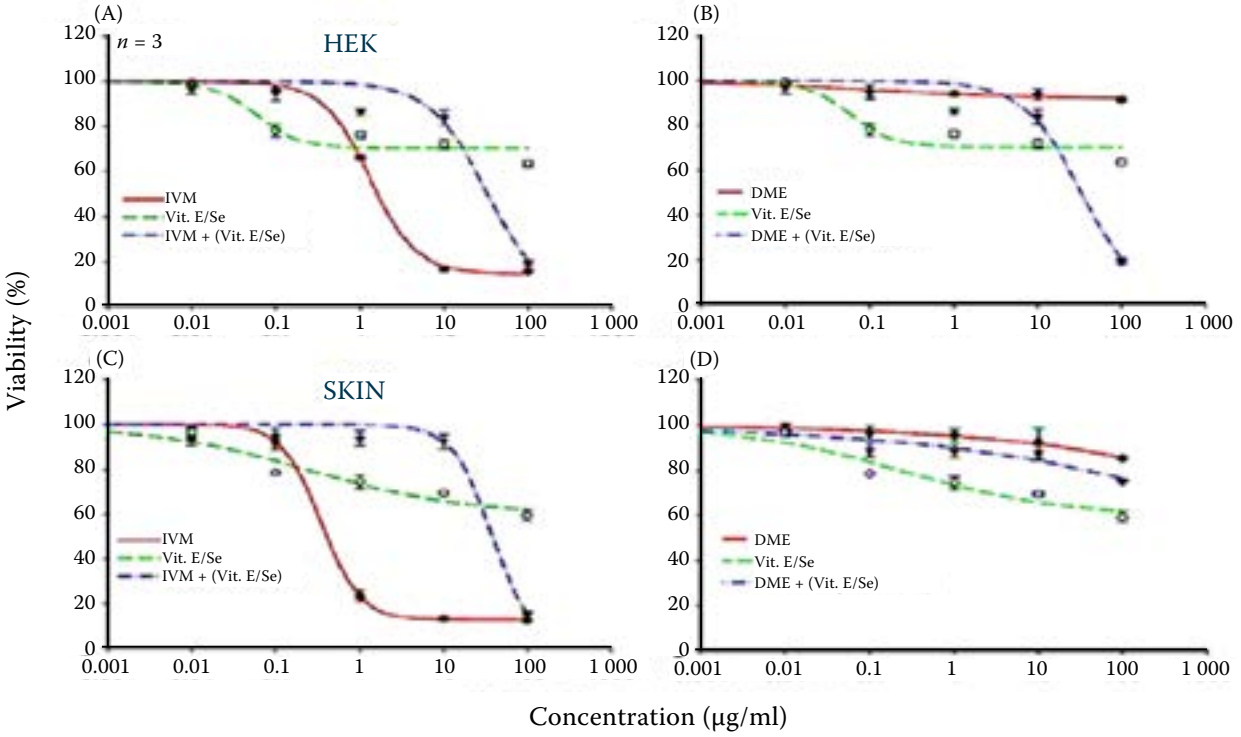

	IC_{50} ± SE µg/ml	
	HEK-293 cells	SKIN cells
Vit. E/Se	15 660.0 ± 9.1	14 380.71 ± 1.50
IVM	0.51 ± 0.01	2.21 ± 0.05
IVM + Vit. E/Se	58.54 ± 0.84	43.60 ± 1.43
DME	298.11 ± 1.61	217.0 ± 93.5
DME + Vit. E/Se	331.13 ± 12.51	518.0 ± 97.11

Figure 3. The response curves of the human embryo kidney (HEK-293) treated with; IVM, Vit. E/Se and IVM+Vit. E/Se (A), and DME, Vit. E/Se and DME+Vit. E/Se (B). The human skin cells treated with; IVM, Vit. E/Se and IVM+ Vit. E/Se (C), and DME, Vit. E/Se and DME+Vit. E/Se (D). The mean values of the IC_{50} (µg/ml ± SE) of the different treatments are shown in the lower table panel

creased up to 331.13 ± 12.51 and 518.0 ± 97.11 µg/ml, compared to each respective value before the treatment with Vit. E/Se, respectively.

The cytotoxicity was explored by the SRB method and revealed by acridine orange/ethidium bromide (AO/EB). According to the IC_{50} values on the human embryo kidney (HEK) and skin cells, the cellular viability, apoptosis and necrosis were detected as shown in Figure 4. They showed cells that were alive, apoptotic or necrotic cells, in response to IVM, DME and Vit. E/Se.

The viable cell count against those apoptotic or necrotic cells is presented in Figure 5. The necrosis was the lowest pathway observed over all the treatments, a favourable marker for drug safety. The avermectins evoked higher levels of apoptosis in the HEK rather than the skin cells (80 *vs.* 30%, respectively), and IVM induced cellular apoptosis and necrosis more than DME in both types of cells. Furthermore, IVM showed high toxic effects on the HEK and skin cells compared to DME.

DISCUSSION

Avermectins, i.e., IVM, are used a potent antiparasitic drugs for both animals and humans causing cytotoxicity and damage to the cellular DNA (Zhang et al. 2016). According to the Food and Drug Administration (FDA), the recommended dose of ivermectin is not toxic up to three doses. Overdoses could induce foetotoxicity or terato-

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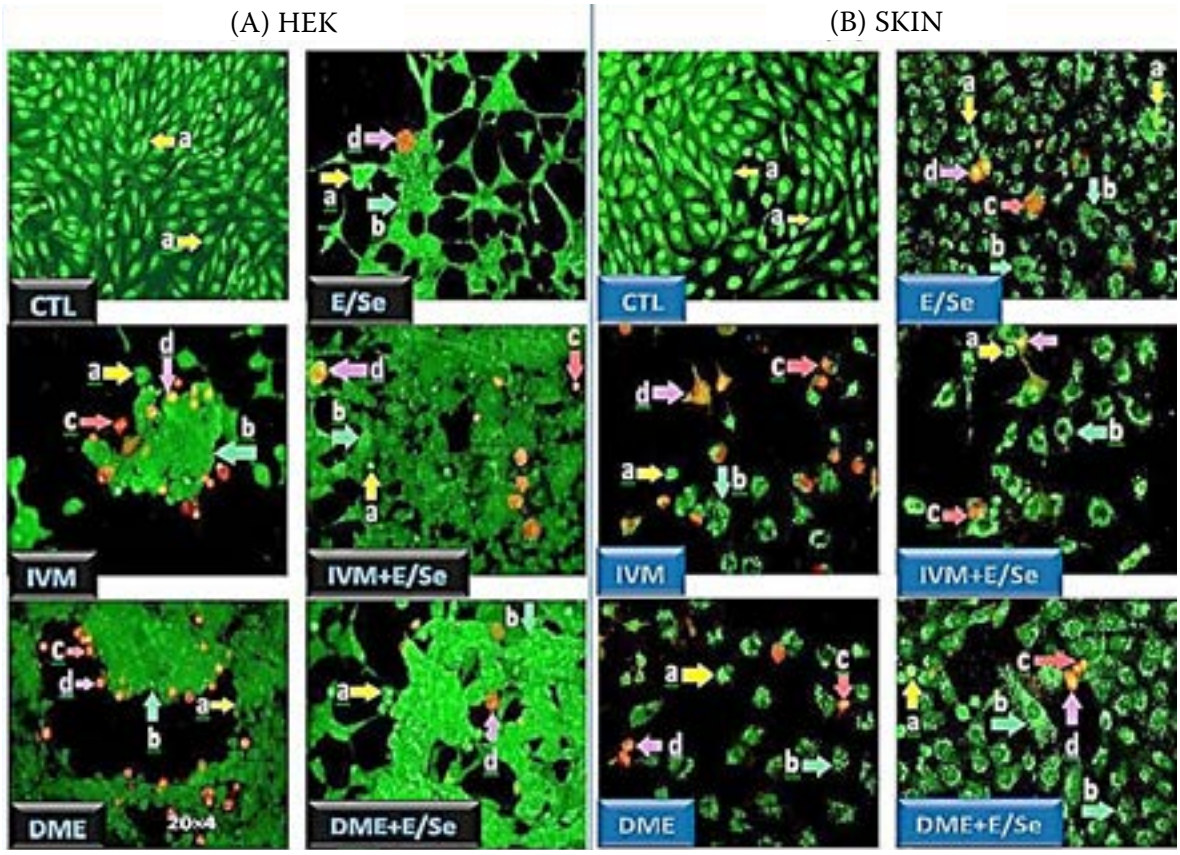

Figure 4. The HEK (panel A) and human skin (panel B) cell damage found by the AO/EB dual staining, in response to IVM and DME, with or without Vit. E/Se, showing; a) regularly shaped live cells with a green nucleus and condensed chromatin, b) early apoptotic cells with fragmented organelles and dense chromatin patches, c) swollen organised necrotic cells, and d) uneven, fragmented, late apoptotic cells

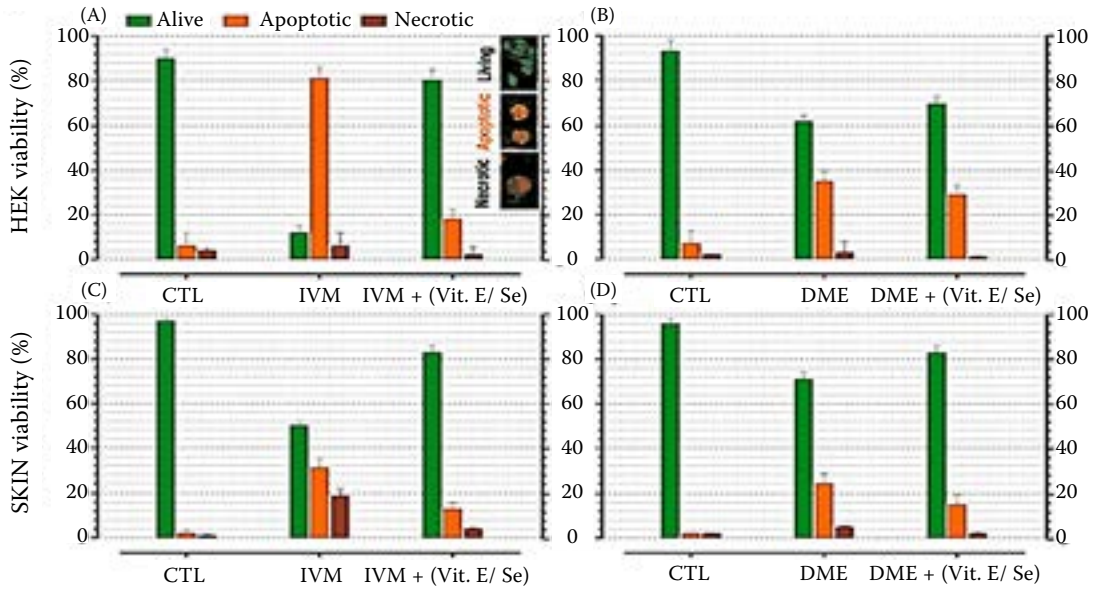

Figure 5. The panels of the human embryo kidney (HEK) and skin cells, treated with; -- (control, CTL), and the IC_{50} of IVM or IVM plus Vit. E/Se are shown in (A) and (D), respectively. The HEK and skin cells treated with; (-- CTL) and IC_{50} dose of DME or DME plus Vit. E/Se are shown in (B) and (C), respectively. The panels show the percentages of the living, necrotic and apoptotic cells stained with AO/EB. The experiment was repeated 3 times for each treatment (n = 3)

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genicity (Merck & Co. 2006). The overdoses were administered in the present study to clearly exhibit the toxic effects of avermectins and, thus, could distinguish the ameliorative role of Vit. E/Se against it. Both IVM and DME revealed a non-significant increment in the red blood cells (RBCs), platelet count and Hb concentration compared to the control (Ismaiel et al. 2017). On the other hand, treatment with IVM or DME tended to significantly increase the white blood cell (WBC) count when compared to the control. The increment in the leukocytic count could result from the inflammatory changes that occurred in different the body organs (Wanji et al. 2017). Our results disagreed with others (Eissa and Zidan 2010). In contrast, the concomitant administration of Vit. E/Se significantly improved the WBC count; they significantly reduced the WBCs than did the IVM or DME administration only, an evidence for stabilising the oxidative balance in the tissues.

The significant increment in the concentrations of the blood urea, creatinine and uric acid in response to IVM or DME, either, were significantly reduced after treatment with Vit. E/Se, referring to the load of the drug medication in the renal tubules. In consistence with previous reports (Khan et al. 2002; Eissa and Zidan 2010), the significant decrease in the serum protein levels in response to the avermectin treatment denoted an impaired liver function which was ameliorated by the concomitant treatment with Vit. E/Se. Hepatocellular damage in response to IVM or DME was revealed by the transaminases; both the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) (Adeyemi et al. 2015). Furthermore, alkaline phosphatase (ALP) was used as a membrane bound enzyme used as a marker for the integrity of the plasma membrane and endoplasmic reticulum (Sharma et al. 2014). In consistence with previous studies, both IVM and DME significantly increased the AST, ALT and ALP activities when compared to the control (El-Far 2013). However, the co-administration of Vit. E and Se with the avermectins significantly decreased those enzymes rather than the IVM or DME treatment only, which showed a non-significant difference to the control. The current results strongly agreed with the previous study that attributed those enzymatic changes in the biochemical and cellular changes to the chronic administration of avermectins (Turkan et al. 2018).

IVM, but not DME, was found to significantly suppress the release of GST and GSH, which is in agreement with Turkan et al. (2018) who found an inhibitory effect of the avermectins on the antioxidants. Several studies stated that Vit. E and Se constitute the primary antioxidant system of the living tissues. Vit. E is the main liposoluble antioxidant acting as a scavenger of ROS/free radicals, and a stabiliser of the cell membranes' permeability (Wang and Quinn 1999) and so it inhibits the oxidation of the cell organelles (Milad et al. 2001). Both Vit. E and Se-containing GSH-Px have the capacity to sweep out the free radicals and catalyse the lipid hydroperoxides into less reactive products (Uboh et al. 2009). Several studies explained the protective role of Vit. E against hepatotoxicity-inducing insecticides (Shokrzadeh et al. 2012). Administration of Vit. E/Se with the both types of avermectins significantly increased the activities of the antioxidant enzymes; GST and GSH-Px, rather than the use of IVM and DME only, in consistence with a previous study which found a stimulant effect of the Vit. E and Se co-administration on the antioxidant levels in blood like GSH-Px levels in the blood inducing cellular protection against the oxidative damages (Ren et al. 2017). GSH-Px and other antioxidants like GST play a pivotal role in the cellular stability against oxidation. Both enzymes maintain the redox status and removal of H_2O_2 during oxidative stress (Song et al. 2018).

The present study emphasises that the repeated administration of avermectins and IVM, in particular, causes significant alterations in the liver and kidney tissues, which coincides with a previous study reported by Utu-Baku (2009). On the other hand, our study unveiled the ameliorative role of Vit. E and Se against the toxicological hazards of both the antiparasitic drugs of the avermectins through the histopathological and cellular vitality. The necrotic changes in the hepatocytes and renal tissue cells confirmed the toxic effects of IVM or DME and their sequential disturbances observed in the serobiochemical indicators (Gaw et al. 1995). Apoptosis and necrosis are the main pathways of cellular death (Ricciarelli et al. 2001; Joshi and Knecht 2013). Necrosis may result from the accumulation of excessive amounts of the media around the cells which prevent the oxygen absorption. The effects of IVM or DME, on the cellular viability and their role in triggering apoptosis were evaluated both *in vivo* and *in vitro*.

https://doi.org/10.17221/74/2019-VETMED

Both types of avermectins were found to suppress the viability of the rat liver cells. They collapse the mitochondrial membrane causing cellular death and, thus, activate the caspase-3 (Zhang et al. 2016). It was consistent with the present study since the hepatic cellular viability showed variable degrees of apoptosis as revealed by caspase-3 beyond treatment with IVM, DME and Vit. E/Se, showing higher levels of caspase-3 positive cells in the avermectins-treated animals, which were lowered in those supplemented with Vit. E/Se. The toxic effect was confirmed *in vitro* on the human embryo kidney (HEK) and skin cell lines showing morphological changes in the nuclear chromatin and obvious clustering of the cell organelles (Ricciarelli et al. 2001). IVM showed higher toxic effects on those cells rather than DME. However, the cytotoxicity was ameliorated with Vit. E/Se, as found in the *in vivo* experiment. Also, IVM induced cellular apoptosis more than DME in both types of cells showing an increased killing effect against the HEK and skin cells.

The ameliorative effect of Vit. E and Se on both types of cell lines was variable. It was clearly detected on the skin cells rather than the HEK cells through the IC_{50} values. Furthermore, the responsive recovery was clearly detected in the case of DME+Vit. E/Se in the skin rather than other treatments. This finding could explain the efficacy of both avermectin drugs. DME is the drug of choice for treatment of ectoparasites; a skin infection, i.e., mange, (Rendle et al. 2007) rather IVM, which is commonly used as potently active drug against a wide range of internal and external nematodes and arthropods (Crump and Omura 2011). The former drug was light, considering the sensitivity of the skin, compared to the latter one that is used against gastrointestinal helminths and liver flukes. The variable effects of those drugs on the HEK and skin cells typically explained the variation in the cellular sensitivity according to the paradoxical reports of the pharmacology (Bellavite et al. 2015). With regard to the variant cell stages of vitality or death, the efficacy of Vit. E/Se against the avermectins showed that Vit. E/Se did not bring a better benefit to the DME treatment, but largely reduces the IVM's toxicity.

In veterinary medication, IVM is used in cattle, sheep, goats, pigs, horses and reindeer as an antiparasitic. It is given subcutaneously as a single dose treatment only. However, in humans, it is used for the treatment of onchocerciasis. The maximum residue limits (MRL) for IVM were previously established in different tissues; fat (100 µg/kg), liver (100 µg/kg) and kidney (30 µg/kg) tissues for all mammalian food producing species (EMA 2014). In comparison to the MRL of IVM in the different tissues compared to their IC_{50} tested *in vitro*, the present findings confirm the cytotoxic effects of IVM, but not DME, on the body tissues. However, future studies are required to prove that the cytotoxicity on the other body tissues like the liver in particular. Although the doses used in the present study, for eight consecutive weeks, were more than that recommended label-doses *in vivo*, the MRL in the body tissues and the IC_{50} of either IVM or DME, should be considered together. The living tissues subjected to the drug MRL of a single dose, and the tissue treated with wide range of concentrations from 0.01 to 1 000 µg/ml (considering the IC_{50}) could confirm the cytotoxicity of either drug in a clear manner.

Ultimately, according to both *in vivo* and *in vitro* experiments, DME was shown as a safe antiparasitic drug rather than IVM. Also, the supplementation of IVM or DME with Vit. E/Se was found to reduce the cytotoxicity and cellular apoptosis. Furthermore, our study presented the beneficial effects of Vit. E/Se which stabilise the plasma membrane and repair the damaged tissues induced by the avermectins. However, Vit. E/Se did not bring a better benefit to the DME treatment, but largely reduce the IVM's toxicity.

The present study strongly agreed with Zhang et al. (2016) who stated a serious risk, not only to animals, but also to human health, by inducing DNA damage and mitochondria-associated apoptosis caused by those antiparasitic drugs. This risk could be achieved in the present study as a result of repeated overdoses and multiple applications out of the recommended label-doses. Furthermore, DME was found safer than IVM. However, the histopathological findings showed that vitamin E and selenium mitigated the cellular changes induced by ivermectin or doramectin, indicating that Vit. E/Se contributed to the protection against both avermectins.

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Conflict of interest

The authors declare no conflict of interest.

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