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Article

Five-day administration of ivermectin is effective in attenuating valproic acid-induced liver toxicity in rats

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Valproic acid (VPA) is an effective and inexpensive anticonvulsant commonly prescribed as an antiepileptic. However, there is a percentage of susceptible patients who experience unwanted effects, such as weight gain and fatty liver. Ivermectin (IVM), a versatile antiparasitic, has shown potential in the treatment of non-alcoholic fatty liver disease. In this study, the potential hepatoprotective effect of ivermectin on valproic acid-induced hepatotoxicity was investigated in rats. VPA (250 mg/kg/day) was orally administered for 14 consecutive days to induce hepatocellular damage in adult Wistar rats, after which the animals were treated with a subcutaneous injection of IVM (0.4 and 1.3 mg/kg/day) for another five days. Animals receiving VPA developed obesity and microvesicular and macrovesicular steatosis. They also had increased levels of plasma triglycerides and aspartate aminotransferase, alanine aminotransferase and alkaline phosphatase enzymes. IVM treatment significantly decreased serum triglyceride and cholesterol levels and attenuated valproic acid-induced hepatic lipid accumulation in rats. These results were confirmed by histopathological examination.

Keywords: Ivermectin. Valproic acid. Weight gain. Fatty liver.

INTRODUCTION

Valproic acid (2-n-propylpentanoic acid) is a first-line anticonvulsant, commonly used in the treatment of different types of epilepsy. It has also been shown to be effective in the treatment of highly refractory syndromes such as West syndrome, bipolar disorder, manic episodes, and in migraine prophylaxis (Zhu *et al*., 2017). Although VPA therapy is effective and generally well tolerated, there are susceptible patients who experience adverse reactions to this treatment (Tolou-Ghamari, Palizban, 2015). Follow-up clinical studies have reported that the most common adverse reactions include unexpected obesity and non-alcoholic fatty liver disease (Guo *et al*., 2019). Other adverse reactions associated with VPA include both cardiovascular and renal disorders. While there is limited evidence of renal toxicity in adult individuals, renal dysfunction may manifest as proximal tubular dysfunction (Nanau, Neuman, 2013).

Experimentally, studies have shown that VPA alters hepatic triacylglycerol and cholesterol biosynthesis, fatty acid catabolism and the expression of lipid transport-related genes (Chang *et al*., 2016). Other characteristic effects of VPA in rats include increased oxidative/nitrosative stress and production of inflammatory mediators such as TNF-α and NF-Kb (Abdelkader *et al*., 2020). Recently, VPA administration (500 mg/kg BW) has been reported to induce proximal kidney tubule injury and renal failure in rats (Maneenin *et al*., 2019).

Despite notable advances in the understanding of the side effects associated with VPA, there are no pharmacological interventions to attenuate the adverse effects associated with VPA therapy. Among the most commonly used therapeutic measures are combined

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programs of diet and moderate aerobic exercise, and increased dietary fiber intake (Pouwels *et al*., 2022). Previous research showed that the administration of both carnitine and ellagic acid can lead to improvements in liver biochemistry, and in necroinflammatory and fibrotic changes associated with VPA-induced hepatotoxicity (Lheureux, Hantson, 2009; Abdelkader *et al*., 2020).

Surprisingly, the antiparasitic IVM has a wide range of effects on diverse organisms besides the endoparasites and ectoparasites it was developed to control (Laing, Gillan, Devaney, 2017). For example, IVM administration has been shown to suppress the proliferation of malignant cells in several types of cancer (Melotti *et al*., 2014), to decrease triglyceride accumulation by inhibiting differentiation of 3T3-L1 preadipocytes *in vitro* (Qi *et al*., 2019), and to regulate serum glucose and cholesterol levels in diabetic rodents (Jin *et al*., 2013a); it has also shown potential in the treatment of non-alcoholic fatty liver disease and insulin resistance (Jin *et al*., 2015b).

In mammals, an important target of IVM is the farnesoid X receptor (FXR), a member of the nuclear receptor superfamily, which is mainly expressed in enterohepatic tissue, and which is involved in bile, cholesterol and glucose homeostasis as well as in the regulation of inflammatory processes; therefore, it has become a pharmacological target for liver disorders (Yang *et al*., 2019). Based on this body of scientific evidence, it was decided to study the potential protective effect of IVM on valproic acid-induced hepatotoxicity in rats.

MATERIAL AND METHODS

Animals

Twenty adult male Wistar rats weighing 165-176 g (seven weeks old) from the laboratory animal facility at Universidad Nacional del Litoral, Santa Fe, Argentina, were used. The animals were housed under controlled environmental conditions (23 ± 2 °C temperature, 60 ± 10% humidity, 12/12 hrs. light/dark cycle). They received food (Ganave-Argentina) and water *ad libitum*. The research protocol was approved (Res. No 0017 CICUAL 23) by the Animal Research Ethics Committee of the Faculty of Medicine of Universidad Nacional del Nordeste (Corrientes, Argentina).

In order to induce hepatotoxicity, VPA (Teva-Argentina) was administered (250 mg/kg/day) for 14 days in accordance with the work of Abdelkader *et al.,* (2020). To assess the potential protective effect of IVM (IVOMEC- Boehringer Ingelheim), one group received a human therapeutic dose of 0.4 mg/kg BW (Arise, Malomo, 2009), while the other group received a dose of 1.3 mg/kg BW (Jin *et al*., 2015) in accordance with previous studies that showed that this dose improved certain metabolic parameters, which are of interest to this study, both in mice fed a high-fat diet and in diabetic mice (Jin *et al*., 2013).

Experimental design

At the start of the experiment, the 20 animals were randomly divided into five groups.

- Control group (n = 4): animals received oral saline solution for 14 days.
- VPA control (n = 4): animals were treated with VPA (250 mg/kg/day) for 14 days and sacrificed 5 days after the end of treatment (to corroborate hepatotoxicity).
- VPA + IVM (n = 4): animals received VPA (250 mg/kg/day) for 14 days and after that period, they were administered with the lower dose of IVM (0.4 mg/kg/day) for 5 days and then sacrificed.
- VPA + IVM (n = 4): animals received VPA (250 mg/kg/day) for 14 days, and after that period, they were administered with the higher dose of IVM (1.3 mg/kg/day) for 5 days and then sacrificed.
- IVM control group (n = 4): animals received the higher dose of IVM (1.3 mg/kg/day) for 5 days and then they were sacrificed.

Biochemical analysis

At the time of sacrifice (24 hrs. after the end of treatment and 8 hrs. after fasting), blood was collected from all anaesthetized animals. The samples were centrifuged at 4000 x g for 15 min to obtain plasma for the assessment of liver function parameters.

Plasma determinations

The parameters assessed in each case were alkaline phosphatase (ALP), alanine amino transferase (ALT), aspartate amino transferase (AST), total cholesterol (TC), HDL cholesterol (HDL-C), LDL cholesterol (LDL-C), and total triglycerides (TG) (Trinder, 1969; Frankel, 1970). The procedures were performed according to the manufacturer's instructions and are described below.

Alkaline phosphatase

In the presence of magnesium and zinc ions, p-nitrophenylphosphate is cleaved by phosphatases into phosphate and p-nitrophenol. The p-nitrophenol released is directly proportional to the catalytic activity of ALP (409 nm).

Alanine aminotransferase

ALT catalyzes the reaction between L-alanine and 2-oxoglutarate. The pyruvate formed is reduced by NADH in a reaction catalyzed by the enzyme lactate dehydrogenase to form L-lactate and NAD+. Thus, the rate of NADH oxidation is directly proportional to the catalytic activity of ALT (340 nm).

Aspartate aminotransferase

AST in the sample catalyzes the transfer of an amino group between L-aspartate and 2-oxoglutarate to yield oxaloacetate and L-glutamate. In the presence of malate dehydrogenase, oxaloacetate reacts with NADH to form NAD+. The rate of NADH oxidation is directly proportional to the catalytic activity of AST (340 nm).

Total cholesterol

Under the action of the enzyme cholesterol esterase, cholesterol esters are cleaved into free cholesterol and fatty acids. Cholesterol oxidase enzyme then catalyzes the oxidation of cholesterol to form cholest-4-ene-3-one and hydrogen peroxide (H_2O_2). In the presence of peroxidase, the H_2O_2 formed results in the oxidative coupling of phenol and 4-amino antipyrine to form a red quinoneimine dye. The color intensity of the dye formed is directly proportional to the cholesterol concentration (512 nm).

HDL cholesterol

The HDL cholesterol test is based on the adsorption of synthetic polyanions on the surface of lipoproteins. The combined action of polyanions and detergent solubilizes HDL cholesterol, excluding LDL, VLDL and chylomicrons. The consecutive action of cholesterol esterase and cholesterol oxidase enzymes catalyzes the oxidation of cholesterol in solution. In the presence of P peroxidase, the H_2O_2 formed reacts with N,N-bis(4-sulfobutyl)-m-toluidine and 4-aminoantipyrine to produce a quinoneimine red dye; the color intensity of which is directly proportional to the HDL cholesterol concentration (552 nm).

LDL cholesterol

HDL, VLDL and chylomicrons are hydrolyzed with the detergent. Cholesterol released from these lipoproteins is immediately reacted by the action of the enzymes cholesteryl esterase and cholesterol oxidase to generate hydrogen peroxide. This is consumed by peroxidase in the presence of 4-aminoantipyrine, and a colorless product is generated. The LDL cholesterol reaction is initiated by the addition of the second detergent, together with N, N bis(4-sulfobutyl)-m-toluidine as a conjugating agent. This releases cholesterol from the LDL particles, which undergo an enzymatic reaction with the conjugating agent to produce a chromatic compound (quinoneimine red), which is directly proportional to the LDL cholesterol concentration (552 nm).

Triglycerides

Triglycerides are hydrolyzed by the enzyme lipoprotein lipase to fatty acids and glycerol. The resulting glycerol is phosphorylated to glycerol-3-phosphate by ATP in a reaction catalyzed by the enzyme glycerol kinase. Oxidation of glycerol-3-phosphate is

catalyzed by the enzyme glycerol phosphate oxidase to form dihydroxyacetone phosphate and hydrogen peroxide. In the presence of peroxidase enzyme activity, hydrogen peroxide carries out the oxidative coupling of 4-chlorophenol and 4-aminophenazone, and forms a quinoneimine red dye that is measured at 512 nm.

Histological studies

Organs such as the liver and the kidneys were then removed, cleaned of adherent tissue, washed with ice-cold saline and weighed. Liver and kidney from each group were fixed by immersion in 10% buffered formalin for 24 hours. After this period, the samples were dehydrated in isopropyl alcohol of increasing strength, then rinsed with xylol and finally embedded in paraffin, and blocks were obtained and cut with a microtome. These histological sections were deparaffinized and stained with haematoxylin and eosin (H&E) to evaluate histopathological alterations. The stained sections were examined under a light microscope (Culling, 1965).

Statistical analysis

The data were expressed as mean ± standard deviation, from three separate experiments performed in triplicate, and the significance of the data between groups was tested by Infostat software (Argentina version 2020). Statistical analyses were carried out using Tukey's multiple comparisons test (after ANOVA). In all cases, the probability level of 95% was considered significant ($p < 0.05$).

RESULTS

Rats treated with either VPA or IVM showed no mortality during the trial. The animals appeared generally healthy, with no signs of disease or lethargy. In addition, no abnormalities were observed in macroscopic assessments of hair, eyes, locomotor activity and behavioral pattern. Food and water intake was normal in all experimental groups.

EFFECT OF TREATMENTS ON BODY WEIGHT

As shown in Table I, a significant increase ($p < 0.05$) in body weight was observed in the VPA group compared to the control group (201.53 ± 11.85; 245.28 ± 12.29). IVM treatment did not change ($p > 0.05$) body weight in healthy animals (209.26 ± 9.31), but at low doses it attenuated the effect of VPA on body weight to values close to those of the IVM-treated control group (223.15 ± 10.79; 226.42 ± 9.31). The changes in relative organ weights are shown in Table I.

TABLE I - Evolution of body weight and relative organ weight (g) during the study period in the different experimental groups

Groups	Body weight (g)		Relative weight of organs (g)		
	Initial	Final	Heart	Liver	Kidney
Control	168.47 ± 10.36	201.53 ± 11.85	0.37 ± 0.04	4.08 ± 0.12	0.96 ± 0.09
VPA	170.85 ± 7.91	245.28 ± 12.29[a]	0.28 ± 0.03[a]	3.13 ± 0.10[a]	0.87 ± 0.11
IVM	174.63 ± 11.32	209.26 ± 9.31[b]	0.38 ± 0.06[b]	3.34 ± 0.08[a]	0.90 ± 0.08
VPA + IVM LD	167.46 ± 9.19	223.15 ± 10.79[b]	0.39 ± 0.04[b]	3.47 ± 0.09[b]	0.88 ± 0.11
VPA + IVM HD	170.72 ± 8.75	226.42 ± 9.31[b]	0.38 ± 0.07[b]	3.56 ± 0.14[b]	0.94 ± 0.05

Data are given as mean ± SD (n=4). VPA: Valproic acid (250 mg/kg). IVM: Ivermectin. IVM LD: group administered with the lower dose of IVM (0.4 mg/kg/day). IVM HD: group administered with the higher dose of IVM (1.3 mg/kg/day). [a] indicates significantly different as compared with control animals ($p<0.05$). [b] indicates significantly different as compared with VPA -treated group ($p<0.05$).

After treatment for 14 consecutive days, the relative kidney weights between the control and VPA groups were not significantly different ($p > 0.05$). This is probably due to the fact that the dose used in this work does not alter the renal structure. However, a significant decrease ($p < 0.05$) in relative liver and heart weights was observed in the treated animals compared with the control group (4.08 ± 0.12, 0.37 ± 0.04; 3.13 ± 0.10, 0.28 ± 0.03). Treatment with IVM did not reverse the effect of VPA on this parameter ($p > 0.05$) (3.47 ± 0.09, and 3.56 ± 0.14, for low dose (LD) and high dose (HD) respectively).

Effect of treatments on plasma markers of liver damage

The results in Table II show that VPA administration resulted in a significant elevation ($p < 0.05$) of serum levels of TC (72.22 ± 6.46, 90.20 ± 10.21), LDL-C (23.57 ± 3.57, 30.10 ± 2.72) and TG (160.67 ± 16.61, 202.88 ± 29.78), as well as a reduction in HDL-C levels (32.90 ± 2.33, 17.45 ± 2.01) compared to the control group.

TABLE II - Effects of valproic acid and/or ivermectin treatments on the concentrations of some blood biochemical parameters in rats

Assay	Control	IVM	VPA	VPA+IVM LD	VPA+IVM HD
TC (mg/dL)	72.22 ± 6.46	100.67 ± 18.02[a]	90.20 ± 10.21[a]	72.10 ± 8.09[bc]	74.37 ± 6.34[bc]
HDL-C (mg/dL)	32.90 ± 2.33	25.33 ± 2.82[a]	17.45 ± 2.01[a]	27.20 ± 2.52[b]	23.51 ± 3.91[b]
LDL-C (mg/dL)	23.57 ± 3.57	51.33 ± 2.13[a]	30.10 ± 2.72[a]	28.17 ± 3.25[c]	31.18 ± 2.88[c]
TG (mg/dL)	160.67 ± 16.61	121.20 ± 19.76[a]	202.88 ± 29.78[a]	153.57 ± 22.31[b]	97.76 ± 10.64[b]
AST (U/mL)	78.73 ± 22.87	67.67 ± 20.64	183.38 ± 47.43[a]	103.02 ± 31.97[b]	90.75 ± 33.03[b]
ALT (U/mL)	51.37 ± 14.92	70.33 ± 16.37[a]	78.67 ± 15.01[a]	53.25 ± 10.29[b]	57.50 ± 14.41[b]
ALP (U/mL)	227.56 ± 95.34	294.27 ± 105.39	466.23 ± 201.24[a]	302.22 ± 143.24	217.85± 101.14[b]

Data are given as mean ± SD (n=4). [a] indicates significantly different as compared with control animals ($p<0.05$). [b] indicates significantly different as compared with VPA -treated group ($p<0.05$). [c] indicates significantly different as compared with VPA-treated group ($p<0.05$). TC = Triglycerides. TC = Total cholesterol. HDL-C = HDL cholesterol. LDL-C = LDL cholesterol. AST = aspartate aminotransaminase. ALT = alanine aminotransaminase. ALP = alkaline phosphatase. VPA: valproic acid (250 mg/kg/day). IVM: Ivermectin (1.3 mg/kg/day). IVM LD: group treated with the lower dose of IVM (0.4 mg/kg/day). IVM HD: group treated with the higher dose of IVM (1.3 mg/kg/day).

IVM treatment also resulted in an elevation of TC (72.22 ± 6.46, 100.67 ± 18.02) and LDL-C levels (23.57 ±3.57, 51.33 ± 2.13) as well as a reduction in HDL-C (32.90 ± 2.33, 25.33 ± 2.82), but the results show a decrease in TG levels (160.67 ± 16.61, 121.20 ± 19.76) ($p < 0.05$) compared to the control group. Furthermore, IVM treatment significantly reduced TC (72.10 ± 8.09, 74.37 ± 6.34) and TG (153.57 ± 22.31, 97.76 ± 10.64) levels in the VPA pre-treated animals, although there was a slight increase in HDL-C levels (27.20 ± 2.52, 23.51 ± 3.91) compared to the VPA group. AST, ALT and ALP activity was significantly higher ($p < 0.05$) in VPA-treated animals in relation to the control group (78.73 ± 22.87, 183.38 ± 47.43; 51.37 ± 14.92, 78.67 ± 15.01; 227.56 ± 95.34, 466.23 ± 201.24) respectively. On the one hand, the results show a significant increase ($p < 0.05$) in ALT activity in the IVM-administered group (51.37 ± 14.92, 70.33 ± 16.37) compared to the control group. On the other hand, IVM treatment significantly reduced ($p < 0.05$) AST, ALT and ALP activity in the VPA pre-treated animals compared to the VPA group (183.38 ± 47.43, 103.02 ± 31.97, 90.75 ± 33.03) (78.67 ± 15.01, 53.25 ± 10.29, 57.50 ± 14.41) (466.23 ± 201.24, 302.22 ± 143.24, 217.85 ± 101.14) respectively.

Effect of ivermectin on liver histopathology

Figure 1A shows the liver of a VPA-treated rat, which is light in color, whereas Figure 1B shows the macroscopic appearance of the liver of a control rat, which has a dark red tone. Figure 1C shows the liver of an animal treated with IVM, which looks just like the liver of the control group. Figures D and E show the organ of the VPA + IVM-treated animals (at lower and higher doses respectively), which is similar in color to the liver of the control group.

The results of the histopathological examination of the organs of rats in the control and treated groups are shown in Figure 1. Moderate hepatic fatty metamorphosis (steatosis), with small cytoplasmic vacuoles in the hepatocytes and some signet ring cells, was observed in the liver of VPA-administered rats (Figure 1a). In contrast, histopathological results showed a liver with preserved architecture, without hepatocellular damage, in both the untreated control group and the IVM-administered group (Figure 1b and 1c). Hepatocytes were found to be neatly arranged to form a hepatic cord around the central vein. Therefore, IVM-administered animals showed a conserved histological structure, similar to the control group.

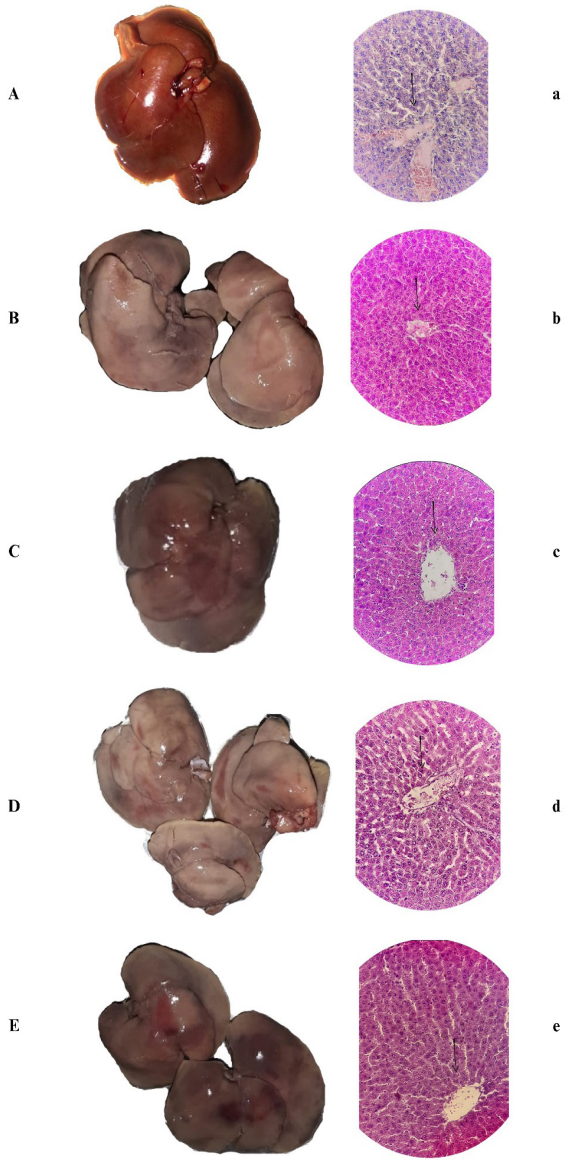

FIGURE 1 - Effects of treatments on the liver tissue. (**A-E**) The liver morphology of rats. (A) shows the macroscopic appearance of the liver of the group treated with VPA, which has a light color. (B) and (C) show the liver of the control group and the group treated with IVM, which has a dark tone. (D) and (C) show the liver of the VPA plus IVM group (low and high dose respectively), which has a similar tone to the control group.

(a-e) Photomicrographs of liver sections from: (a) VPA group showing the distorted hepatic lobules associated with edema and inflammatory cells infiltration; (b) control and (c) IVM groups, showing intact hepatic lobular architecture (black arrow); (d) VPA plus IVM low dose group (0.4 mg/kg/day), showing a slight accumulation of adipocytes between the hepatic cords; and from (e) VPA plus IVM high dose group (1.3 mg/kg/day), showing restoration of the histological structure of the liver tissue. (H & E × 40). VPA: Valproic acid. IVM: Ivermectin.

The results of the histopathological examination of the groups receiving VPA plus IVM are also shown in Figure 1 (d and e). Under light microscope, there were no obvious abnormalities in the liver tissue structure in relation to the untreated control group. Likewise, the effects of IVM on several metabolic parameters correlated with liver histology: while the lower dose of IVM reduced lipid accumulation (Figure 1d), the higher dose of IVM drastically reduced lipid accumulation, where large lipid droplets almost completely disappeared (Figure 1e).

However, no histological changes in heart structure were observed in any of the animals (data not shown). This suggests that the reduction in relative heart weight in VPA-administered animals may be an adverse reaction associated with altered glucose and lipid metabolism or, possibly, rapid body weight gain. Similarly, a preserved architecture was observed in the kidneys (data not shown). In all treated groups, the vascular system in the renal cortex was preserved. The proximal convoluted tubule was found to be brush-bordered cuboidal epithelium; and the cuboidal epithelial cells of the distal convoluted tubule were arranged in an apparently orderly fashion, and this finding is similar across the study groups. The results obtained in this study suggest that repeated administration of IVM does not compromise the integrity of the kidney and liver and, therefore, does not adversely affect their normal functions. Nor does it aggravate the health status of animals previously treated with VPA.

DISCUSSION

VPA, a potent antiepileptic drug, is prescribed for seizure control, and as a mood stabilizer in bipolar disorders. It is now used worldwide because its beneficial effects are considered to outweigh the risk of liver injury associated with VPA therapy (Guo *et al*., 2019). However, it is recognized that the administration of VPA rapidly induces hepatotoxicity in rodents accompanied by body weight gain (Abdelkader *et al*., 2020). Similarly to previous studies, a significant increase in body weight and a reduction in relative liver and heart weights were observed in VPA-treated animals (Table I). Histological analysis showed that these animals developed alterations

in hepatic lobular architecture, associated with hepatocyte degeneration and inflammatory cell infiltration (Figure 1a). In terms of plasma markers of liver damage, the VPA hepatotoxicity model is characterized by increased enzyme activity of transaminases and ALP. Similarly, in this study, animals administered with VPA showed increased AST, ALT and ALP activities (Table II). These findings paralleled the results of previous studies by Abdelkader *et al.* (2020).

The effects of VPA administration on lipid metabolism have been known for some years. Studies in patients receiving VPA therapy and in animal models have shown elevated plasma levels of TC, LDL-C and TG (Nanau, Neuman, 2013). Similarly, in this study, animals treated with VPA showed an increase in these parameters compared to the control group (Table II). It has been reported that most cardiovascular adverse events associated with VPA are due to the modulatory effects of the drug on glucose and lipid metabolism (Nanau, Neuman, 2013). The results of this study are consistent with these findings, as a reduction in relative heart weight was observed without histopathological changes, suggesting a long-term indirect effect of the drug on cardiovascular health, which could be manifested through symptoms or signs that were not studied here. Furthermore, some studies have reported that VPA administration induces nephrotoxicity in rats (Maneemin *et al.,* 2019). In the present study, the administration of VPA did not modify the renal tissue or alter the relative weight of this organ, compared to the control group (Table I). This is probably due to the fact that the dose administered was not sufficient to alter the renal structure in the treated animals.

In contrast, IVM administration to healthy animals did not change their body weight compared to the control group, but decreased their relative liver weight, probably due to the loss of white adipose tissue, without altering the morphology of the organ (Table I). These results are similar to those observed by Dong *et al.* (2020), who demonstrated that 14-day administration of IVM to Wistar rats modifies the hepatosomatic ratio of the animals, although it does not affect their weight. Macroscopic analysis showed that the livers of the IVM-treated animals had more red tissue similar to the control group, while the livers of the VPA group were paler and whitish in color, like fat (Figures 1 A, B and C). In turn, histopathological analysis showed that there were no obvious pathological changes in the liver tissue structure (Figure 1 c). In relation to the behavior of plasma markers of liver damage, it was observed that IVM administration to healthy rats did not change the enzymatic activity of AST and ALP, but increased plasma ALT levels (Table II). Previous studies in rats have reported elevated ALT and AST levels at both high and therapeutic doses of IVM (Arise, Malomo, 2009). No explanation for these findings has been found, but as IVM is metabolized through the liver, an elevation of transaminases could be an expected result. In fact, other studies have reported similar findings indicating that repeated administration of IVM for 5 days does not compromise the integrity of liver or kidney tissue, thus not adversely affecting their normal functions (Dong *et al.*, 2020).

Unexpectedly, treatment with this drug significantly increased plasma levels of both TC and LDL-C and decreased HDL-C and TG levels compared to the untreated control group (Table II). These findings are consistent with those observed by other authors, who have shown that IVM administration at doses similar to those used in this study can induce an increase in TC and a reduction in TG levels in rats (Arise, Malomo, 2009; Jin *et al*., 2015). In the absence of further evidence, it is likely that the increase in TC and LDL-C at baseline may be the result of adaptive, treatment-associated metabolic changes that are of little relevance to possible drug toxicity (Haschek, Rousseaux, 2010).

On the other hand, the animals treated with VPA plus IVM significantly decreased their body weight compared to the animals in the VPA group, although neither of the two drugs had any effect on food intake. In addition, a significant increase in relative liver weight was observed in the animals given VPA plus IVM compared to the group given VPA alone (Table I). Furthermore, treatment with VPA plus IVM attenuated the increase in ALT, AST and ALP levels compared to the animals in the VPA group (Table II). These results, correlated with the histopathological findings of the liver biopsy study that showed preserved architecture, demonstrate that IVM administration counteracts VPA-induced tissue damage,

with minimal histopathological changes and restoration of normal liver architecture (Figure 1d and e). This could be due to the lipotropic effect of IVM, as found in other studies in *in vitro* and *in vivo* conditions, but other factors such as the anti-inflammatory effect of the drug could also be considered (Jin *et al*., 2015; Yang *et al*., 2019). Likewise, in animals treated with VPA plus IVM, plasma TC and TG concentrations decreased significantly compared to the VPA-only or IVM-treated groups (Table II). While the administration of both IVM and VPA reduced HDL-C levels, it increased LDL-C levels with respect to the controls. In turn, the treatment with VPA plus IVM induced an increase in plasma concentrations of HDL-C compared to animals treated with VPA. However, it attenuated the increase in LDL-C levels with respect to IVM-only treated rats. This apparent negative effect on plasma lipids may be due to the fact that this study did not assess fecal elimination of bile salts, in which case the results may have been different and indicated a net negative cholesterol balance. Based on these results, it is possible to suggest that the action of IVM on TC and TG levels is dependent not only on the dose used but also on the health status of the animal.

In mammals, a major target of IVM has been reported to be the FXR, a nuclear hormone receptor involved in bile, cholesterol and glucose homeostasis as well as in the regulation of inflammation (Jin *et al.,* 2015). Bile acids are known to be endogenous ligands of FXR, and their activation inhibits the transcription of the limiting enzyme in the biosynthesis of these compounds, cholesterol-7α-hydroxylase. Considering its repressor effect on bile salt synthesis, pharmacological antagonism of FXR should stimulate the conversion of cholesterol into bile salts, increase their secretion into bile and their elimination from the body via the fecal route. Thus, a possible mechanism for this protective effect could be pharmacological antagonism of FXR, which could generate a negative cholesterol balance (Yang *et al*., 2019).

In this regard, IVM has been reported to be an antagonist of this receptor and, concordantly, its administration in diabetic mice reduces serum glucose and cholesterol levels and improves insulin sensitivity (Jin *et al.,* 2013). However, it is not clear whether the effects observed in this study can be explained by the same mechanisms. As the effect of IVM on body weight and TG level was very pronounced, it seems likely that other mechanisms may explain these findings. Recent studies suggest that IVM administration modifies several signaling pathways involved in the synthesis, uptake and oxidation of fatty acids, and in the expression of lipogenesis-related genes. In addition, IVM was reported to inhibit 3T3-L1 preadipocyte adipogenesis and triglyceride accumulation *in vitro* (Qi *et al*., 2019). Therefore, alterations in these signaling pathways, as well as other factors such as the regulation of inflammation, should be studied as possible mechanisms for the effects found.

CONCLUSION

The administration of IVM to animals treated with VPA reduces cholesterol and triglyceride levels. It also attenuates VPA-induced hepatotoxicity while preserving hepatic architecture and functionality. However, this study has several limitations such as the size of the population studied and the lack of observations with more complex equipment, such as electron microscope; consequently, it was not possible to observe and record more specific changes at the cellular level. In view of the results obtained, it would be advisable to carry out complementary studies in order to further investigate the mechanisms involved in the effect of this drug on, for example, the different inflammatory pathways involved in VPA-induced hepatotoxicity.

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