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Antioxidant and hepatoprotective effect of *Jatropha dioica* against the valproic acid-induced damage in an in vivo model

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Abstract

Background Understanding liver diseases is important worldwide due to their prevalence. Apart from liver disease arising from hepatitis C viral infection, most chronic liver diseases currently have no cure. Several therapeutic alternatives, including some natural products, have been proposed to treat liver diseases. The natural product *Jatropha dioica* has been reported to possess antioxidant activity and, by extension, could have hepatoprotective activity. Accordingly, our aim was to test the hypothesis that an extract of *J. dioica* is protective against liver damage induced by valproic acid (VPA).

Methods Twelve male and twelve female Wistar rats were sorted into four groups: control, non-toxicity, valproic acid control (VPA-C), and *J. dioica* + VPA (JdVPA). *J. dioica* (300 mg/kg, given orally) was used as treatment, followed by a concomitant injection of VPA (500 mg/kg, i.p.) for the first 4 days to induce liver injury. To evaluate the severity of the injury, liver function tests were performed. In addition, oxidative stress biomarkers were quantified, as well as measures of the expression of the genes *Actb, Il6,* and *Nfkb1*.

Results The VPA-C group showed a significant increase in alanine aminotransferase (ALT), aspartate aminotransferase (AST), and malondialdehyde (MDA), a decrease in superoxide dismutase (SOD), and a reduction in glutathione (GSH) vs the control group. The JdVPA group showed a significant decrease in ALT, AST, and MDA and an increase in GSH and SOD vs the VPA-C group. Gene expression of *Il6* and *Nfkb1* did not show any statistically significant differences between study groups. Histologically, VPA presented an inflammatory infiltrate, which decreased in the JdVPA group.

Conclusion The extract of *J. dioica* at the administered dose did not display toxicity and was capable of ameliorating the liver injury generated by VPA in biochemical and oxidative stress biomarkers, which suggests its potential hepatoprotective activity.

Keywords *Jatropha dioica*, Valproic acid, Chemical- and drug-induced liver injury, Plant extract

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Background

Drug-induced liver injury (DILI) is of great global importance due to its high prevalence and the increasing incidence of drug-induced hepatic toxicity (DILT) [1]. DILI presents unique challenges due to the idiosyncratic mechanisms involved [2]. Although liver damage caused by DILI may vary depending on the specific mechanisms involved, there are common damage mechanisms shared with other forms of liver injury [3, 4]. To study these mechanisms and develop therapeutic strategies, murine models of liver damage have been established, allowing these lesions to be recreated in the laboratory [5–7].

DILT is one of the methods used to study therapeutic alternatives. Drugs such as amiodarone, acetaminophen, and valproic acid (VPA) have been used in rats and mice, as well as in vitro models [5, 6, 8, 9]. While compounds such as carbon tetrachloride have also been used, this method has proven too toxic for the tissue studied, as well as for the researchers [10].

Several therapeutic alternatives have been proposed to treat DILT in rats. Among them, extracts, and substances from plants, such as *Silybum marianum*, *Cordia africana*, *Malvaviscus arboreus*, *Vaccinium arctostaphylos*, and *Curcuma longa*, are believed to mainly possess anti-inflammatory and antioxidant properties [11–16]. By contrast, species from the *Jatropha* genus, such as *Jatropha gossypiifolia* and *Jatropha elliptica*, have shown diverse biological activities, chiefly antioxidant, anti-inflammatory, antihyperglycemic, antiherpetic, and antimicrobial capabilities [17–24]. As an example, a methanolic extract of *Jatropha curcas* seeds has displayed anti-inflammatory effect, quantified through the inhibition percentage of hemolysis and the inhibition of radicals by superoxide dismutase (SOD), catalase and lactoperoxidase in carrageenan induced damage of a murine model; the observed effect was attributed to flavonoid and phenolic compounds present in said extract [25]. In addition, J. mollisima and J. gossypiifolia have recently demonstrated hepatoprotective activity in models of liver damage induced by toxic agents [26].

Regarding *Jatropha dioica*, Silva-Belmares et al. and Solis-Cruz et al. have reported the antimicrobial and antiherpetic activity of this plant's extract, respectively [17, 23]. Other reports include the antihyperglycemic [24], chemoprotective [27] and nephroprotective [28] activities of this natural product. Extracts of *J. dioica* leaves, branches, and roots have revealed phenolic and flavonoid compounds with antioxidant activity [27, 29–34]. The nephroprotective activity of the hydroalcoholic extract of *J dioica* root has also been reported in a diabetic rat model [35]. The 2,2-diphenyl-1-picrylhydrazyl (DPPH) testing showed that aqueous, methanolic, and hydroalcoholic root extracts displayed significant antioxidant activity [27, 31]. Additionally, DPPH and 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulfonic acid) (ABTS) demonstrated a strong antioxidant capacity in hexanic and ethyl acetate extracts of *J. dioica* leaves and roots; the latter displayed greater antioxidant activity regardless of the extraction solvent used [36]. The observed activity was attributed to the presence of alkyl esters of ferulic acid, diterpene ketones, and phenols [36]. Another study reported a high content of phenolic compounds and the antioxidant activity of J. *dioica* analyzed by three methods based on radical scavenging DPPH, ABTS and by inhibition of lipid oxidation [37]. In addition, another study reported the presence of phenolic compounds and antioxidant activity measured by ABTS and DPPH associated with histopathological improvement of liver tissue in hyperglycemic rats [30]. Through the trolox total antioxidant capacity assay, Ramírez-Moreno et al. reported higher concentrations of gallic acid equivalents in a hydroalcoholic extract obtained from the root compared to the stem [19]. Moreover, rutin, catechin, epicatechin, chlorogenic, and syringic, gallic, and p-hydroxybenzoic acids have been found in an aqueous extract of *J. dioica* leaves [30]. In a paracetamol-induced toxicity model in rats, gallic acid has demonstrated hepatoprotective and antioxidant activity by decreasing tumor necrosis factor-alpha (TNF-α and malondialdehyde (MDA) levels [38]. On the other hand, the anti-inflammatory and antioxidant activity of gallic acid has also been reported in fluoxetine-induced liver damage in rats, improving serum biochemical parameters such as hepatic catalase and SOD, serum and hepatic MDA, hepatic vitamin C, TNF-α, and histologic parameters [39].

As previously mentioned, a mechanism involved in drug-induced liver damage is oxidative stress. On the other hand, various compounds have been identified in *J. dioica* extracts, which are attributed to the widely reported antioxidant activity of this plant. Therefore, the aim of the present study was to evaluate the hepatoprotective effect of *J. dioica* against the damage induced by VPA.

Materials and methods

Plant material

J. dioica var. sessiliflora (Hook) was collected from Villaldama Municipality of Nuevo León, Mexico, and was authenticated at the Institutional Herbarium of the Biological Sciences School at Universidad Autónoma de Nuevo León (UANL) by Consuelo González de la Rosa. Voucher specimens (UAN-24077) have been deposited.

Extraction and isolation

Dried and powdered roots of *J. dioica* (1 kg) were extracted with ethanol/water 8:2 (3 1 L) at room temperature to give 45 g of crude extract upon evaporation *in vacuo* and freeze-drying (48 h at 2.5 10^{-4} bars). The extract was analyzed using thin-layer chromatography and high-performance liquid chromatography methodology previously reported [40].

Animals

This study included 12 male and 12 female Wistar rats, weighing 210–290 g provided by the animal facility of the Department of Physiology at the School of Medicine, Universidad Autonoma de Nuevo León. These rats were kept under controlled environmental conditions, including temperature and standard 12 h cycles of light and dark. Water *ad libitum* and rat pellets acquired from Nutrimix de México, S.A. de C.V. (Mexico City, México) were provided. Ethical guidelines described in the Mexican Official Norm NOM-062-ZOO-1999, as well as the international guidelines of the Basel Declaration, were followed during the implementation of the reported procedures. In addition, this project received approval from the Research Ethics Committee and Research Committee UANL(CONBIOÉTICA-19-CEI-001–20160404) with the registration number: HI22-00003.

Experimental design

The rats were randomly paired with the following groups:

- Control group, $n = 6$. Rats were treated with a 1 mL of saline solution 0.9% (SS) containing 3% Tween 20 (USB Corporation, Cleveland, OH, USA), once per day for 7 days by oral gavage. Furthermore, rats were treated with 1 mL of SS after the oral dose, once per day for the first 4 days by intraperitoneal administration.
- Non-toxicity (JdTox) group, $n = 6$. Rats were treated with 300 mg/kg of *J. dioica* dissolved in the solution used in the control group once per day for 7 days by oral gavage. This 300 mg/kg dose was used in the administration to experimental animals based on the literature [41]. Furthermore, 1 mL of SS was administered once per day for the first 4 days by intraperitoneal administration after the oral dose.
- Valproic acid control (VPA-C) group, $n = 6$. Rats were treated with the same oral administration as the control group, once per day for 7 days by oral gavage. The rats were then treated with a solution of 500 mg/kg of VPA in the form of sodium valproate (Sigma-Aldrich, St. Louis, Missouri, USA) in SS once per day for the first 4 days by intraperitoneal administration.
- *J. dioica* + VPA (JdVPA) group, $n = 6$. Rats in this group were treated with 300 mg/kg of *J. dioica* dissolved in the solution used in the control group once per day for 7 days by oral gavage. Furthermore, 500 mg/kg of VPA dissolved in SS was administered once per day for the first 4 days by intraperitoneal administration.

Rats were anesthetized using xylazine (10 mg/kg, Sedaject, Vedilab, Mexico) and ketamine (100 mg/kg, Anesket, PiSA Agropecuaria, Mexico) via intraperitoneal administration.

After abdominal trichotomy and asepsis, a midline incision was made; subsequently, rats were euthanized by aortic puncture and exsanguination followed by collection and storage of blood, which was centrifuged, and the serum was separated and stored. Representative liver samples were cut and stored in formalin for eventual histological evaluation. In addition, more liver tissue was resected and stored at −80 °C.

Biochemical markers

Serum activities of liver function tests were evaluated, including alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), and alkaline phosphatase (ALP), as well as the serum concentrations of gamma-glutamyl transferase (GGT), total bilirubin (TB), direct bilirubin (DB), total protein (TP), and glucose. These measurements were performed using an ILab Aries analyzer (Instrumentation Laboratory, Italy).

Oxidative stress biomarkers

The activities of SOD and reduced glutathione (GSH) and concentrations of MDA were measured in 200 mg of mechanically homogenized liver tissue along with their respective lysis buffers (radioimmunoprecipitation assay buffer for MDA, 4-2-hydroxyethyl-1-piperazineethanesulfonic acid buffer for SOD, and potassium buffer for GSH) in an ice bath. MDA, the final product of lipid peroxidation, was assessed through a thiobarbituric acid-reactive substance assay kit (Cayman Chemical Company, USA). After homogenization, the solution was centrifuged at 1600*g* for 10 min at 4 °C, the supernatant was placed on a microplate reader, and absorbance of the product was measured at 540 nm. The SOD activity was determined by tetrazolium salt WST-1 that interacting with superoxide, causes a reduction to WST-1 formazan (chromogen) using the commercial SOD assay kit (Cayman Chemical Company). Measurements were made at a wavelength of 460 nm.

GSH activity was quantified by a reduction of 5'5-dithiobis (2-nitrobenzoic acid) to 5-thio-2-nitrobenzoic acid (TNB) and the glutathione disulfide formed is recycled

by glutathione reductase and NADPH measuring the change in absorbance at a wavelength of 412 nm using the commercial GSH assay kit (Sigma-Aldrich, St. Louis, Missouri, USA)

The measurements of the previous markers were normalized to protein concentrations in the homogenates through Bradford's method [42], and this reagent was prepared following the method employed in previous literature. MDA concentrations are expressed in mol/mg of protein, SOD activity is expressed as U/mg of protein, and GSH concentration is expressed as nmol/mg of protein.

Histological evaluation

After recollection, liver tissue samples were preserved in a 10% buffered formalin solution at a pH of 7.4. Routine paraffin processing was performed with subsequent 4⌈m microtome cuts. The tissue sections were deparaffinized and processed for their ulterior blind evaluation of the hematoxylin-eosin stained sections. Liver tissue injury was assessed by observation of vacuolization, hydropic degeneration, inflammatory cell infiltration, sinusoid congestion, central vein congestion, and portal vein congestion of varying degrees: no damage = 0, mild damage = 1, moderate damage = 2, and severe damage = 3 [8, 43].

Quantitative qRT-PCR

We used quantitative real time-polymerase chain reaction (qRT-PCR) to measure the expression of *Actb, Il6,* and *Nfkb1.* RNA extraction was performed on 50 mg of stored liver tissue using a TRIzol testing reagent (Invitrogen, Thermo Fisher Scientific, USA). To quantify RNA concentrations, a Microdrop Multiskan GO (Thermo Fisher Scientific, USA) was used. The genetic material was kept at −80 °C until qRT-PCR was performed. Subsequently, we used TaqMan Fast 1-Step Master Mix (Thermo Fisher Scientific, USA). As a housekeeping gene, ®-actin (*Actb*) was used. The following TaqMan probes were used: *Actb* (ID: RN00667869_M1, Applied Biosystems), *Nfkb1* (ID: RN01399572_M1, Applied Biosystems), and *Il6* (ID: RN01410330_M1, Applied Biosystems). Each reaction was performed per the provided instructions, using 100 ng of RNA in an individual reaction. We used the $2^{-\Delta\Delta Ct}$ method to quantify fold changes in expression for the evaluated genes.

Statistical analysis

Using GraphPad Prism 8.0.1 software, normality of the data was assessed via a Shapiro–Wilk test. To determine significant differences between groups, a parametric one-way analysis of variance with a Tukey *post hoc* test or a nonparametric Kruskal–Wallis test with a Dunn *post hoc* test was performed. Fold changes in gene expression were analyzed using $2^{-\Delta\Delta Ct}$ method. Mean ± standard deviation was used if normality tests indicated a normal distribution. The results were expressed as median with interquartile range if data displayed a non-normal distribution; $p < 0.05$ was considered statistically significant.

Results

Serum biochemical markers

To determine whether VPA administration induces liver injury, we evaluated the serum levels of several biochemical markers related to liver function. Administration of VPA led to a statistically significant increase in the serum levels of ALT (55.00 [45.20–64.00] vs 130.00 U/L [100.00–140.00], $p = 0.0336$) and AST (133.80 ± 34.30 vs 243.30 ± 32.00 U/L, $p < 0.0001$) compared with the control group. The remaining biochemical markers such as serum LDH, ALP, glucose, DB, TB, and TP displayed no significant statistical differences between any of the groups (Fig. 1).

The serum levels of ALT (55.00 [45.20–64.00] vs 61.00 [44.00–66.00] U/L) and AST (133.80 ± 3 4.30 vs 113.00 ± 19.60 U/L) in the JdTox group did not display statistically significant differences compared with the control group. In addition, the difference in serum ALT levels was found to be statistically significant between the VPA-C and the JdVPA groups (130.00 [100.00–140.00] vs 45.50 [36.50–58.00] U/L, $p= 0.0015$); this was also true for serum levels of AST (243.30 ± 32.00 vs 104.00 ± 13.10 U/L, $p< 0.0001$). The serum levels of LDH showed a weak statistically significant difference between the JdTox and JdVPA groups (1022.00 ± 466.20 vs 431.20 ± 146.90 U/L, $p = 0.0498$). However, the remaining biochemical markers displayed no significant differences between the study groups (Fig. 1).

Oxidative stress biomarkers in liver tissue

In the VPA-C group significantly reduced the levels of GSH compared with the control group (0.25 ± 0.04 vs 1.26 ± 0.36 nmol/L, $p< 0.0001$); furthermore, the JdVPA group displayed significantly increased levels of GSH compared with the VPA-C group (0.71 ± 0.15 vs 0.25 ± 0.04 nmol/L, $p = 0.0221$). Additional statistically significant differences between groups were control vs JdVPA (1.26 ± 0.36 vs 0.71 ± 0.15 nmol/L, $p = 0.0071$) and JdTox vs VPA-C (1.01 ± 0.20 vs 0.25 ± 0.04 nmol/L, $p = 0.0007$) as shown in Fig. 2.

The VPA-C group displayed an increase in MDA in liver tissue compared with the control group (66.80 ± 14.41 vs 171.00 ± 34.99 ⌈mol/L, $p = 0.0012$); in addition, the liver tissue levels of MDA were significantly decreased in the JdVPA group compared with the VPA-C

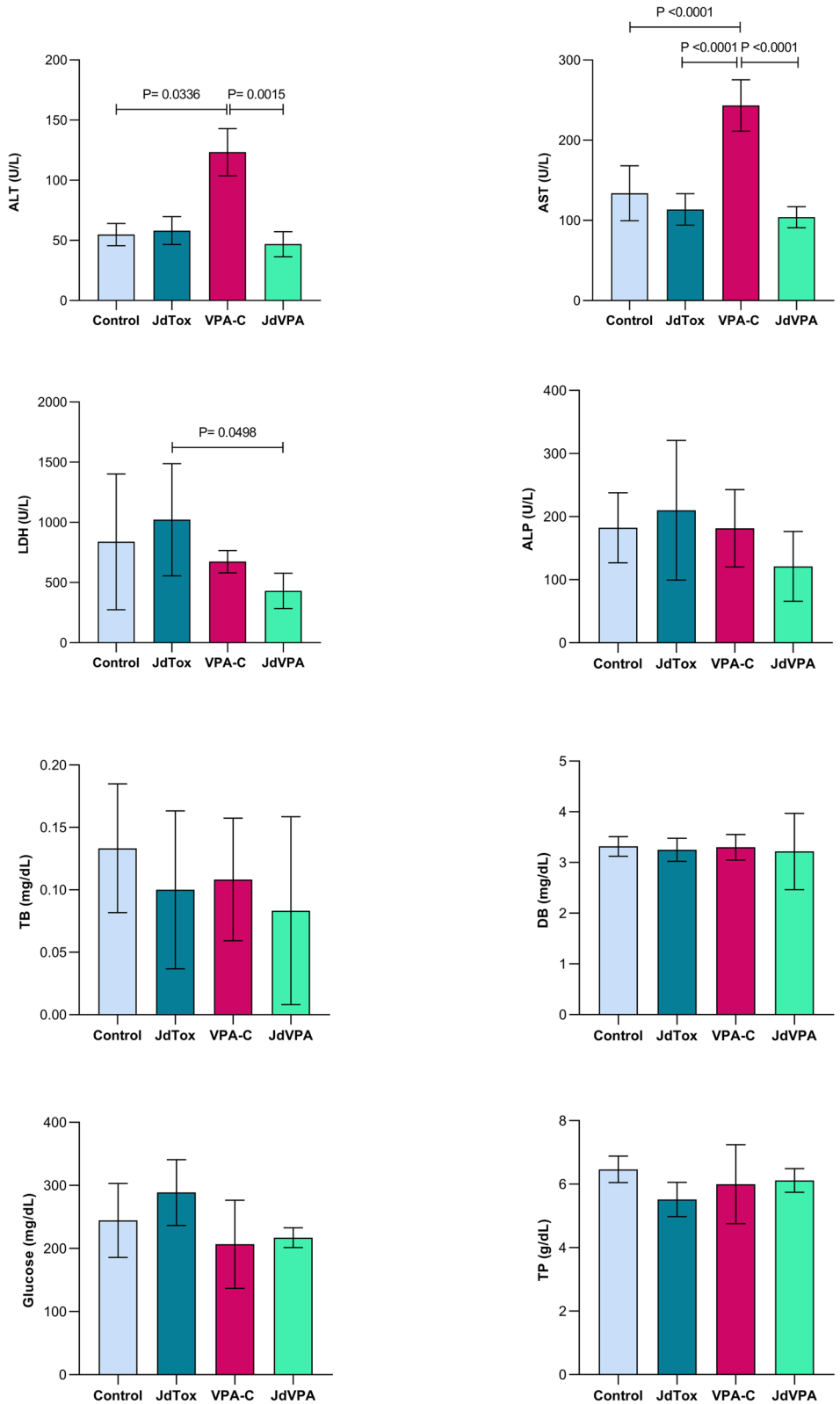

Fig. 1 Biochemical markers in the study groups. Alanine aminotransferase (ALT), aspartate aminotransferase (AST), lactate dehydrogenase (LDH), alkaline phosphatase (ALP), total bilirubin (TB), direct bilirubin (DB), glucose, and total protein (TP). Kruskal–Wallis with Dunn *post hoc* test for ALT and a one-way ANOVA with Tukey *post hoc* test for the remaining markers

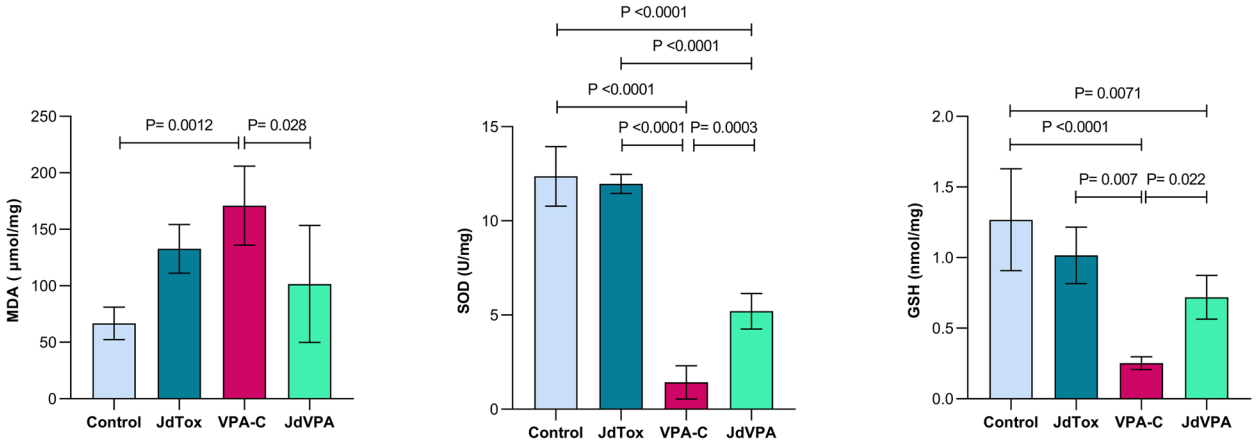

Fig. 2 Oxidative stress biomarkers in the study groups. Malondialdehyde (MDA), superoxide dismutase (SOD), and reduced glutathione (GSH). One-way ANOVA with Tukey *post hoc* test was used

Table 1 Histopathologic parameters in the study groups

Parameter	Control	JdTox	VPA-C	JdVPA
Vacuolization	0[0–0.75]	0[0-0]	0[0-0]	0[0-0]
Hydropic degeneration	0[0–0.5]	1[1,2]	1[0–1.5]	0[0-0] *
Inflammatory cell infiltration	1[1-1]	1[0–2.5]	1[0.5–2.5]	1[1–1.5]
Sinusoidal congestion	0[0-0-5]	0[0-0]	0[0–1.5]	0[0-0]
Hepatic vein congestion	1[0–1]	1[0–1]	1[0–1]	1[0–1]
Portal congestion	0[0–1]	0[0–1]	1[0.5–1]	1[0–1]

* p= 0.0401 vs JdTox. Kruskall-Wallis test with Dunn *post hoc* was performed. Data are expressed as median with interquartile range

group (171.00 ± 34.99 vs 101.60 ± 51.77 ⌈mol/L, p = 0.0238) (Fig. 2).

SOD levels evaluated in liver tissue were significantly decreased in the VPA-C group compared with the control group (12.36 ± 1.58 vs 1.42 ± 0.87 U/mg, p< 0.0001) and JdVPA group (5.20 ± 0.94 vs 1.42 ± 0.87 U/mg, p = 0.0003). The control vs JdVPA (12.36 ± 1.58 U/mg, p< 0.0001), JdTox vs VPA-C (11.96 ± 0.50 vs 1.42 ± 0.87 U/mg, p< 0.0001), and JdTox vs JdVPA (11.96 ± 0.50 vs 5.20 ± 0.94 U/mg, p< 0.0001) group comparisons were also statistically significant (Fig. 2).

Histopathological evaluation

The JdVPA group displayed decreased hydropic degeneration compared with the JdTox group (0.00 [0.00–0.00] vs 1 [1.00–2.00], p = 0.0401) (Table 1), while the remaining parameters showed no statistically significant differences between groups. An increase in infiltration of inflammatory cells in the VPA-C group was observed in microscopy compared with the control group, which decreased in the JdVPA group but was not statistically significant (Fig. 3).

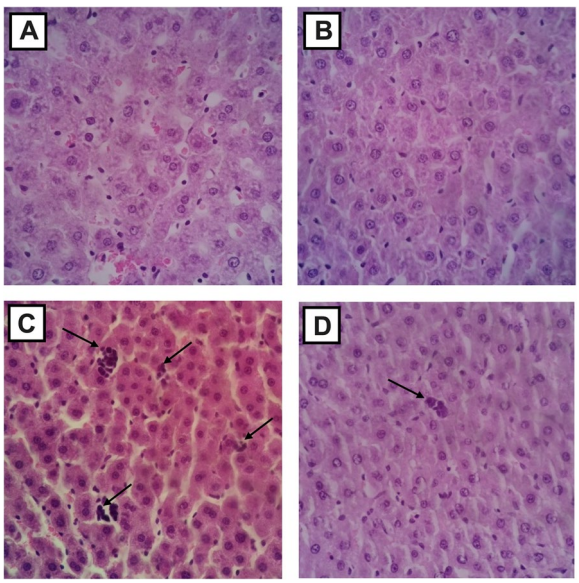

Fig. 3 Representative liver histology from each study group. **A** Control group, **B** JdTox group, **C** VPA-C group, and (**D**) JdVPA group. Inflammatory cell infiltration is not observed in control group, JdTox, and JdVPA micrographs as intensely as in VPA-C. Hematoxylin and eosin staining, all images are at 400x zoom. The arrows indicate the presence of inflammatory cells, showing an increase in the damage group (C) that is not significant in the statistical study

Gene expression

Il6 gene expression did not show any statistically significant differences between the control and VPA-C groups (0.87 [0.64–1.55] vs 1.73 [0.82–3.16]) and VPA-C vs JdVPA groups (1.73 [0.82–3.16] vs 1.18 [1.13–1.81]). *Nfkb1* behaved similarly (Table 2).

Table 2 Gene expression of inflammation markers in the study groups

Molecular marker	Control	JdTox	VPA-C	JdVPA
[b] *Il6* ($^{-\Delta\Delta CT}$ from Control)	0.87 [0.64–1.55]	2.18 [0.73–2.77]	1.73 ± [0.82–3.16]	1.18 [1.13–1.81]
[a] *Nfkb1* ($^{-\Delta\Delta CT}$ from Control)	1.00 ± 0.24	1.19 ± 0.65	0.88 ± 0.23	0.89 ± 0.30

[a] One-way ANOVA with Tukey *post hoc* test. Data are expressed as mean with standard deviation

[b] Kruskal-Wallis test with Dunn *post hoc* was performed. Data are expressed as median with interquartile range

Discussion and conclusions

The *Jatropha* genus is a member of the *Euphorbiaceae* and possesses a broad spectrum of biological activities. This is the first study to specifically examine the hepatoprotective effects of *Jatropha dioica* extract in an experimental model of liver injury induced by VPA. Previous reports of *J. dioica* at doses of up to 300 mg/kg have shown no liver toxicity. Studies performed on mice with peripheral blood micronuclei (BALB-C strain) using aqueous *J. dioica* root extract at doses of 30, 60, 100, and 300 mg/kg did not display any cytotoxic or genotoxic effects [44]. Similarly, ethanolic and aqueous extracts of leaves and roots at a dose of 450 µg/mL showed low cytotoxicity in rat 3 T3/NIH fibroblasts after 24 h exposure [45]. The absence of genotoxicity was reported when an aqueous extract of *J. dioica* at a dose of 60, 100, and 300 mg/kg was orally administered to pregnant rats during the final stage of their gestation [41]. Rats that received 300 mg/kg of *J. dioica* extract displayed no elevation of both transaminase enzymes and no differences between the control and JdTox groups in oxidative stress biomarkers. This finding has proven similar to the nontoxic nature of this extract reported in previous studies under different testing conditions.

Liberation of intracellular enzymes present in the cytoplasm such as AST, ALT, and LDH is an indicator of acute hepatocellular necrosis. Furthermore, an increase in ALP has been related to biliary dysfunction [46]. In the present study, we demonstrated that VPA induced acute liver injury by significantly increasing the levels of ALT and AST, as reported by Elyamany et al. and Calitz et al. [47, 48]. However, we observed no significant changes in serum levels of LDH and ALP, where the previous authors did. This divergence may be due to a prolonged exposure time in their studies. The remaining biochemical markers related to liver synthesis such as TB, DB, and TP presented no statistically significant changes among groups. Korglu et al. made similar observations [8].

It has previously been shown that treatment with plant extracts from the *Jatropha* genus confers a hepatoprotective effect in drug-induced liver toxicity by decreasing ALT, AST, ALP, GGT, TB, and DB levels, and reinstating ALB synthesis [21, 49]. Administration of *J. dioica* at a dose of 300 mg/kg ameliorated the injury caused by VPA administration identified through a decrease in serum levels of AST and ALT. The remaining biochemical markers showed no significant alterations among groups, which may be due to the acute and short treatment nature of our model. Changes in these parameters that have been reported previously involved prolonged exposure times to this toxic agent [50].

Oxidative stress has been proposed as one of the mechanisms of in vitro and in vivo liver toxicity induced by VPA determined by an increase in reactive oxygen species and the formation of free radicals by a plethora of mechanisms such as decreased SOD activity [51, 52], depletion of GSH reserves, which is the main intracellular nonenzymatic reducing agent [53], and an increase in the concentrations of lipid peroxidation products such as MDA [54, 55]. The metabolism of VPA is carried out through CYP2EI and may disrupt the mitochondrial electron transport chain, which is involved in the oxidative stress-inducing capacity of VPA [56]. In our model, the administration of VPA was able to induce a decrease in GSH and SOD levels, consistent with findings by Elyamany et al. and Nazmy et al., who observed a similar effect [48, 57]. By contrast, other studies have reported an increase in lipid peroxidation induced by VPA [47, 57, 58], as observed in the present study with an evident increase in MDA levels in liver tissue.

Interestingly, various studies in which agents with antioxidant capacity were used as a treatment against VPA administration revealed an increase in GSH reserves and SOD activity but a decrease in MDA concentrations [47, 57, 58], which agrees with our results obtained with *J. dioica*. This suggests that the observed effect can be attributed to the presence of compounds with potential antioxidant activity in *J. dioica*, such as diterpenes, coumarins, and phenols [18, 27, 59]. It has been reported that metabolites extracted from natural products, such as diammonium glycyrrhizinate isolated from licorice root, attenuate liver damage induced by VPA by decreasing inflammation and oxidative stress through the prevention of erythroid nuclear factor 2 (Nrf2) reduction in the cytosol and suppression of its nuclear translocation, which in turn increases the activity of antioxidant enzymes such as GSH and SOD [60]. Therefore, it is proposed that future research evaluate

whether the *J. dioica* extract modifies Nrf2 pathway, which regulates cellular responses to oxidative stress and has been associated with VPA-induced toxicity by reducing intracellular antioxidants [61]

Histopathological damage induced by VPA has been described as an increase in hydropic degeneration and liver congestion at a dose of 500 mg/kg for 4 and 14 days [8, 49], findings not observed in the present study. Hydropic degeneration presented a significant difference between the JdTox and JdVPA groups, but none of the other parameters displayed differences between groups at the same dose and exposure time. By contrast, a non-statistically significant decrease in inflammatory cell infiltration was observed in the JdVPA group compared with the VPA-C group.

It is recognized that oxidative stress can induce an inflammatory response in liver damage caused by VPA [62]. However, in the case of inflammation, several studies have reported the elevation of various markers at the plasma and liver tissue levels, such as NF-κβ and cytokines dependent on it, including IL-6, TNF-α, and IL-1. The elevation of these markers has been detected at the mRNA and protein levels, but at different time points than those evaluated in this study, at 7 and 14 days following intraperitoneal or oral administration of VPA at doses ranging from 250 to 500 mg/kg/day [43]. However, in our study, no increase in the expression of these genes was observed in any of the analyzed groups, which could be explained by the shorter VPA administration period compared to previous studies [43, 47, 63–66]. To conclude, VPA administration induced acute liver injury, as evidenced by an increase in both transaminase enzymes. Furthermore, VPA induced a state of oxidative stress in liver cells, as shown by altered SOD and GSH activities and MDA concentrations. The extract of *J. dioica* at the administered dose did not display toxicity and was capable of ameliorating the liver injury caused by VPA in biochemical and oxidative stress biomarkers, which suggests its potential hepatoprotective activity. The findings obtained in this study open the door to future research that could identify the molecules responsible the molecular mechanism and signal pathways for the hepatoprotective effects of the evaluated extract.

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Clinical trial number
Not applicable.

Authors' contributions
C-P. P and T-M. R: these authors contributed equally to the study conception, design, execution, analysis, interpretation of data, drafting, final approval of the version to be published; M-P. DP: conception, design, execution, analysis, interpretation of data, drafting, final approval of the version to be published; R-G. VM, T-G.L, R-R. DR and A-G. G: execution, analysis, interpretation of data drafting, final approval of the version to be published; M-H. OH, E-C. CB and S-C. GY: execution, analysis; M-E. LE, P-R. E and C-E. I: interpretation of data drafting, final approval of the version to be published.

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Data availability
All datasets used and analyzed during the current study are available from the corresponding author on reasonable request

Declarations

Ethics approval and consent to participate
Ethical guidelines described in the Mexican Official Norm NOM-062-ZOO-1999 were followed during the implementation of the reported procedures. In addition, the study was reviewed and approved by the Research Ethics Committee of the 'Dr. José Eleuterio González' University Hospital of the Universidad Autónoma de Nuevo León, with registration number: HI22-00003. The institutional ethics committee is registered with the National Bioethics Commission of the Ministry of Health under registration number CONBIOÉTICA-19-CEI-001-20160404.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

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