IJPSR (2021), Volume 12, Issue 12 **(Research Article)**

INTERNATIONAL JOURNAL OF PHARMACEUTICAL SCIENCES AND RESEARCH

Received on 27 January 2021; received in revised form, 29 April 2021; accepted, 29 May 2021; published 01 December 2021

HEPATOPROTECTIVE ACTIVITY OF *DORSTENIA BRASILIENSIS* AGAINST ACUTE HEPATITIS INDUCED BY ACETAMINOPHEN AND CARBON TETRACHLORIDE IN MICE

A. M. Velázquez, E. M. G. Diarte, A. K. Galeano, A. J. Burgos-Edwards, N. L. Alvarenga, O. Y. Heinichen, Y. Montalbetti, M. A. Campuzano-Bublitz, M. L. Kennedy, M. C. Hellión-Ibarrola and D. A. Ibarrola *

Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Asunción, Campus UNA, 2169, San Lorenzo, Paraguay.

Keywords:

Dorstenia brasiliensis, Acetaminophen, Carbon tetrachloride, Liver, Transaminases, Alkaline phosphatase

Correspondence to Author:
Derlis A. Ibarrola

Departamento de Farmacología, Facultad de Ciencias Químicas, Universidad Nacional de Asunción, Campus UNA, 2169, San Lorenzo, Paraguay.

E-mail: dibarrol@qui.una.py

ABSTRACT: The aim of this study was to assess the influence of extract of *D. brasiliensis* (CEDb) on acute liver injuries induced by both acetaminophen, and carbon tetrachloride in mice as an initial step to validate its popular use. A liquid-chromatography method coupled to Mass Spectrometry showed the presence of coumarins dorstenin, bergapten, and psoralene in CEDb. Swiss albino male mice were pre-treated orally with CEDb and then injected with paracetamol and carbon tetrachloride. Animals were sacrificed, and serum was separated for measuring the activity of transaminases and alkaline phosphatase. Increased serum level of aspartate transaminase (AST), alanine transaminase (ALT) and alkaline phosphatase (ALP) induced by both acetaminophen (350 mg/kg i.p.) and CCl_4 (1% in sunflower oil v/v, i.p.) administered to mice were attenuated by oral pre-treatment with CEDb (50.0, 100.0, 200.0 and 300.0 mg/kg). In the paracetamol-induced liver injury, the influence of doses of CEDb configured a dose-dependent-like effect. Moreover, in carbon tetrachloride-induced liver injury, a non-dose-dependent effect was observed. Based on these results, we concluded that *D. brasiliensis* provoked a protective effect on chemically-induced acute liver injury in mice. The active compound(s) and mechanism of protection is unknown and encourages us to pursue complementary studies.

INTRODUCTION: The liver is a vital organ that is responsible for the detoxification of various metabolites, protein synthesis, and the production of several enzymes for digestion, among other functions [1].

QUICK RESPONSE CODE

DOI: 10.13040/IJPSR.0975-8232.12(12).6384-92

This article can be accessed online on www.ijpsr.com

DOI link: http://dx.doi.org/10.13040/IJPSR.0975-8232.12(12).6384-92

While many drugs lead to liver injury in patients, the data about their potential hepatoxicity is not easily accessible and thus often underestimated.

Due to insufficient and often misleading information, it is not clear if hepatotoxicity is related to enzyme elevations in clinical trials and/or clinically apparent liver injury. Some drugs, such as chlorpromazine, halothane, or isoniazid, have clearly been demonstrated to cause liver injury [2-5]. Several herbal products, including *Astragalus membranaceus* [6], *Phyllanthus amarus* [7], and *Aeschynomene elaphroxylon* [8], have been studied

for liver health. We previously investigated the hepatoprotective effect of *Prosopis ruscifolia* [9], which showed an interesting action preventing toxicity induced by acetaminophen in mice.

Dorstenia brasiliensis (Moraceae) is a medicinal plant locally named taropé. The plant decoction is used as an antiparasitic, antiseptic, and treatment of ear and teeth pain by indigenous group Paî Tavyterâ [10]. The leaves are used as febrifuge, diuretic, emmenagogue, contraceptive, antirheumatic. Also, roots are used to treat sore throat, dyspepsia, and liver disorders [11]. The presence of furanocoumarins has been reported in this plant, including psoralen as the major constituent alongside bergaptene and sterols such as sitosterol, stigmasterol, 3-0-β-glucosylsitosterol, and sucrose [12]. The presence of dorstenic acid A and B (triterpenoids), isopimarane-type diterpenoid, and six different types of coumarins were shown after root chemical analysis [13].

This study, focalized in *D. brasiliensis*, was conducted as a part of a pharmacological validation of the natural product that is used with a putative hepatoprotective effect in the traditional medicine of Paraguay. The purpose of this study was to evaluate the influence of oral administration of *D. brasiliensis* on acute experimental hepatitis induced by both acetaminophen and carbon tetrachloride in mice.

MATERIALS AND METHODS:

Plant Material and Extract Preparation: Roots of *D. brasiliensis Lam. (Moraceae)* were collected in Itá Azul, Guairá, Paraguay. A voucher specimen was authenticated at the herbarium of Facultad de CienciasQuímicas (FCQ), Universidad Nacional de Asunción and was deposited under code R Degen 3877. Fresh samples were dried in an oven at 38°C and ground to a fine powder. 931g of this powder was extracted with ethanol using a conventional reflux method for 1 h, then filtered and evaporated under reduced pressure. The ethanolic extract of *D. brasiliensis* (CEDb) yielded 85.5 g (9.18%) and was stored at room temperature in a desiccator until use in all biological tests.

Analysis of Extracts by Liquid Chromatography Coupled to Mass Spectrometry (LC-MS): The samples were dissolved in LCMS grade acetonitrile (Merck KGaA, Darmstadt, Germany) at a concentration of 10 mg / mL and filtered with a Nylon sample filter of 0.22 µ pore diameter (Microclar, Bs. As., Argentina).

They were subsequently injected into a liquid chromatography coupled to a tandem mass spectrometer (Xevo-TQD, Waters Corporation, Milford, MA, USA) with an electrospray ionization source (ESI). The following conditions were used: column Kinetex EVO C18 (100 x 2.1 mm x 1.7 µm, Phenomenex, Torrance, CA, USA), flow 0.3 mL/min, gradient elution using a mixture of water (with 0.1% formic acid) and acetonitrile (with 0 0.1% formic acid). All reagents were LCMS grade, (Merck KGaA, Darmstadt, Germany). Gradient conditions: initial 100% A, maintaining these conditions for 1 min, then decreasing A to 0% at 7.75 min and maintaining those conditions until 10 min, then increasing A to 50% at 15 min, increasing again to 95% at 18 min and maintaining these conditions until the end (20 minutes).

The conditions of the mass spectrometer included: ionization in the positive and negative mode, a cone ramp voltage of 18.80 to 30 V, a capillary voltage of 3.20 (+) and 3.90 (-) kV, respectively, source temperature of 150 ºC, desolvation temperature of 500 ºC, and desolvation gas flow of 1000 L / h.

Reagents and Drugs: Chemicals of analytical grade were used for the pharmacological assay. Sodium chloride (WAKO Pure Chemical Industries LTD. Japan), carbon tetrachloride (Biopack), paracetamol, silymarin (Sigma Chemical Company Mo.), sodium pentobarbital (Nembutal Abbott Laboratory, Japan), Propylenglycol, and ethanol (submitted to glassware distillation before use) were acquired locally.

Animals and Ethical Considerations of *in-vivo* Testing: Swiss albino male mice weighing 25 and 35 g were obtained from the Bioterium of the Facultad de Ciencias Químicas. The animals were kept in a room with controlled light/dark cycles (12 h), temperature (23±2 °C), and relative humidity (50-60%) to minimize factors affecting behavior from the environment. The animals received commercial foods and were fasted overnight before the experiments having free access to drinking water during the trials. All experimental practices performed in the present work were directed in

agreement with the International Standards of Animal Welfare, and the protocol was previously approved by Institutional Comite de Éticaen Investigación of FCQ (CEI-188-15).

Acute Toxicity: The oral acute toxicity evaluation was performed using the fixed-dose procedure (FDP) proposed by OECD Guide 425 [14, 15, 16] with mice. Swiss albino male mice weighing 20 - 35 g were treated with of CEDb in a stepwise procedure, using the FDP (500.0; 1000.0 and 2000.0 mg/kg, *p.o.*) in different groups of 5 mice searching the LD50. The animals were observed for lethality during the first 24 hours and daily for 14 days. After this period, mice were euthanized, and the internal organs were evaluated macroscopically by comparing them with the corresponding organs of the control group.

Acetaminophen-induced Hepatotoxicity and Treatment: The animals were randomly allocated into seven different experimental groups of six mice each. Drug-induced acute hepatotoxicity was provoked by an intraperitoneal injection of 350.0 mg/kg of acetaminophen (APAP= N-acetyl-p-aminophenol or paracetamol) as described in Wu *et al.*, (2008) with small modifications [17]. Briefly, two groups received vehicle (2.5% of ethanol in distilled water) and five other groups received silymarin (100.0 mg/kg p.o.) and CEDb (50.0, 100.0, 200.0 and 300.0 mg/kg p.o.) as pre-treatments. Thirty minutes later, all groups except the vehicle group were administered intraperitoneally with 350.0 mg/kg of acetaminophen (APAP). After 4 hours, all animals were submitted to total blood collection according to individual timing. Previously, a deep pentobarbital (50.0 mg/kg, i.p.) anesthesia was induced and an intracardiac puncture was done, followed by cervical dislocation. Serum was separated and measured aspartate transaminase (AST = GOT), alanine transaminase (ALT = GPT) and alkaline phosphatase (ALP).

Carbon Tetrachloride-induced Hepatotoxicity and Treatment: The animals were assigned randomly into seven different groups of six mice each. Acute hepatotoxicity was provoked by oral administration of 1% carbon tetrachloride (v/v) dissolved in sunflower oil as described in Sun *et al.*, (2018) [18] with some modifications. Two groups received the vehicle (2.5 % of tween 80 in distilled water) and distilled water (0.1 mL/10g), respectively. The other five groups received silymarin (100.0 mg/kg p.o.) and CEDb (50.0, 100.0, 200.0, and 300.0 mg/kg p.o.) as pre-treatments once a day for five days. One hour after the last administration, the control group was treated orally with sunflower oil (0.1 mL/10g), and all other groups were administered orally with 1 % carbon tetrachloride (0.1 mL/10g). After 4 hours, all animals were submitted to total blood collection according to individual timing. Previously a deep pentobarbital (50.0 mg/kg, i.p.) anesthesia was induced, and an intracardiac puncture was done, followed by cervical dislocation. Serum was separated, and aspartate transaminase (AST = GOT), alanine transaminase (ALT = GPT), alkaline phosphatase (ALP), total protein, and albumin levels were measured.

Statistical Analysis: The results were expressed as mean ± standard deviation (S.D), and a One-way ANOVA followed by Dunnett's multiple comparisons test was performed using GraphPad Prism version 7.0 for Windows, GraphPad Software, La Jolla California USA, (www.graphpad.com). A level of probability (p) less than 0.05 was considered as statistically significant.

RESULTS:

Acute Toxicity and Effect on General Behavior in Mice: Doses of up to 3000.0 mg/kg (p.o.) of CEDb did not provoke lethality in mice during 24 h or in the 14 subsequent observation days. Consequently, the lethal dose (LD_{50}) of CEDb is considered superior to the dose mentioned above. All animals were sacrificed and the thoracic and abdominal organs were examined by direct visual inspection. None denoted changes in color, size or morphological conditions when compared to the control group. Doses higher than those mentioned above were not used because they are considered excessive and non-rationale. Furthermore, a weak effect on the general behavior of mice submitted to the influence of CEDb was observed. Oral doses of 500 mg/kg provoked a clear increase in motility, exploratory behavior, grooming behavior, and respiratory rate. Comparatively, 1000.0 and 2000.0 mg/kg p.o. of CEDb provoked a decrease in motility and respiratory rate. Also, increased piloerection, passivity, palpebral ptosis, tail erection, and grooming behavior were observed (data not shown).

Phytochemical Analysis and Identification of the Components of the Extracts by LCMS: From the extract of *D. brasiliensis* (CEDb), coumarins dorstenin, bergapten, and psoralene were identified **Fig. 1**. The chromatogram showed three peaks at 6.60, 6.86 and 7.40 min. Their molecular ions were 187.14, 217.18 and 369.39 $[M^{+}H]^{+}$ corresponding to the psoralene, bergapten and dorstenin that were previously isolated in this species[12] **Fig. 2**.

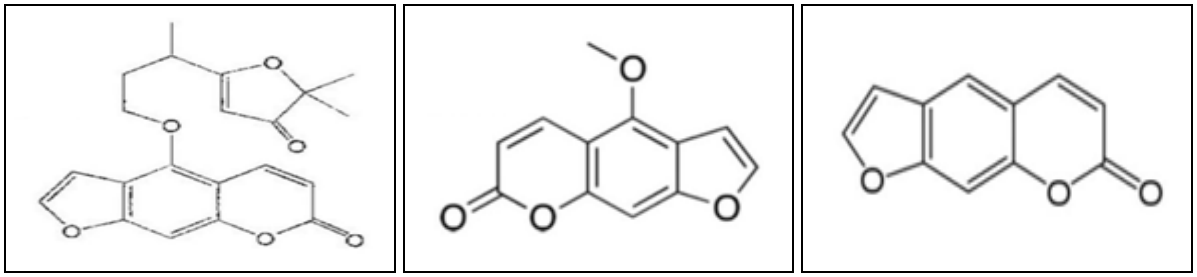

FIG. 1: STRUCTURES OF DORSTENIN, BERGAPTEN AND PSORALEN COMPOUNDS ISOLATED FROM *D. BRASILIENSIS* (CEDb)

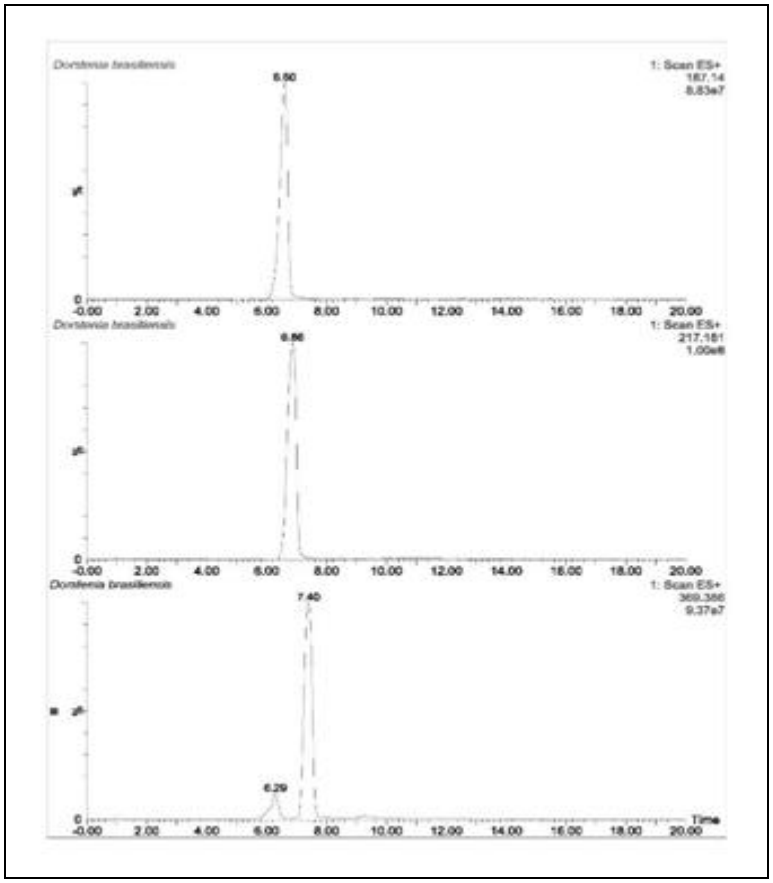

FIG. 2: LC-MS CHROMATOGRAMS SHOWING THREE PEAKS CORRESPONDING TO PSORALEN, BERGAPTEN AND DORSTENIN, RESPECTIVELY

Effect of CEDb on Acetaminophen-induced Hepatotoxicity in Mice: A clear hepatoprotective effect was denoted after examining the serum level of GPT (ALT), GOT (AST), and ALP of mice treated with different doses of *Dorstenia brasiliensis* (CEDb). CEDb administered orally to mice (200.0, 234±78 and 300.0 mg/kg, 211±55.9 U/L; **p<0.0021), significantly reduced the APAP-induced elevation of the serum level of GOT (U/L) as far as 49.7%. Similarly, 100.0 mg/kg of silymarin (203.9 ± 68 U/L; positive protecting control) induced a significant protective effect on liver damage visualized by reduced serum GOT level in comparison to positive pathological control (419.6 ± 240.9 U/L) by 51.4%. The group of animals treated with APAP (350 mg/kg) denoted an increase of GOT serum level by 456%, which is significantly different from vehicle-treated animals

(92 ± 10.2 U/L; ****p< 0.0001), thus validating the methods used **Fig. 3**. Interestingly, 200.0 and 300.0 mg/kg of CEDb provoked a similar hepatoprotective effect to silymarin. Lower doses (50.0; 402±48.5 and 100.0, 377.6.2±80 mg/kg, U/L) did not affect GOT serum level.

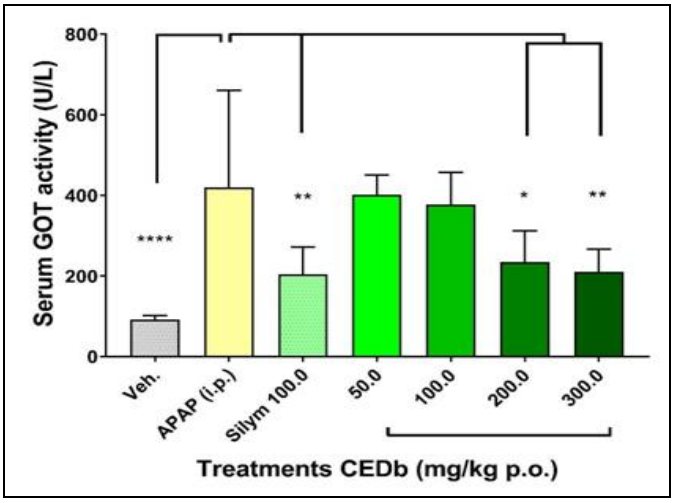

FIG. 3: INFLUENCE OF THE *D. BRASILIENSIS* (CEDb) ADMINISTERED ORALLY TO MICE, ON SERUM GOT LEVEL (U/L) AFTER ACUTE HEPATITIS INDUCED BY PARACETAMOL (APAP 350 mg/kg). Each bar represents the mean ± SD (n= 6). Statistical analysis was performed using ANOVA followed by Dunnett's Multiple Comparison Test. ****p<0.0001; ***p<0.0002; **p<0.0021; *p<0.0332 significantly different from vehicle.

Likewise, the elevation of serum level of GPT induced by APAP was significantly attenuated in mice orally pre-treated with CEDb (50.0; 70.25± 11.7, 100.0, 66.4±13.6; 200.0, 74±21.5 and 300.0 mg/kg, 52±25.31U/L) and silymarin (63.1±5.6 U/L). The most potent liver-protecting effect of 60 % was observed with 300.0 mg/kg of CEDb in comparison to APAP-induced GPT increase in mice **Fig. 4**. Similarly, 100.0 mg/kg of silymarin (63.1±5.6 U/L; positive protecting control) induced a significant protective effect on liver damage visualized by reduced serum GPT level in comparison to pathological control (129.2±31.3 U/L) by 51%. On the contrary, the group of animals treated with APAP (350 mg/kg) denoted an increase of GPT serum level by 382% significantly different from vehicle (33.8±7.3 U/L; ****p< 0.0001) treated animal, thus validating the methods used. Additionally, the same doses of CEDb significantly diminished the ALP increase induced by APAP in mice in a similarly effective way as silymarin **Fig. 5**. The elevation of serum level of ALP induced by APAP (326.8±5.4 U/L) was significantly attenuated in mice orally pre-treated with CEDb (200.0, 198.6±43.2 and 300.0 mg/kg, 170.8±37.3 U/L) and silymarin (176.6±94.4 U/L). However, lower doses (50, 307.5±93.5 and 100.0 mg/kg, 222.9±75.2) were not effective in attenuating liver injury.

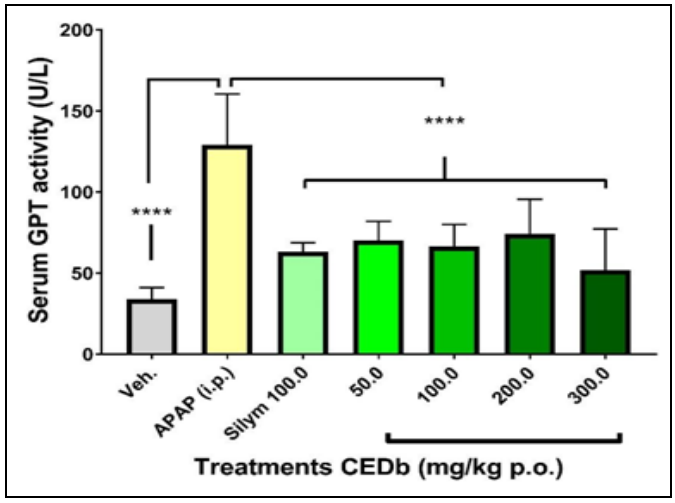

FIG. 4: INFLUENCE OF *D. BRASILIENSIS* (CEDb) ADMINISTERED ORALLY, ON SERUM GPT LEVEL (U/L) OF MICE AFTER ACUTE HEPATITIS INDUCED BY ACETAMINOPHEN (APAP 350 mg/kg). Each bar represents the mean ± SD (n= 6). Statistical analysis was performed using ANOVA followed by Dunnett's Multiple Comparison Test. ****p< 0.0001 significantly different from vehicle

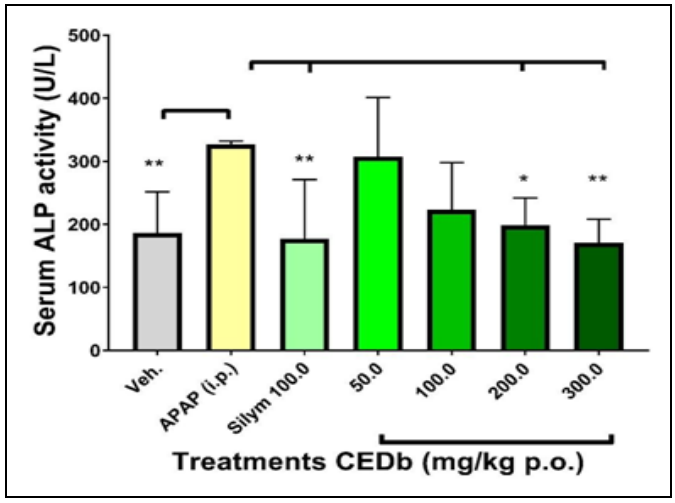

FIG. 5: INFLUENCE OF *D. BRASILIENSIS* (CEDb) ADMINISTERED ORALLY TO MICE, ON SERUM ALP LEVEL (U/L) AFTER ACUTE HEPATITIS INDUCED BY PARACETAMOL (APAP 350 mg/kg). Each bar represents the mean ± SD (n= 6). Statistical analysis was performed using ANOVA followed by Dunnett's Multiple Comparison Test. **p< 0.0021; *p< 0.0332; significantly different from vehicle

Effect of CEDb on Carbon Tetrachloride-Induced Hepatotoxicity in Mice: A strong liver protective effect was demonstrated due to increased

serum levels of GOT, GPT, and ALP induced by CCl_4 in mice that were attenuated with different oral doses of CEDb. CEDb (50.0; 210.3±113, 100.0; 298.5±157.9; 200.0, 314±164.3 and 300.0 mg/kg, 310.8±152.9 U/L; ****p<0.0001) administered orally during five days to mice significantly reduced the CCl_4-induced elevation of the serum level of GOT by 70 %. Similarly, 100.0 mg/kg of silymarin (287±81.77 U/L; positive protecting control) induced a significant protective effect on liver damage as denoted by reduced serum GOT level in comparison to pathological control (701.4±225.1 U/L) by 59 %. Additionally, the group of animals treated with CCl_4 (1%) demonstrated an increase of GOT serum level by 590%, which is significantly different from the vehicle-treated animal (118.9±33.46 U/L; ****p< 0.0001), thus validating the method used **Fig. 6**. The dose of 50.0 mg/kg of CEDb provoked a remarkably more intense (70%) protection than silymarin (59%).

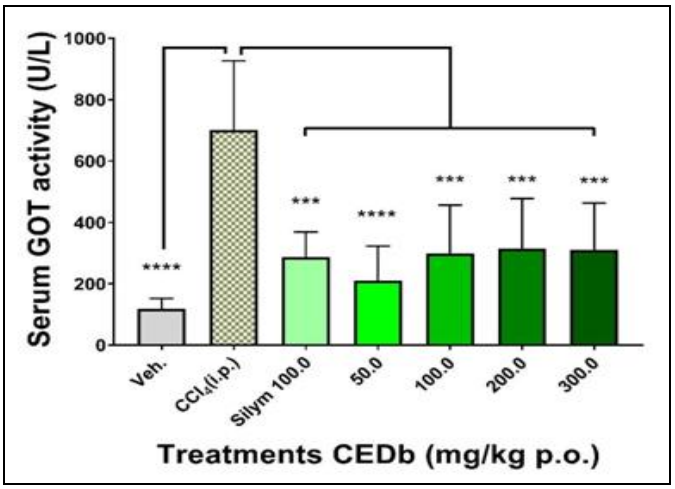

FIG. 6: INFLUENCE OF *D. BRASILIENSIS* (CEDb) ADMINISTERED ORALLY TO MICE, ON SERUM GOT LEVEL (U/L) AFTER ACUTE HEPATITIS INDUCED BY CARBON TETRACHLORIDE (CCl_4 1%). Each bar represents the mean ± SD (n= 7). Statistical analysis was performed using ANOVA followed by Dunnett's Multiple Comparison Test. ****p< 0.0001; ***p< 0.0002; significantly different from vehicle.

Likewise, unquestionably CEDb (50.0; 228.6± 122.4; 100.0; 378.6±45.13; 200.0, 453±113 and 300.0 mg/kg, 491.3±116.9 U/L) administered (5 days) orally to mice significantly reduced the CCl_4-induced elevation the of serum level of GPT (820.7±434.9 U/L) by 72%. Similarly, 100.0 mg/kg of silymarin (373.4±66.46 U/L; positive protecting control) induced a significant protective effect on liver damage as visualized by the 54.5% reduced serum GPT level in comparison to the pathological group (820.7±434.9 U/L). The group of animals treated with CCl_4 (1%) denoted an increase of GPT serum level by 1780%, which is significantly different from the vehicle-treated animal (46.14±19.38; ****p< 0.0001), thus validating the method used **Fig. 7**. The dose of 50.0 mg/kg of CEDb provoked more effective protection than silymarin (72% *vs*. 54.5%). However, five days of treatment with higher doses of CEDb (100.0; 378.6±45.13; 200.0, 453±113 and 300.0 mg/kg, 491.3±116.9 U/L) showed a lower protection capacity (40-54%) considering the GPT level increased after liver injury induced by CCl_4.

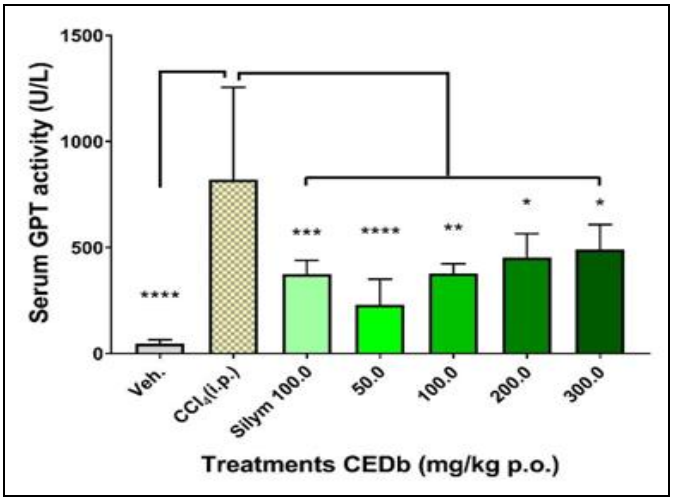

FIG. 7: INFLUENCE OF *D. BRASILIENSIS* (CEDb) ADMINISTERED ORALLY TO MICE, ON SERUM GPT LEVEL (U/L) AFTER ACUTE HEPATITIS INDUCED BY CARBON TETRACHLORIDE (CCl_4 1%). Each bar represents the mean ± SD (n= 7). Statistical analysis was performed using ANOVA followed by Dunnett's Multiple Comparison Test. ****p< 0.0001; ***p< 0.0002; **p< 0.0021; *p< 0.0332 is significantly different from vehicle

Correspondingly, five days of oral pre-treatment of mice with CEDb (50.0; 214.7±65.15, 100.0; 193.5±48.99; 200.0, 244±34.88 and 300.0 mg/kg; 223.7±60.61 U/L **p<0.0021) significantly reduced the CCl_4-induced elevation of the serum level of ALP as much as 43%. Furthermore, silymarin (219±48.73 U/L **p<0.0021; positive protecting control) induced a significant protective effect on liver damage as visualized by the reduced serum ALP level in comparison to the pathological control (338.7±38.93 U/L) by 35% **Fig. 8**. Likewise, the serum ALP level increased 180% (338.7±38.93) after liver injury induced by CCl_4 when compared with the vehicle treated group (188.3±68).

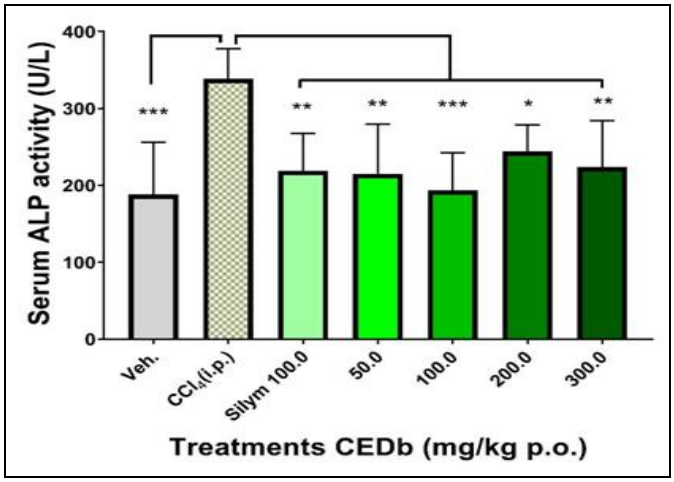

FIG. 8: INFLUENCE OF *D. BRASILIENSIS* (CEDb) ADMINISTERED ORALLY TO MICE, ON SERUM ALP LEVEL (U/L) AFTER ACUTE HEPATITIS INDUCED BY CARBON TETRACHLORIDE (CCl_4 1%). Each bar represents the mean ± SD (n= 7). Statistical analysis was performed using ANOVA followed by Dunnett's Multiple Comparison Test. ***p< 0.0002; **p< 0.0021; *p< 0.0332 significantly different from vehicle.

DISCUSSION: This report is unique in characterizing the influence of crude extract of *D. brasiliensis* (CEDb) in two different trials of chemically-induced acute hepatitis in mice. CEDb reduces serum transaminase and alkaline phosphatase levels in mice submitted to paracetamol and carbon tetrachloride-induced liver injury. Usually, measuring transaminases and alkaline phosphatase serum levels are the initial steps for evaluation of hepatic conditions after viral or chemically-induced injuries [18]. As expected, significant elevation of such enzymes induced by APAP and CCl_4 were observed in this study. Therefore, using these models, a significant reduction in the APAP and CCl_4-induced elevation of serum levels of these parameters was observed in mice treated with CEDb. These results therefore reveal a significant protective effect of CEDb in contrast to the respective APAP and CCl_4 treated groups. For the first time, our findings showed a protective effect of *D. brasiliensis* using two different liver injury models in mice. The mechanism of the attenuating effect induced by *D. brasiliensis* in mice submitted to acute chemically-induced liver injury is unknown. However, the presence of psoralene, bergapten, and dorstenin, as denoted in this study, could induce a minimizing oxidative stress in the liver in a similar mechanism as mentioned by several studies obtained from literature. According to Domitrović and Potočnjak, (2016) [19], in APAP-induced hepatitis some triterpenoids (ginsenosides) increase superoxide dismutase, glutathione peroxidase, catalase activity and restore the glutathione level. Some alkaloids (berberine) also suppress oxidative stress in CCL_4-induced liver injury. Moreover, bergapten and derivatives are associated with the maintenance of lipid homeostasis in experimental hepatocellular carcinoma [20].

Medicinal plants with a similar protective effect against chemically-induced hepatitis in rodent models are abundant and can currently be found in the literature. For example, *Artemisia asiatica* [21], *Cassia fistula* [22], *C. occidentalis* [23], *Litchi chinensis* [24], *Alhagi sparsifolia* [25,] and *Sorbus pohuashanensis* [26] among others, have demonstrated similar effects. The damage to the structural integrity of the liver drives out cytoplasmic enzymes, releasing them into circulation after injury or death and thus rising serum levels of transaminases (GPT, GOT) and ALP [21]. Therefore, treatments using phytopharmaceuticals or plants are common practices in traditional medicine with the hope of reversing liver ailments conditions in humans0 [27]. Citrus flavonoids, red wine polyphenols, and several plant extracts were studied against the massive liver injuries induced by APAP and showed a positive effect on transaminase, oxidative stress, and DNA fragmentation in rats [28]. Mice with chronic liver injury and fibrosis induced by CCl_4 were markedly ameliorated by polydatin (a glucoside of resveratrol) through inhibition of oxidative stress and inflammation. Levels of serum transaminases (GPT and GOT) and inflammatory mediators responsible for fibrosis were clearly modified by polydatin, reducing chronic oxidative stress and inflammation-induced with CCl_4 treatment [29].

Bieski *et al.*, (2012) [13] had previously shown the presence of dorstenic acid A and B (triterpenoids), isopimarane-type diterpenoid, and six different types of coumarins in roots of *D. brasiliensis*. Due to the presence of furanocoumarins in the species of *Dorstenia*, some authors have suggested its use in psoriasis and vitiligo. A few pharmacological studies have demonstrated analgesic and anti-inflammatory activities of *D. brasiliensis* in animal models [30]. Additionally, *D. brasiliensis* may possess some biologically active compounds similar to

other *Dorstenia* species from the same genus and may thus share a similar pharmacological profile. Some authors have investigated its potential use as an antivenom [31], among other activities.

Nevertheless, some differences in results are noted between the two mice models submitted to CEDb treatment. Firstly, in the paracetamol-induced liver injury, the influence of doses of CEDb configure a dose-dependent- like an effect. Moreover, in carbon tetrachloride-induced liver injury, a non-dose-dependent effect was observed. We have no knowledge about this response and speculate that it may be due to absorption difficulties (poor bioavailability of CEDb), and/or the dose and type of toxic agents (APAP and CCL_4) and/or sensitivity of animals and/or the CEDb treatment design (single and multiple dosing).

The influence of pharmacokinetic factors (such as absorption [32] or oxidative metabolic inhibition of CPY450), may affect the final protection intensity, especially in CCL_4 treated mice that receive multiple doses of CEDb [21, 24]. In addition, we have no information regarding how sensitive or resistant the albino mice utilized are to hepatotoxin (APAP and CCL_4). Therefore, based on results demonstrated by this study, it is logical and necessary to advance with complementary research to clarify chemical components, the molecular mechanism implicated in the pharmacological activities, and gather histopathological evidence of effectiveness against chemically liver injury.

CONCLUSION: Based on results, the crude extract obtained from *D. brasiliensis* demonstrated an evident protective effect on chemically-induced acute liver injury considering the transaminase and ALP serum levels in two models of liver injury. The active compound(s) and protection mechanism remain unclear and will be revealed soon in a new series of chemical and pharmacological studies.

ACKNOWLEDGEMENT AND FINANCIAL SUPPORT: This research work was performed under the financial support of Facultad de Ciencias Químicas de la Universidad Nacional de Asunción and Consejo Nacional de Ciencia y Tecnología CONACYT (Grant code 14-INV-325). We want to thank to Departamento de Botánica from Facultad de Ciencias Químicas for supporting us with identification of plant material.

CONFLICTS OF INTEREST: The authors declare that they have no conflicts of interest.

AUTHORS' CONTRIBUTIONS: All authors collaborated in the laboratory work, data analysis, animal care, drafting the work, and revising it critically. All the authors have read the final manuscript and approved the submission.

REFERENCES:

1. Pandit A, Sachdeva T and Bafna P: Drug-Induced Hepatotoxicity : A Review. J Appl Pharm Sci 2012; 02(05): 233-43.
2. Björnsson ES: Hepatotoxicity by Drugs : The Most Common Implicated Agents. Int J Mol Sci 2016; 17(224): 1-7.
3. Chalasani N and Björnsson E: Risk Factors for Idiosyncratic Drug-Induced Liver Injury. Gastroenterology 2010; 138(7): 2246-59.
4. Bjornsson ES and Hoofnagle JH: Categorization of Drugs Implicated in Causing Liver: critical assessment based on published case report. Hepatology 2016; 63(2): 590-603.
5. Hunt CM, Papay JI, Stanulovic V and Regev A: Drug Rechallenge Following Drug-Induced Liver Injury. Hepatology 2017; 66(2): 646-54.
6. Zhang Z, Wen Q and Liu C: Hepatoprotective effects of astraglus root 1990; 30: 145-49.
7. George A, Udani JK and Yusof A: Effects of *Phyllanthus amarus* PHYLLPRO TM leaves on hangover symptoms : a randomized, double-blind, placebo-controlled crossover study. Pharm Biol 2019; 57(1): 145-53.
8. Hashem MM, Salama MM, Mohammed FF, Tohamy AF and Deeb KS: Metabolic profile and hepatoprotective effect of *Aeschynomene elaphroxylon* (Guill. &Perr.). PLoS One 2019; 14(1): 1-24.
9. Soverina MS, Campuzano-bublitz MA, Centurión JR, Galeano AK and Kennedy ML: Preliminary evaluation of hepatoprotective and nephroprotective effects of *Prosopis ruscifolia* Griseb. leaves extract in mice. J Appl PharmSci 2019; 9(12): 37-41.
10. Céspedes De Zárate C, Fogel Pedroso, Ramón Bruno Soria Rey N and Valdez Ayala SC: Etnomedicina de los Pueblos MbyaGuarani y Pai Tavytera. AGR S.A 2016; 169-70.
11. González de García MG, González Villalba YP and Degen de Arrúa RL: *Dorstenia brasiliensis* Lam. (Moraceae): caracterización morfoanatómica de una especie polimórfica, empleada con fines medicinales en Paraguay. J Pharm Pharmacogn Res 2019; 7(02): 116-25.
12. Kuster RM, Bernardo RR, Da Silva AJR, Parente JP and Mors WB: Furocoumarins from the rhizomes of Dorstenia brasiliensis. Phytochemistry 1994; 36(1): 221-23.
13. Bieski IGC, Santos RF, Oliveira RM De, Espinosa MM, Macedo M, Albuquerque UP and Oliveira Martins DT: Ethnopharmacology of Medicinal Plants of the Pantanal Region (Mato Grosso, Brazil). Evidence-based Complement Altern Med 2012; 1-36.
14. OECD. Acute Oral Toxicity – Fixed Dose Procedure (chptr). OecdGuidel Test Chem 2001; (December):1–14.
15. Stallard N and Whitehead A: 2004. A statistical evaluation of the fixed dose procedure. Altern Lab Anim 32(S2): 13-21.
16. OECD. Test No. 425: Acute Oral Toxicity: Up-and-Down Procedure. Test 2008; (October):1–21.

17. Wu YL, Piao DM, Han XH and Nan JX: Protective Effects of Salidroside against Acetaminophen-Induced Toxicity in Mice. Biol Pharm Bull 2008; 31(8): 1523-29.
18. Sun J, Wen X, Liu J, Kan J, Qian C, Wu C and Jin C: Protective effect of an arabinogalactan from black soybean against carbon tetrachloride-induced acute liver injury in mice. Int J Biol Macromol 2018; 117: 659-64.
19. Domitrović R and Potočnjak I: A comprehensive overview of hepatoprotective natural compounds: mechanism of action and clinical perspectives. Arch Toxicol 2016; 90(1): 39-79.
20. Pattanayak SP, Bose P, Sunita P, Siddique MUM and Lapenna A: Bergapten inhibits liver carcinogenesis by modulating LXR/PI3K/Akt and IDOL/LDLR pathways. Biomed Pharmacother 2018; 108: 297-308.
21. Ryu BK, Ahn BO, Oh TY, Kim SH, Kim WB and Lee EB: Studies on protective effect of DA-9601, Artemisia asiatica extract, on acetaminophen- and CCl4-induced liver damage in rats. Arch Pharm Res 1998; 21(5): 508-13.
22. Bhakta T, Banerjee S, Mandal SC, Maity TK, Saha BP and Pal M: Hepatoprotective activity of Cassia fistula leaf extract. Phytomedicine 2001; 8(3): 220-24.
23. Yadav JP, Arya V, Yadav S, Panghal M, Kumar S and Dhankhar S. *Cassia occidentalis* L.: A review on its ethnobotany, phytochemical and pharmacological profile. Fitoterapia 2010; 81(4): 223-30.
24. Bhoopat L, Srichairatanakool S, Kanjanapothi D, Taesotikul T, Thananchai H and Bhoopat T: Hepatoprotective effects of lychee (*Litchi chinensis* Sonn.): A combination of antioxidant and anti-apoptotic activities. J Ethnopharmacol 2011; 136(1): 55-66.
25. Aierken A, Jiang Z, Maimaitimin K, Shayibuzhati M and Zhang X: Protective effect of *Alhagi sparsifolia* against acetaminophen-induced liver injury in mice. Trop J Pharm Res 2018; 17(4): 641-6.
26. Yin Y, Zhang Y, Li H, Zhao Y, Cai E, Zhu H, Li P and Liu J: Triterpenoids from fruits of *Sorbus pohuashanensis* inhibit acetaminophen-induced acute liver injury in mice. Biomed Pharmacother 2019; 109: 493-502.
27. He Q, Kim J and Sharma RP: Silymarin Protects Against Liver Damage in BALB / c Mice Exposed to Fumonisin B 1 Despite Increasing Accumulation of Free Sphingoid Bases. Toxicol Sci 2004; 80: 335-42.
28. Ray SD, Patel N, Shah N, Nagori A, Naqvi A and Stohs SJ: Pre-exposure to a novel nutritional mixture containing a series of phytochemicals prevents acetaminophen-induced programmed and unprogrammed cell deaths by enhancing BCL-XL expression and minimizing oxidative stress in the liver. Mol Cell Bioc 2006; 293(1-2): 119-36.
29. Zhao X, Li R, Liu Y, Zhang X, Zhang M, Zeng Z, Wu L, Gao X, Lan T and Wang Y: Polydatin protects against carbon tetrachloride-induced liver fibrosis in mice. Arch Biochem Biophys 2017; 629: 1-7.
30. de Morais Lima GR, de Albuquerque Montenegro C, de Almeida CLF, de Athayde-Filho PF, Barbosa-Filho JM and Batista LM: Database survey of anti-inflammatory plants in South America: a review. Int J Mol Sci 2011; 12(4): 2692-2749.
31. Ruppelt BM, Pereira EFR, Goncalves LC and Pereira N: Pharmacological screening of plants recommended by folk medicine as antisnake venom I Analgesic and antiinflammatory activities. Mem do Inst Oswaldo Cruz 1991; 86(2): 203-05.
32. Theodosiou E, Stamatis H and Kolisis F: Bioavailability of silymarin flavonolignans : drug formulations and biotransformation. Phytochem Rev 2014; 13: 1-18.

How to cite this article:
Velázquez AM, Diarte EMG, Galeano AK, Burgos-Edwards AJ, Alvarenga NL, Heinichen OY, Montalbetti Y, Campuzano-Bublitz MA, Kennedy ML, Hellión-Ibarrola MC and Ibarrola DA: Hepatoprotective activity of *Dorstenia brasiliensis* against acute hepatitis induced by acetaminophen and carbon tetrachloride in mice. Int J Pharm Sci & Res 2021; 12(12): 6384-92. doi: 10.13040/IJPSR.0975-8232.12(12).6384-92.