

Full Length Research Paper

Toxicological potential of ethanol extract of stem bark and *Croton heliotropiifolius* (Euphorbiaceae)

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***Croton heliotropiifolius* is an endemic specie in northeast of Brazil. It is renowned for its medicinal properties, larvicidal, insecticidal and cytotoxic activity. This study evaluated the phytochemical screening of ethanolic extract stem bark of *C. heliotropiifolius* (EECroton) and the toxicological potential in Swiss albino mice. Phytochemical analysis were performed by thin-layer chromatography. The EECroton at 2000 mg/kg (orally) was used for acute toxicity on Swiss albino mice evaluated during a 14-day period. Histomorphometric analysis of liver and renal tissue along with hematological and serum enzyme levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), creatinine and urea were measured. The phytochemical investigation of EECroton revealed the presence of coumarins, flavonoids triterpenes and absence of alkaloid, saponins and condensed tannins. The EECroton (2000 mg/kg: o.v.) showed moderate toxicity and the results of serum hematological and enzymatic levels were similar in the respective groups. Histopathological and histomorphometric analyzes confirmed a low of level of toxicity, showing organization of the structural units of the cells, nuclei and sinusoidal capillaries of the hepatocytes as well as renal corpuscles without presenting morphological damage to their organs. These data suggest that the presence of phenolic compound in EECroton demonstrate low order of toxicity.**

Key words: *Croton heliotropiifolius*, medicinal plants, phytochemical, acute toxicity, histomorphometric.

INTRODUCTION

The use of medicinal plants is a common habit among the population and, currently, the interest in the use and

trade of medicinal plants and herbal products has increased in Brazil. According to the World Health

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Organization, millions of people use traditional medicine as the primary and, sometimes, the only source of healthcare (WHO, 2013). However, some medicinal plants can produce compounds that are harmful to the organism.

The indiscriminate use of these plants has increased concerns regarding the presence of toxic substances and their provocation of adverse effect (Monteiro et al., 2010; Saad et al., 2006). The study of medicinal plants is important not only for the confirmation of therapeutic uses, but also for the identification of potentially toxic, carcinogenic or teratogenic components (Silva et al., 2017, 2016). *Croton heliotropiifolius* Kunth is an endemic specie in Northeast of Brazil frequently found within “caatinga”, brejo, resting and “cerrado” vegetations. It is known by unofficial names such as “velame”, “velaminho” and “velame-de-cheiro due to the presence of trichrome. Previous studies have described the presence of alkaloids, polyphenols and reducing agents in *C. heliotropiifolius*, including its medicinal use for relief of stomach pain, vomiting, diarrhea and as an antithermic (Randau et al., 2004). The essential oil has been described as larvicidal against *Aedes aegypti* (Doria et al., 2010). The methanolic extract of *C. heliotropiifolius* showed moderate toxicity against the microcrustacean *Artemia salina* and a low to medium percentage of cell growth inhibition on tumor lines tested (Silva et al., 2018, 2017). The Human Toxicity Potential (HTP) is an important test to be performed early in the study of medicinal herb. It is recommended that new herbs and those with unknown actions shall be analyzed in a controlled cellular environment as free of complex interactions inherent in an organism. The methodologies are aimed at analyzing the cellular behavior, display several advantages such as low costs, easy and quick execution and controlled cell environment (Freshney, 2000).

The acute toxicity test is the initial step to investigate the systemic toxic potential of a substance administered in a single dose (Lorke, 1983). This test can suggest the choice of dose for additional toxicity tests and provide relevant information about the risks that this substance can cause harmful effect to animal and human health (Scandelai et al., 2019). To carry out the test, the Organization for Economic Cooperation and Development (OECD) recommends that female mice or rats and about three animals be used at each stage of the experiment, where the occurrence or non-occurrence of death of the treated animals will determine the need for further steps. Due to knowledge that chemical analysis and the potential for human toxicity as well as histopathological investigation are important tests for the medical use of plants, this work aimed at evaluating the phytochemical screening of the ethanolic extract stem bark of *C. heliotropiifolius* (EECroton) and the acute toxicity assays, with emphasis on the histopathology study of the hepatic and kidney tissues in Swiss albino mice.

METHODS

Chemicals

Ethanol, distilled water, 10% Carboximetilcelulose, formalin, sodium chloride (0.9% NaCl), hematoxylin, eosin, and acetaminophen were supplied by Merck (Germany).

Plant material

Barks *C. heliotropiifolius* (Euphorbiaceas) were collected in February 2018 in the urban area of Garanhuns city, Pernambuco Brazil, State of Brazil (Latitude- 8.89074, Longitude- 36.4966 8 53” 27” Sul, 36° 29 48 Oeste). The plant was identified by Dardano de Andrade de Lima herbarium in Agronomic Research Institute of Pernambuco (IPA). It was registered in the referred herbarium under the catalog number 90440 in which an exsiccate was deposited.

Extraction and phytochemical screening

Stem bark of *C. heliotropiifolius* (1.3 kg) were washed in running water, pulverized and extracted with ethanol (EOH) at room temperature for 48 h. Thereafter, the solvent was filtered and evaporated at 35°C under reduced pressure. The ethanolic extract of stem bark of *C. heliotropiifolius* (EECroton) yielded 54.5 g. The presence of alkaloids, triterpenoids, flavonoids, coumarins, and saponins was tested by thin layer chromatography (TLC). The mobile phase used for flavonoids was ethyl acetate-formic acid-acetic acid-water (AcOEt-HCOOH-H₂O 100:11:11:27v/v), while for coumarins ether-toluene-acetic 10% (50:50:50 v/v) was used. The visualization reagents used for flavonoids and alkaloids were respectively etilborilaminoester acid (Neu) and Dragendorff. For coumarins, the visualization method used was UV light on 365 nm (Wagner and Bladt, 1996). The presence the saponins was tested by mechanical shaking of the extract and visualization of foam (Simões et al., 2004). Formation of foam for 15 min was considered as positive for the presence of saponins (Dewick, 2002). The presence of tannins was investigated by addition of iron chloride (0.5 M) to the fresh dilution of dried extract in saline solution (0.9% NaCl) which was prepared on the day of experiments.

Determination of acute toxicity

Experimental animals

Twelve healthy female Swiss albino mice (*Mus musculus*), aged 40 days, weighing on average 35 to 45 g were acquired from the Aggeu Magalhães Research Center (Pernambuco, Brazil). Animals had free access to water and feed. They were kept under standard environmental conditions of temperature, humidity 12:12 light-dark cycle. The Committee on Animal Research and Ethics of UFPE approved the experiments under opinion No. 500/12, process N° 0072/2018.

Experimental design and treatment arrangement

Swiss albino mice were randomly divided into two groups (n=3 animals/group) and orally treated during fourteen days according to their respective group: Control- treated with Carboximetilcelulose (1%), and treatment- ethanolic extract stem bark of *C. heliotropiifolius* (EECroton) (2000 mg/kg, v.o.). After administration, the animals were observed in the first hours and then every 24 h daily (OECD, 2001). Then the hippocratic screening method was

Table 1. Phytochemical prospection of the ethanol extract of the bark of *C. heliotropifolius*.

Classes of metabolites	Results
Alkaloids	-
Cumarins	+
Flavonoids	+
Saponins	-
Tannins	-
Triterpenes	+

Table 2. Evolution of water and feed consumption of mice in the control and EECroton groups.

Group	Evolution	
	Water	Feed
Control	32.75 (5.93)	18.75 (2.36)
EECroton	20.18 (5.36)	12.68 (2.93)
p-value ¹	0.003	0.003

¹p-value of Mann-Whitney test. N= 3 (each group).

used, which examines important aspect of animals such as: The state of consciousness, disposition, activity and coordination of the motor system and muscle tone, activity of the central and autonomic nervous system. The consumption of water, feed and weight of the animals was also observed. Later on the fourteenth day, blood was gained through cardiac puncture, and serum was separated for subsequent determination of hematological and serum enzyme levels of serum glutamate oxaloacetate transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) creatinine and urea. Following this, they were subjected to euthanized cervical dislocation for collection of liver sample.

Hematological and transaminase levels

Hematological (erythrocytes, hematocrit, hemoglobin, HCV, HCM, CHCM and leukocytes) and serum enzyme levels (serum glutamate oxaloacetate transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) creatinine and urea of control and treated group of Swiss albino mice submitted to treatment with extract prior to analyses using commercial kits according to the manufacturer's protocols. Blood samples were centrifuged (3,000 rpm for 15 min at 4°C) to separate serum, which was stored at -20°C for analysis of enzymatic dosages.

Histopathological and histomorphometric analysis of liver and renal

Renal and hepatic tissue samples from control and the treated group were fixed in formaldehyde (2.5%) and stained with hematoxylin and eosin (HE) (Masson 1956). Thirty histological sections were analyzed through four random fields. The histological sections were digitized using a digital video camera (Leica DFC 280, Wentzler, Germany) coupled to a light microscope (Leica Microscopy & Systems GmbH, Wentzler, Germany) under 40x final magnification. Morphometric evaluation of the area and perimeter of the hepatocyte as well as diameter renal corpuscle was performed using the Scion Image software (Scion Corporation, New York, U.S.A.).

Statistical analysis

The results for the hematological and transaminase levels of the area along with perimeter of hepatocytes and diameter of the corpuscle renal were expressed as mean \pm SEM (standard error mean) and analyzed by the Shapiro-Wilk test method of overall significance level ($p < 0.001$). The comparison between the control groups was performed by applying the Mann-Whitney test.

RESULTS

Phytochemical screening

The result of phytochemical prospection of the ethanol extract of stem bark of *C. heliotropifolius* (EECroton) is shown in Table 1. The EECroton showed the presence of coumarins, flavonoids, and triterpenes and the absence of alkaloids, saponins and tannin.

Determination of acute toxicity

After the end of the toxicological assays, it was verified that the EECroton in daily doses of 2000 mg/kg; v.o., showed to have some action on the central nervous system and also peripheral action in the pre-established doses. As stimulating action, behavioral reactions, such as agitation and increase in heart frequency were observed in all doses. As depressive action, exhaustion effect common to all doses was verified. In all doses, it was observed that soon after their application, there was abdominal contraction and dragging of the posterior legs when moving around. Table 2 shows the results of evolution of water and feed consumption of the EECroton control groups. The control showed a significant difference (32.75; 18.75) in water and feed consumption compared to EECroton (20.18; 12.68) respectively. The distribution comparison test was significant (p -value¹-0.003), indicating that the control group had higher water consumption and feed demand.

Tables 3 represents the weight evolution of the mice in the 14-days period and the weight assessment of the liver and kidney organs of the control groups treated with EECroton. The evolution of weight and mice as well as the relative weight of the liver and kidney organs of the control group compared to those treated with EECroton were similar.

Hematological levels

Table 4 shows the hematological levels (erythrocytes, hematocrit, hemoglobin, HCV, HCM, CHCM and leukocytes) in Swiss albino mice treated with EECroton. Results of group control were close to those treated with EECroton.

Levels of transaminases

Table 5 shows the serum levels of urea, creatinine, serum glutamate oxaloacetate transaminase (SGOT),

Table 3. Weight evolution of the mice and relative Weight of live kidney organs (Albino Swiss mice in the control and treated EECroton groups).

Group	Weight mice (g)		Relative weight of organs	
	Initial	Final	Live	Kidney
Control	37.8 ± 0.7	30.1 ± 1.0	2.00 ± 0.0	0.51 ± 0.0
EECroton	31.0 ± 0.8	28.0 ± 1.9	1.68 ± 0.0	0.48 ± 0.0

Values are expressed as mean ± SEM (*n* = 3 each group).

Table 4. Hematological (erythrocytes, hemoglobin, hematocrit, HCV, HCM, CHCM and leukocytes) in Swiss albino mice treated with EECroton (2000 mg/kg; v.o).

Hematological	Normais	Control	EECroton
Erythrocytes (10 ⁶ /mm ³)	9.0- 11.3	8.6±0.74	8.8±0.69
Hemoglobin (g/dL)	3.5-17.0	10.2±1.00	11±0.97
Hematocrit (%)	45-55	45.3±4.20	44±4.43
VCM (µg)	47-55	52.8±1.83	49.6±1.80
HCM (pg)	13-16	11.9±0.43	12.5±0.25
CHCM (%)	29-34	22.5±0.60	25.3±0.52
Leukocytes (10 ³ /mm ³)	2- 10	3.2±0.74	6.1±1.32

Table 5. Serum levels of urea, creatinine, Serum glutamate oxaloacetate transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT) in Swiss albino mice treated with EECroton.

Group	Dose (mg/kg)	Urea (mg/dL)	Creatinine (mg/dL)	SGOT(mg/L)	SGPT (U/L)
Control	15 mL/kg	57.5±7.78	0.49±0.03	187.9±39.6	69.0±8.39
EECroton	2000 mg/kg	54.2±10.5	0.33±0.06	184.6±23.5	110.9±19.0

Results are expressed as mean ± S.E.M; *Statistically significant compared to animals treated with (*p* < 0.05).

serum glutamic pyruvic transaminase (SGPT) in Swiss albino mice treated with EECroton. The animals of the control group showed high levels of urea (57.5±7.78), creatinine (0.49±0.03). SGOT (187.9±39.6) and SGPT (69.0±8.39) compared to animals treated with EECroton: Urea (54.2±10.5), creatinine (0.33±0.06) SGOT (184.6±23.5) and SGPT (110.9±19.0) which reduced serum enzyme levels. The animals of the control group showed reduced levels of SGPT (69.0±8.39) compared to animals treated with EECroton SGPT (110.9±19.0).

Histopathological and histomorphometric analysis of liver

Hepatic tissue samples collected from each group on the fourteenth day were evaluated at 400x magnification. A photomicrograph of each group is shown in Figure 1(A and B). Histological analysis of hepatic tissue after treatment with oral 2000 mg/kg of EECroton and control receiving Carboximetilcelulose (25 mg/kg) showed normal hepatic tissue. Lobular central vein (CV) radiated

out of the vein, with well-reorganized hepatocyte (HC) in the structural units of cells among which are the well-arranged sinusoidal capillaries (S) without leukocyte infiltration (L).

Area and perimeter of hepatocyte

The areas and perimeters of hepatocyte of the two groups were randomly compared and are shown in Table 6. The areas and perimeters of control (0.98 (0.31)) and (1.07(0.10)) showed no significant difference compared to those of EECroton at 2000 mg/kg; v. o (1.10 (0.15)) and (1.20 (0.15)) (*P*<0.001).

Histopathological and histomorphometric analysis of renal tissue

A photomicrograph of each group is shown in Figure 2A and B. Histological analysis of renal tissue after treatment with EECroton (2000 mg/kg; v.o) and the control group

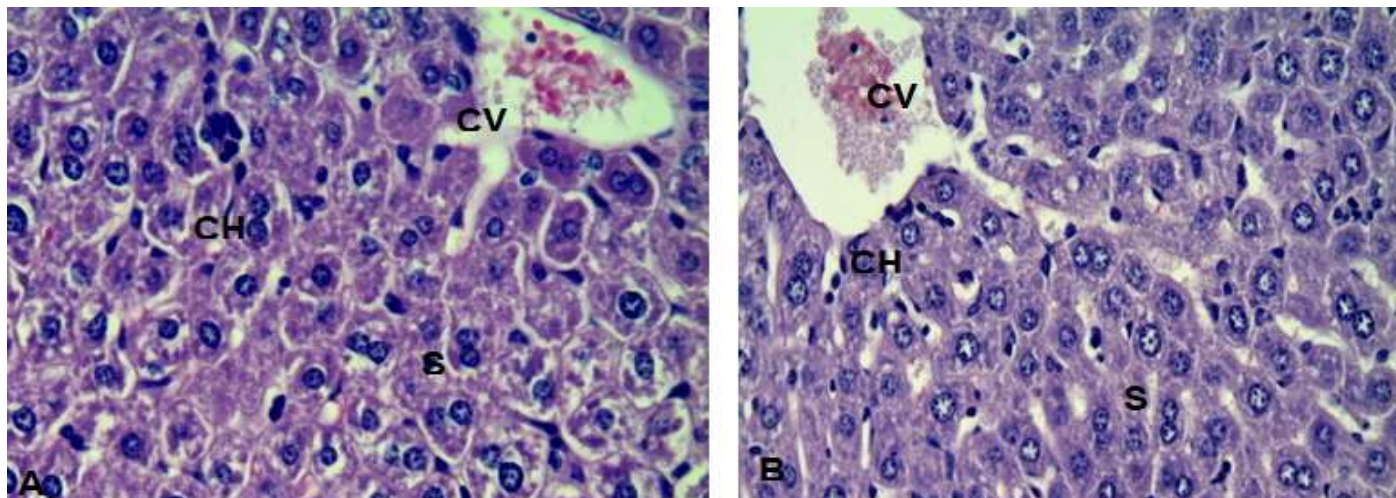


Figure 1. Photomicrograph of hepatic tissue of Swiss albino mice (Magnification x400). A = control group/administered Carboxymethylcellulose; B = treated group hepatic tissue/administered EECroton (2000 mg/kg v.o). Formalin fixed, HE- stained. S, Sinusoid capillaries; CV, Central veins; CH, Hepatocytes cord.

Table 6. Histomorphometry of cells liver of Swiss albino mice (area and perimeter).

Group	Hepatocyte	
	Area	Perimeter
Control	0.98 (0.31)	1.07 (0.10)
EECroton	1.10 (0.15)	1.20 (0.15)
p-value ¹	0.028	0.018

Control = hepatic tissue/administered Carboxymethylcellulose; EECroton = hepatic tissue/administered extract. Data were subjected to ¹p-value according to Mann-whitney test (P < 0.001). N- 200 cells liver.

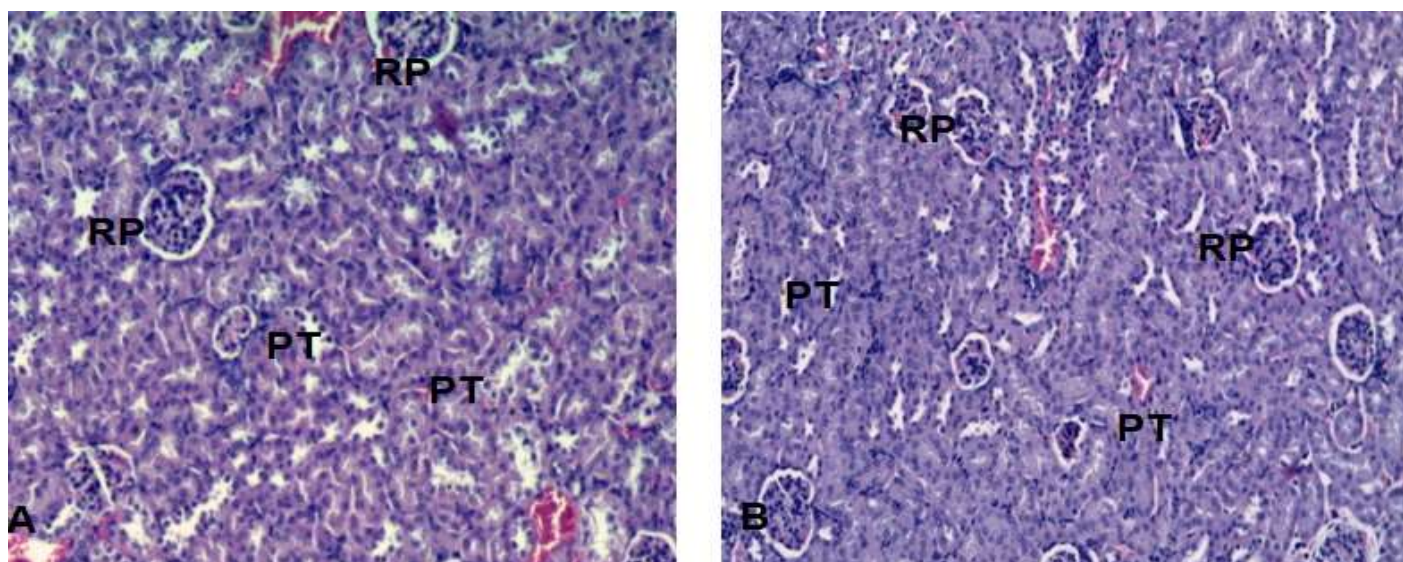


Figure 2. Photomicrograph of renal tissue of Swiss albino mice (Magnification x400). A = Control group renal tissue/administered Carboxymethylcellulose; B = Treated group renal tissue/administered EECroton (2000 mg/kg v.o). Formalin fixed, HE- stained. PT, Proximal tubules; RP, Renal corpuscle.

Table 7. Histomorphometry of renal corpuscles of Swiss albino mice (area and perimeter).

Group	Renal corpuscle	
	Area	Perimeter
Control	2.42 (0.20)	2.57 (0.21)
EECroton	2.38 (0.33)	2.51 (0.37)
p-value ¹	0.584	0.584

Control = renal tissue/administered Carboximetilcelulose; EECroton = renal tissue/administered extract; Data were subjected ¹p-value according to Mann-whitney test (P <0.001). N- 200 renal corpuscle

receiving Carboximetilcelulose (25 mg/kg) showed normal renal tissue (Proximal tubules (PT) and Renal Corpuscles (RC)).

Area and perimeter of renal corpuscles

The areas and perimeters of renal corpuscles of the two groups were randomly compared and are shown in Table 7. The areas and perimeters of control (2.42 (0.20)) and (2.57 (0.21)) showed no significant difference compared to those of EECroton at 2000 mg/kg dose (2.38 (0.33) and 2.57 (0.37) ($P < 0.001$)).

DISCUSSION

Studies focusing on *C. heliotropiifolius* have a predominant presence of alkaloid, polyphenols and reducing compounds (Randau et al., 2004). This species is useful in relieving stomach pain and dysentery and also acts as an antipyretic (Randau et al., 2004). This work aimed to evaluate the phytochemical screening of the stem bark *C. heliotropiifolius* (EECroton) and the acute toxicity assays *in vivo*, with emphasis on the histopathology study of the hepatic and kidney tissues in Swiss albino mice. Results from this study showed the EECroton have chemical profile similar to its genre and to majority of Euphorbiaceae family members. Thin-layer chromatography (TLC) was used to identify the presence of coumarins, flavonoids and terpenes and absence of alkaloids, saponins and tannins. Randau et al. (2004) in a similar study using the methanol extract of the stem of *C. heliotropiifolius*, also reported the presence of flavonoids and triterpenes and absence of coumarins. Polyphenols such as coumarins, is a secondary metabolite whose concentration in the plant is influenced by factors such as incident UV-A (315-400 nm) and UV-B (280-315 nm) radiation levels, as well as the age and position of the organ storage in the plant (Craveiro and Silveira, 1982). In this study, the presence of coumarins is probably due to the plant's storage criteria. In the study by Silva et al. (2017), the presence of chemical compounds such as flavonoids in leaves and flowers, coumarins stem and in

lower concentrations in the flowers has been identified. Flavonoids have their cytotoxic activity established in different tumor cells (Santos et al., 2013). The data indicate the presence of steroids or triterpenes as essential or volatile oils. The therapeutic interest in this class of secondary compounds is given by the importance of the cardiotonics glycosides, which are part of this group; and by the interest on sitosterol, stigmasterol, espirostanicas saponins, which serve as raw material, primarily for the production of contraceptives, steroids and anti-inflammatory (Cunha et al., 2014; Santos et al., 2013). The alkaloids and saponins were not identified in the species under study, corroborating the reports by Silva et al. (2017). In addition, an alkaloid was identified in the root skin of *C. heliotropiifolius* (Cordell et al., 2001). Among the classes of chemical compounds found in plants are the alkaloids; it is estimated that this class covers more than 4,000 compounds, which account for about 15-20% of known natural products (Verpoorte, 1986; Cunha et al., 2009; Matias et al., 2010). Experiments showed the extract did not contain condensed tannins although they have been detected in such specie (Randal et al., 2004). Hydrolysable tannins are made up of esters of glycolic acids and glycated ellagic acids (Randal et al., 2004; Costa-Lotufo et al., 2003). The toxicity of several secondary metabolites, present in vegetal have been described (Silva et al., 2012). The methanol extract of *C. heliotropiifolius* showed moderate toxicity to the micro crustacean *Artemia salina* (Silva et al., 2018). After the end of the toxicological assays, it was verified that the EECroton showed to have some action on the central nervous system and peripheral action in the pre-established doses. The results obtained make it clear that there was a low toxicity under the conditions evaluated. This can be confirmed within 14 days using the observed parameters. Significant decrease related to water consumption was observed in the group treated with EECroton, but this fact did not cause changes in the weight evolution of the animals. The weight evolution and consumption of water and feed are used to investigate the toxicity of a sample under study on the gastrointestinal system or even on the central nervous system if there is a change in more than one of the

parameters in question. Studies reporting examination of acute toxicity in vivo in mice with the species *C. heliotropiifolius* Kunth were not found in the literature, which makes it difficult to compare the results with others under the same conditions. The result of the acute toxicity test in vivo Swiss albino mice submitted to EECroton at a daily dose of 2000 mg/kg v.o. presented a low toxicity order during 14 days of observation. According to Leite (2003) who used the species *Indigofera suffruticosa*, the aqueous extract of *I. suffruticosa* leaves has demonstrated a low toxicity order. In toxicological activity, the LD₅₀ has not been determined, the extract can be considered practically non-toxic. The aqueous extract of *I. suffruticosa* leaves at different doses of 300 to 2,000 mg/kg i.p did not cause a mortality rate during 72 h of observation in the preliminary trial. Hematological parameters are very important, since the hematopoietic system is very sensitive to the activities of toxic agents. Hematological analyzes are performed with a focus on erythrocytes (erythrocytes), leukocytes and thrombocytes (Muriel and Rivera-Espinoza, 2008). The results of hematological levels (erythrocytes, hematocrit, hemoglobin, HCV, HCM, CHCM and leukocytes) in Swiss albino mice of group control were close to these treated with EECroton. Serum enzymes levels act as markers reflecting both hepatocellular necrosis and their release into the blood after damage to cell membrane (Renteria et al., 2007; Shanmugam et al., 2013). For the assessment of liver function, two enzymes are of great importance, serum glutamate oxaloacetate transaminase (SGOT) and serum glutamic pyruvic transaminase (SGPT). The action of these enzymes has been used as an indicator of hepatocellular damage. SGOT catalyzes the conversion of aspartate to oxaloacetate, as it is found in several organs and tissues, including the liver, heart (myocardium), skeletal muscle, among others (Muriel et al. and Rivera-Espinoza, 2008). It is present in the cytoplasm and also in the mitochondria, so its elevation indicates deeper cellular damage. SGPT catalyzes the conversion of alanine to pyruvic acid, is found in greater concentration in the liver and kidney, its origin is predominantly cytoplasmic, causing it to rise rapidly when liver damage occurs, which makes it a sensitive marker of liver function (Janbaz and Gilan, 2000). Mice pretreated with EECroton showed no changes in serum enzyme levels of ALT, AST and bilirubin, compared to control group which demonstrated low level of toxicity. However, the control groups treated with EECroton showed a significant increase in serum urea concentration might be an indication of a decrease in the glomerular filtration rate. Drug toxicity resulting from inadequate drug excretion or its metabolites can lead to impaired renal function. Thus, the assessment of blood levels of urea and creatinine is an excellent indicator of renal toxicity. Particularly, the accumulation of these nitrogenous substances in the blood can indicate kidney

failure (Schossler et al., 2001). The kidneys excrete urea, the product of protein metabolism. Renal tubules reabsorb 40% of this product; therefore, blood levels of this parameter are an indication of renal function and can serve as an index of glomerular filtration rate (Schossler et al., 2001). Theoretically, creatinine is the most suitable for checking kidney function, since the amount of creatinine present in the kidneys is more constant, but it is not reabsorbed in the renal tubules like urea (Emanuelli et al., 2008). However, some factors not associated with renal dysfunction, such as gastrointestinal hemorrhage, corticosteroid therapy and a protein-rich diet can increase urea production (Emanuelli et al., 2008). The liver is an organ of importance and plays a significant role in the metabolism and detoxification of exogenous toxin and therapeutic agents (Ramachandra et al., 2007). Histological observations are important tools for the detection of morphological changes in the liver caused by bioactive compounds (Silva et al., 2014; Lima et al., 2019). From the data of that study, histological analysis was performed and it showed that there was no structural alteration in the liver tissue of mice treatment (daily doses 2000 mg/kg v.o.) EECroton. The results of the histomorphometric analysis of the area and perimeter of the hepatocyte of mice treatment with EECroton was compared with control groups without extract, but no statistical significance was observed. A similar result was found with the species *I. suffruticosa* belonging to the Fabaceae family (Silva et al., 2014; Lima et al., 2019). Accordingly, Silva et al. (2014) demonstrated that the aqueous extract of *I. suffruticosa* preserved the liver architecture, and suggested its use as an alternative protective agent of the liver tissue. The result of the histomorphometric analysis of liver tissue after subchronic treatment with extract by infusion and maceration of the groups G1, G2 and G4 were similar and showed no degraded areas or leukocyte infiltration compared to G3, which shows a marked destruction of liver architecture (Silva et al., 2014). Histopathological and histomorphometric analyzes confirmed methanolic extract of *I. suffruticosa* leaves hepatoprotective activity, showing reorganization of structural units of cells, nuclei and sinusoidal capillaries of hepatocytes, reducing the damage on liver tissue and increasing organ regeneration rate (Lima et al., 2019). The histomorphological analysis of renal tissue shows the maintenance of cellular architecture in-group treated with EECroton and the group control; however, EECroton group present increase in serum urea levels. The histomorphometric analysis of the diameter renal corpuscles did not show statistical difference between the groups. Studies on histology and renal morphology are common in literature, revealing important roles especially when it is necessary to assess new substances with nephrotoxic potential. However, no published article was found on renal tissue histomorphological assessment of mice treated with *C. heliotropiifolius* extract. Similar studies using the same

species of *I. suffruticosa* showed that the aqueous extract obtained by maceration, when used in mice with sarcoma 180 treatments, did not induce any histomorphometric changes in the proximal convoluted tubules and renal corpuscles (Santana et al., 2015). In this study, no histological alterations were identified in the renal tissue of mice treated with daily dose of EECroton (2000 mg/kg v.o.) when compared to control. Also, there were no changes in the renal corpuscles diameter. The kidney is a very important organ and plays an important role in blood pressure regulation and fluid volume in acid-base balance, and in certain hormones formation and release (Gartner and Hiatt, 2008).

Conclusion

This phytochemical study of EECroton showed the presence of coumarins, flavonoids and terpenes and absence of alkaloids, saponins and tannins. In acute toxicity in vivo in mice under the conditions tested, it showed moderate toxicity, showing absence of changes in hepatic and renal tissues, being confirmed in hematological levels and serum enzymes, in addition to presenting the structural units of tissues in hepatocytes and renal corpuscles as well organized. These data suggest that EECroton has low order of toxicity.

CONFLICT OF INTERESTS

The authors have not declared any conflict of interests.

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