IOSR Journal Of Pharmacy and Biological Sciences (IOSR-JPBS)
e-ISSN:2278-3008, p-ISSN:2319-7676. Volume 20, Issue 4 Ser. 3 (July. – August. 2025), PP 19-30
www.Iosrjournals.Org

Toxicity Evaluation Of The Ethanolic Extract Of Cnidoscolus Quercifolius Pohl Bark

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Summary:

Cnidoscolus quercifolius is a xerophytic plant that is used ethnobotanically to treat injuries, ulcers, dysentery and pain through the rustic preparation of teas and ethanolic bottles. Despite its use by local inhabitants in Brazil, there is still a lack of research into its toxicity factors, which is of fundamental importance for its safe consumption by the population. This study sought to assess the toxicity of the ethanolic extract of the plant's bark. The results showed a low hemolytic factor, a median lethal dose of 97.96 ppm in Artemia salina assay, an insecticidal effect of 100 % at 2.5 mg/mL, phytotoxicity at 1 mg/mL, deaths were seen only in intraperitoneal administration (LD_{50}=117,1 mg/Kg), but alterations in urea and creatinine along with the morphology of red blood cells and tissue cells in organs such as the liver, spleen and kidneys were found via both intraperitoneal and oral, which leads to a warning that its consumption can cause damage. From this elucidation, it can be seen that its consumption presents risks, and it is necessary to ascertain its therapeutic potential in conjunction with safety dosages.

Keywords: *Faveleira, ethnobotany, Toxicity, Medicinal plants.*

Date of Submission: 07-08-2025 Date of Acceptance: 17-08-2025

I. Introduction

Cnidoscolus quercifolius, commonly *known* in Brazil as "faveleira" or "white nettle" and belonging to the *Euphorbiaceae* family, is a plant native to dry regions with a wide distribution in Latin America, mainly in Mexico and Brazil. This plant is found in greater quantities in Brazilian northeast, and is characteristic of the Caatinga phytogeographic domain (Coelho et al., 2012; Trindade; Lameira, 2014; Novaes et al., 2021). Its constituents include secondary metabolites such as phenols, tannins, flavonoids, terpenes, saponins and tocopherols (Morais et al., 2016; Paredes et al., 2016; Ribeiro et al., 2020), as well as fatty acids, carbohydrates (Paredes et al., 2016), lipids and a high protein content with antioxidant and anti-inflammatory activity similar to other fruits such as almonds (Medeiros; Aloufa, 2016).

The use of *C. quercifolius* by the traditional population due to the presence of these compounds has led to its growing application in various pathological conditions and dysfunctions such as abdominal pain, dysentery, pain, inflammation (Novaes et al., 2021; Oliveira-junior et al., 2019), oxidative stress (Santos et al., 2017), anti-leishmania (Fernandes et al., 2020), antiproliferative, in superficial wounds and for peptic ulcers (Paredes et al., 2016; Ribeiro et al., 2021; Novaes et al., 2021).

In view of the ethnobotanical value of plants in the Caatinga region with popular use in the preparation of teas, bottles, syrups and oil, *C. quercifolius* is also included with high pharmacological importance (Souza et al., 2016; Teixeira et al., 2019) with the root, stem, leaves, flower, fruit and bark being used, having high nutritional value for animals in periods of drought and humans in the preparation of cornmeal after grinding with flour and rapadura (Medeiros; Aloufa 2016). As a result of the indiscriminate use of medicinal plants without preliminary research into their toxicity, it is possible to note the growing risk of exposure to chemical interactions of their constituents in the body that tend to cause damage in the short and long term, requiring analyses that guarantee safety in their consumption/administration (Rosa et al., 2014; Oliveira et al., 2016).

By means of an experimental analysis using different models, this study aims to establish toxicity parameters in different routes from the ethanolic extract of *Cnidoscolus quercifolius* with the use of ethanol, making an important contribution to its vast therapeutic potential, which is suffering from issues such as the lack of botanical knowledge of its biome in the face of modern therapeutic techniques (Novaes et al., 2021). Thus, the aim of this study is to evaluate the toxicity of *C. quercifolius bark* ethanolic extract.

II. Methodology

Obtaining raw materials and extraction

Cnidoscolus quercifolius (Faveleira) was collected from Sítio Serra dos bois located in the city of Taquaritinga do Norte - Pernambuco/Brazil (7° 53' 17" South, 36° 5' 33" West). One specimen was deposited and identified by Dr. Marlene Carvalho de Alencar Barbosa under registration number 85312 in the Geraldo Mariz Herbarium of the Biosciences Center (CB) of the Federal University of Pernambuco (UFPE). It is registered with the National Genetic Heritage Management System (SISGEN) under the number A191B7E. The bark was removed using a blade and pulverized after drying in a 40° C oven, a *Willye 920* type forage machine. Extraction was carried out using a soxhlet apparatus with the solvent used according to the elutropic scale from the most apolar (hexane) to the most polar (absolute ethanol) following the boiling temperature of each solvent. The extracts were then concentrated by rotaevaporation and the yields were determined.

Hemolytic assay

The methodology proposed by Oliveira et al. (2012) was followed, with some modifications. Blood was collected by venipuncture from a healthy volunteer donor in a 3.2% sodium citrate tube. The whole blood was centrifuged at 2,500 rpm for 10 min and the RBC precipitate was washed three times in PBS (0.01M, pH 7.4). A 0.5% suspension in PBS was prepared from the washed red blood cells. 400 µL of the ethanolic extract (CqEE) (2-0.062 mg/ml) was added to 1.1 mL of the RBC suspension. The samples were then incubated at 37°C in a water bath for 60 min and then centrifuged. Triton X-100 at 0.1% was used as a positive control and the negative control (PBS). The absorbance was measured at 540 nm and the percentage of hemolysis was determined according to the formula: % hemolysis= (Absorbance (Abs) of sample-Abs of untreated) / (Abs of positive control-Abs of untreated) x 100.

Toxicity to *Artemia salina*

This was done according to the methodology of Meyer et al., 1982, by counting the number of dead individuals 24 hours after being incubated with different concentrations of CqEE (1000-15.625 ppm, 5ml). The positive control was 0.1% potassium dichromate and the negative control was artificial seawater. The results were expressed as percentage mortality and the LC_{50} determined by non-linear regression.

Larvicide test with *Tenebrio molitor*

Larvae of the species *Tenebrios molitor* used samples of the extract diluted in 1.5 mL of distilled water, making a serial dilution in triplicate using 6 species from breeding. Plates were purchased and adapted for air passage. With the insertion of film paper, 5 groups were separated, 1 of which was the control, 1 vehicle and 3 concentrations, 1.25, 2.5 and 5%. After this, around 50 microliters were applied to the prothoracic region, where their survival rate was analyzed after being protected from light for 72 hours (Fazolin et al., 2007).

Phytotoxicity test

This test was carried out according to the methodology described by Torres et al. (2018). CqEE at different concentrations (2 - 0.25 mg/ml, 1 ml) diluted in buffer were added to eight achenes *of Lactuca sativa* L. (Feltrin Lettuce Great Lakes 659). MES/NaOH buffer with 0.5% DMSO was used as a negative control and glyphosate herbicide (Citromax®) was added as a positive control. The plates were incubated for 7 days under a 12-hour photoperiod at 25-32ºC and then placed at -22ºC for 24 hours. The seedlings were scanned and the images were used to measure root and hypocotyl length in the ImageJ® program. In addition, the relative germination percentage of the seeds (GRS), the relative root growth (CRR) and the germination index (GI) were calculated according to the formulas of Hoekstra et al. 2002.

Assessment of acute oral and intraperitoneal toxicity

All animal experiments were approved by the Animal Research Ethics Committee of the Federal University of Pernambuco - CEUA-UFPE, under process number 0013/2021, using 4-month-old adult *Mus musculus* Albino Swiss mice. The animals were obtained from the vivarium of the Keizo Asami Immunopathology Laboratory (LIKA-UFPE) and brought to the vivarium of the Biochemistry Department (UFPE), where they were housed for a week before the tests began. The animals were kept under controlled temperature and humidity, with food and water *ad libitum* and a 12/12 hour light/dark photoperiod. *In vivo*

toxicity tests were carried out *in* accordance with OECD guidelines (2001). Oral doses of 50mg/kg, 200 mg/kg, 500 mg/kg and 1000 mg/kg and intraperitoneal doses of 100 mg/kg, 500mg/kg and 1000 mg/kg of EWC were administered (5 mice in each group), with the negative control group being administered 0.9% saline.

The animals were observed at 24, 48 and 72 hours and every day until the 14th day for changes in body weight, behavior and physique. On the 14th day, blood was collected via the retro orbicular plexus after anesthesia using xylazine (10mg/kg) and ketamine (115 mg/kg). The whole blood was used to make a smear which was stained by rapid panoptic and used to measure cell morphological changes and the serum was used to measure glucose, total cholesterol, urea and creatinine using a commercial diagnostic kit (Labtest Diagnóstica LTDA, Vista Alegre, Brazil). The animals were then euthanized and the kidneys, liver, heart and spleen removed for later histological analysis.

Statistical analysis

Test results were expressed as mean±standard deviation. Statistical analyses were carried out using the one-way ANOVA test followed by comparative analysis with Tukey's post-test. All values with $p<0.05$ were considered statistically significant. The LC_{50} was calculated by non-linear regression and all the data was analyzed using GraphPad Prism 8.0, USA.

III. Results And Discussion

The study of toxicity factors is of paramount importance for determining the parameters of reliability and safety in the use of organic materials, whether for consumption or as means of producing new materials (Lalitha et al., 2012; Oliveira et al., 2016). The hemolytic test is carried out as means of cytotoxic screening, in view of the possibility of some phytochemical components affecting the integrity of red blood cells, preliminarily indicating the safety or otherwise of a given natural product. For CqEE, the highest percentage found was 18.71 % at the highest concentration tested (1 mg/ml), with no additional hemolysis from 0.5 mg/ml downwards. Previously, the ethanolic extract of the leaves of this plant showed a lower hemolytic percentage (6.98%) at a higher concentration (4 mg/ml) (Gomes et al., 2014).

In a phytochemical screening, phenols, tannins, flavonoids and steroids/triterpenoids were found and also in increasing order of quantity flavonoids, condensed tannins and polyphenols (Fernandes et al., 2018). Within the genus, only a protein-rich fraction of *C. urens* was tested for its hemolytic activity and showed almost no hemolysis. Thus, *C. quercifolius* and others in the genus appear to have low cytotoxicity for erythrocytes, when directly in contact (Menezes et al., 2014).

In *Artemia salina*, CqEE caused 100 % of deaths at the concentration of 250 ppm (0.25 mg/ml), as can be seen in Figure 1. The LC50 was determined to be 97.96 ppm (0.09796 mg/ml). According to Meyer and Cols. (1982), a CL50 < 1mg/ml is considered toxic and a CL50 > 1mg/ml is not toxic, therefore CqEE is classified as toxic in the *A. salina* toxicity test.

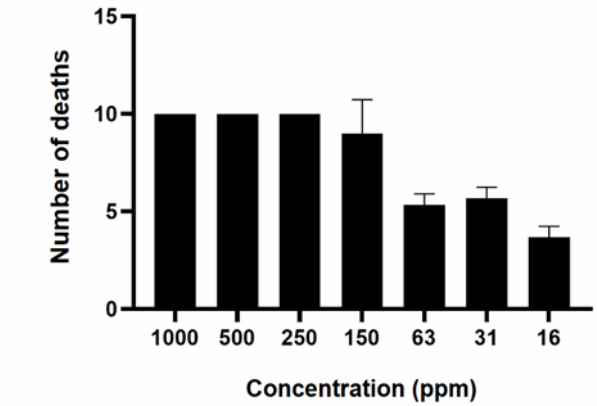

Figure 1 - Number of deaths in the *Artemia salina* toxicity assessment of the ethanolic extract of *Cnidoscolus quercifolius* bark at different concentrations after 24h

In a study with the methanolic extract of the roots of this same species, a lower CL50 was found (84.76 µg/ml), but not for the extracts of leaves (1079.78 µg/ml) and root bark (341.45 µg/ml). Phenols, tannins, flavones, flavonols, xanthones, flavanones were reported for all these extracts in a phytochemical screening, and were related to these low levels of toxicity (Paredes et al., 2016). *C. chayamansa* is another species within the genus whose aqueous extract of mature leaves was also tested on *Artemia salina* and found to be safe for consumption (Soto et al. 2015). As a model for accessing toxicity, *Artemia salina* is considered an easy,

accessible, fast and convenient test that is widely used to evaluate different plants under various extraction methods (Nunes et al., 2006; Selvi et al. 2018).

CqEE was also evaluated for its larvicidal activity against larvae of *Tenebrio molitor*, known as the mealworm. This larvicidal activity study model is efficient and important for evaluating the environmental toxicity of natural products that are intended to be used for pest control (Spochacz et al. 2018). In the results, CqEE promoted larval mortality of 94.40 % at the concentrations of 5 and 2.5 mg/mL, not differing from the positive control, and 22.22 % mortality for 1.25 mg/mL as can be seen in Table 1.

Table 1 - Toxicity of the ethanolic extract of *Cnidoscolus quercifolius* bark on *Tenebrio molitor* larvae after 72 hours

Groups tested						
				CqEE concentration (mg/ml)		
	Positive control	**Negative control**	**Vehicle**	**5.0**	**2.5**	**1.25**
Mortality (%)	100	0.00*	0.00*	94.4	94.4	22.22*

Positive control, bleach; Negative control, Group without application; Vehicle, 0.9% saline solution + tween 80. *p < 0.001 vs positive control, one-way ANOVA followed by Tukey's Test.

The methanolic extract of roots and aerial parts of *Myrtillocactus geometrizans*, and isolated sterols (peniocerol and macdougallin), significantly affected the larval growth of *T. molitor*, and the lipidic content was associated with this potential (Céspedes et al. 2005). However, *Solanum nigrum* fruit extracts and pure glycoalkaloids (solamargine, solasonine) did not cause any larval deaths, causing only sublethal effects that can damage development and metabolism (Spochacz et al. 2018). In addition, the ethanolic extract of *Nicotiana tabacum* leaves showed a CL50 of 21.1 mg/ml which seems to be better than that presented here (Sentosa et al. 2019).

In the evaluation of phytotoxic activity, it was observed that the growth of the hypocotyl was significantly reduced in all the concentrations tested (Figure 2d) when compared to the buffer (positive control), while only the concentrations of 2.0 mg/ml and 1 mg/ml produced the same effect (Figure 2e). It was also possible to see in the scanned images of the seedlings that the higher concentrations of CqEE (Figure 2a) affect their growth and that this effect is lessened as the concentration decreases (Figure 2b) when compared to the buffer (Figure 2c).

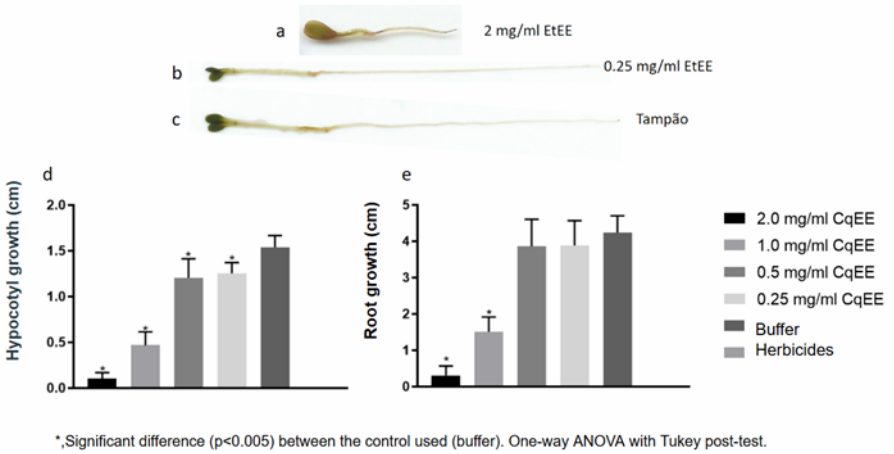

Figure 2 - Effect of different concentrations of ethanolic extract of the bark of *Cnidoscolus quercifolius* (CqEE) on the growth of lettuce (*Lactuca sativa* large lakes).*p < 0.005 vs buffer. One-way ANOVA with Tukey's post-test.

Observing the results of the relative germination percentage of the seeds, root growth and germination index, it was possible to see that the extract largely interferes with the development of the hypocotyl and root (Table 2). Analyzing the germination index (GI), the concentrations of 2 mg/mL and 1 mg/mL were classified

as very phytotoxic and phytotoxic, respectively, and the others were described as non-phytotoxic according to Souza et al. (2017).

Table 2 - Relative seed germination (RSG), relative root growth (RRG) and germination index (GI) for the toxicity test with ethanolic extract of *C. quercifolius*

Samples	**GSR (%)**	**RRG (%)**	**GI (%)**
2 mg/ml CqEE	39.13	7.24	2.83
1mg/ml CqEE	84.78	35.58	30.16
0.5 mg/ml CqEE	95.65	91.08	87.11
0.25 mg/ml CqEE	93.47	91.65	85.66

CqEE, Ethanolic extract of the bark of *Cnidoscolus*

In view of the results obtained in an assisted manner and by controlled experimental intervention, the use of low concentrations of the ethanolic extract of the bark of *Cnidoscolus quercifolius* in the root region of the lettuce did not interfere with its development. Therefore, the higher concentrations can be used as herbicide. Studies of its phytotoxic potential have already been carried out using faveleira seeds with distilled water and alcohol, showing no significant results (Henrique et al., 2021).

The evaluation of *in vivo* toxicity in animal models is of fundamental importance in the development of new therapies, as they have similarities with human physiology and can be substituted during tests to validate new therapies. Furthermore, their technical practicality in relation to other organisms such as rabbits and pigs makes them the toxicity evaluation model of choice (Bednarczuk et al., 2010). In the evaluation of the *in vivo* toxicity of EQC, the mice showed a gradual increase in body mass over the time of observation (Table 3). The lack of changes in the animals' body weight that could indicate any interference or toxicity of the extract may be related to the nutritional value already reported for *C. quercifolius*, which is rich in lipids, phenols, carotenoids, unsaturated fats, saponins, flavonoids, terpenes and anthocyanins. These constituents give this plant anti-inflammatory and antioxidant capacity, as well as combating the accumulation of fat in adipose tissue (Paredes et al., 2016; Chabra et al., 2018; Ribeiro et al., 2020; Oliveira-Junior et al., 2020).

Table 3 - Average body weight of mice submitted to the acute toxicity test (14 days of observation) with different forms of administration of the ethanolic extract of the bark of *Cnidoscolus quercifolius*

Groups	Dose (mg/Kg)	Average initial body weight (g)	Average final body weight (g)	Difference between initial and final weight (g)
	Control	32.76 ± 1.14	37,26 ± 1.18	4,5 ± 1.94
Oral	50	30.244 ± 1.20	36,82 ± 1.07	6.57 ± 1.35
	200	28.66 ± 2.66	34.86 ± 1.62	6.2 ± 2.95
	500	31.94 ± 1.10	38.18 ± 0.31	6.24 ± 0.99
	1000	30.85 ± 3.42	38.375 ± 0.68	4.44 ± 3.22
Intraperitoneal*	Control	38.3 ± 5.77	47.63 ± 2.59	9.33 ± 7.42
	100	37. 23 ± 3.52	39.93 ± 1.41	2.7 ± 4.7

* Mice from 500 and 1000 mg/kg dose groups all died. No significant differences in one-way ANOVA analyses followed by Tukey's post-test and t-test analysis.

In the evaluation of signs of toxicity after oral administration of the extract, no abnormalities were seen (agitation, motor alterations, abdominal writhing, lethargy, piloerection, respiratory alterations, bleeding, changes in urine and feces). No animal deaths were observed either, as can be seen in Table 4.

Table 4 - Analysis of signs of acute oral toxicity using the ethanolic extract of *Cnidoscolus*

	Groups (mg/kg)				
	50	200	500	1000	Control
Abnormalities	0/5	0/5	0/5	0/5	0/5

Deaths	0/5	0/5	0/5	0/5	0/5

Control, saline solution.

In the evaluation of toxicity via the intraperitoneal route (Table 5), deaths were already observed on the 2nd day of experimentation in the higher dosage groups (500 and 1000 mg/ml). Therefore, the extract is toxic by this route at the highest concentrations, in contrast to oral administration, which showed no alterations. Oral administration involves digestive metabolization, which may probably have degraded the constituent of this extract that is causing the toxicity, which possibly does not occur for intraperitoneal administration (Silva et al., 2015; Oliveira et al., 2016; Surendra et al., 2016).

Table 5 - Analysis of the intraperitoneal toxicity of the ethanolic extract of *Cnidoscolus quercifolius*

	Groups (mg/kg)			
	Control	100	500	1000
Abnormalities	0/3	0/3	1/5	0/5
Deaths	0/3	0/3	5/5	5/5

Control, saline solution.

According to Covelli (2015) and the OECD guidelines (2001), no macroscopic/visible changes were observed in the mice that compromised quality of life during oral administration, in contrast to the intraperitoneal route which led to death at the highest dosages and slight apathy during the first hours of observation. This, in turn, is due to the presence of secondary compounds/metabolites such as flavonoids and tannins that generate hemolytic effects via the oxide reductase pathway, altering the shape of the erythrocyte membrane, which may be similar to the consumption of other materials such as silver derivatives that cause numbness and death (Kim et al., 2012; Silva et al., 2015; Surendra et al., 2016; Cardoso de Almeida, 2014).

In the histological analysis of the intraperitoneal group that did not survive, it is possible to highlight the reason for the high mortality rate, where the liver externally showed a thin capsule made up of mesothelium and parenchyma with hepatocytes arranged disorderly around the central lobular vein. However, the animals treated with 100 mg/mL showed an intense amount of lipid droplets and binucleated hepatocytes as seen in figure 3.

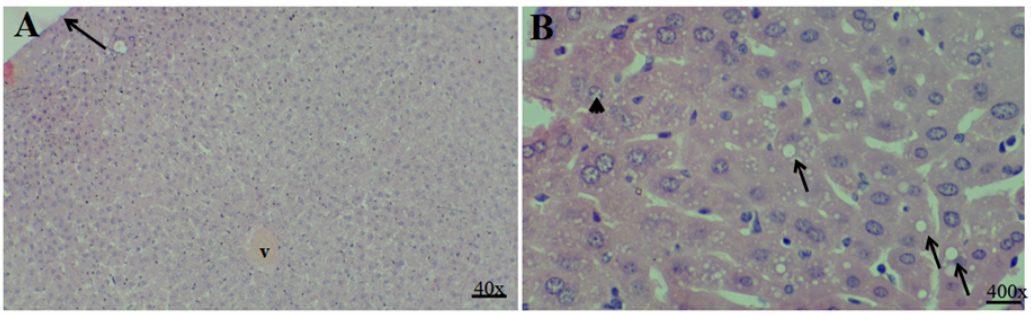

Figure 3 - Photomicrograph of the liver of mice after intraperitoneal administration of *Cnidoscolus quercifolius* extract. A, Control group; B, group with 100 mg/dL of the extract.

The kidneys of all the experimental groups had a capsule covering the organ and well-defined cortical and medullary regions. The cortical region showed renal glomeruli and proximal and distal convoluted tubules. However, the animals treated with 100 mg/mL showed very cellularized renal glomeruli, with no clear demarcation of the subcapsular space, as well as vacuolization and cell proliferation between the renal tubules in the cortical region.

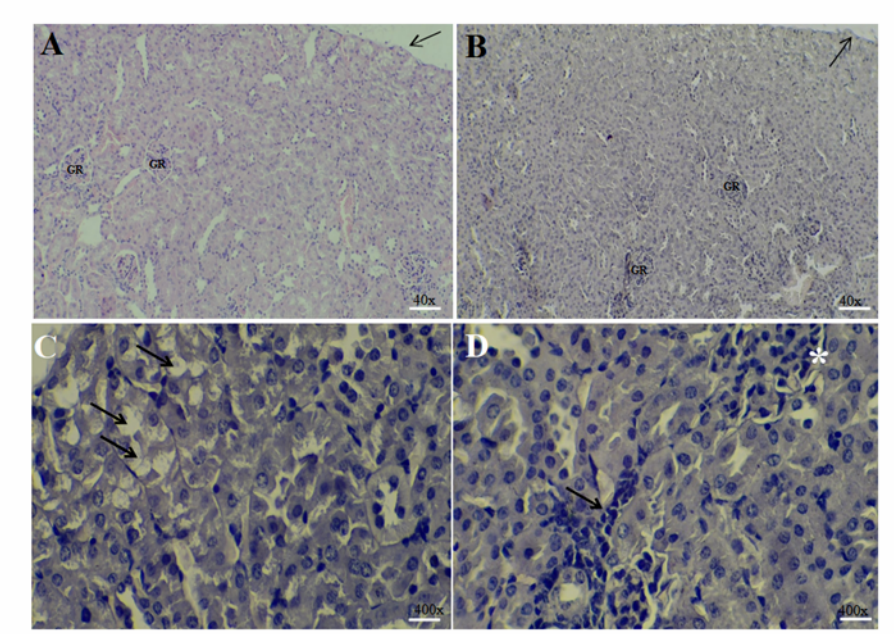

Figure 4 - Photomicrograph of kidney slides from the intraperitoneal group. A;B, Control group; C;D, Group treated with the extract.

Histological analysis of the spleen of all the experimental groups showed the capsule lining the outside of the organ, the red pulp and the well-defined white pulp. The white pulp is defined by the lymphoid nodule consisting of the germinal center and central arteriole. However, the animals treated with 100 mg/mL showed a disorganization of the lymphoid nodule with cells proliferating into the red pulp. They also showed a greater number of megakaryocytes.

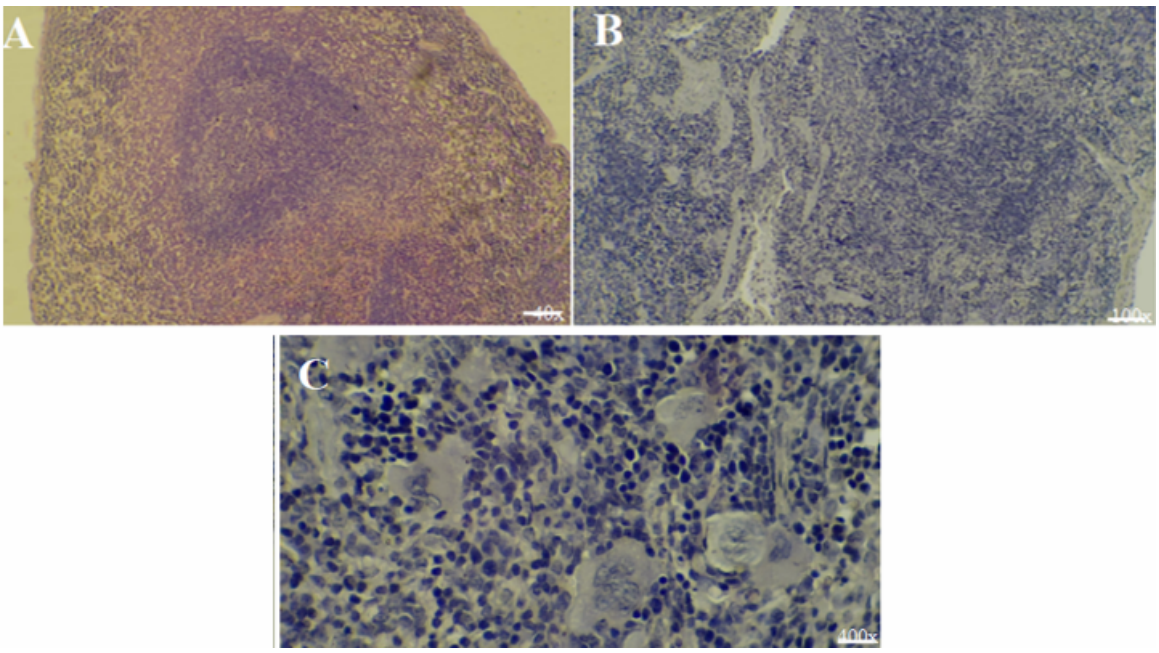

Figure 5 - Photomicrograph of the kidneys after intraperitoneal administration of the plant extract.

Many of the systemic changes can be expressed in weight, serving as a preliminary indication of the toxicity factor (Junior et al., 2012). During the experiment, there were no significant changes in weight from the lowest to the highest concentrations, which was different when compared to the ingestion of substances with anorexigenic potential and which bring about irritative changes that induce a greater chance of mortality (Lutz et al., 1993; Guo et al., 2016). The results also showed no predisposition to secondary infections due to the

presence of exudation. The hemolytic test showed similar results, and in this cytotoxic screening the highest concentrations resulted in cell death.

Photomicrographs of the blood samples collected at the end of the observation period for oral and intraperitoneal administration showed the presence of morphological changes in the red blood cells, which are indicative of toxicity and are responsible for hemolysis and consequent death, as demonstrated in the 500 mg/kg and 1000 mg/kg intraperitoneal administration groups. In the controls of the intraperitoneal group, no change was seen in the size and shape of the red blood cells, in contrast to the 50 mg/kg group with the presence of anisocytosis and rouleaux red blood cells (Figure 3) which is due to a possible increase in plasma proteins whose origin comes from intense inflammation which may have caused death at the highest concentrations without there being a process of elimination of the causative agent which was possibly inactivated by the oral route (Silva; Monteiro, 2020).

There were also no changes in the control group administered orally (Figure 6A), but as the concentrations increased, some signs of toxicity were noticed. In the 50 mg/kg oral group (Figure 6B) there was anisocytosis, possibly due to vitamin deficiency, together with acanthocytes. The 200 mg/kg oral group (Figure 6C) showed the same structures, as well as dacryocytes, which can be explained by their origin outside the bone marrow, which gives them their distinctive shape, which can indicate damage due to systemic changes, together with codocytes and Heinz Bodies, an indication of oxidative damage to hemoglobin (Dacie; Lewis, 1995; Hoffbrand et al., 2001).

In the 500 mg/kg oral group (Figure 6D), it was possible to see the presence of spherocytes due to changes in the plasma membrane proteins which predispose to the appearance of anaemias which weaken and cause complications in the proper distribution of nutrients. The 1000 mg/kg oral group (Figure 6E), which was the highest dosage tested, showed the presence of dacryocytes, spherocytes and a high prevalence of echinocytes per area due to changes that cause renal insufficiency (Dacie; Lewis, 1995).

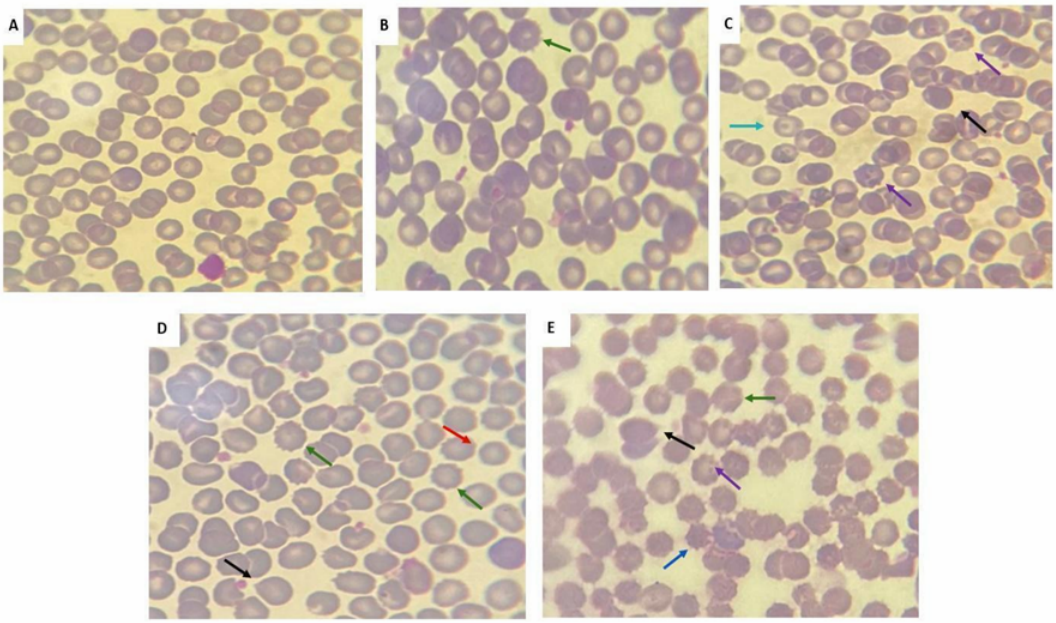

Figure 6 - Photomicrograph of red blood cells after oral administration of different concentrations of the ethanolic extract of *Cnidoscolus quercifolius* (magnification 1000X). A, Control; B, 50 mg/kg; C, 200 mg/kg; D, 500 mg/kg; E. 1000 mg/kg. Dark green arrow, acanthocytes; light green arrow, codocytes; black arrow, dacryocytes; purple arrow, Heinz bodies; red arrow, spherocytes; blue arrow, echinocytes.

Red blood cells (RBCs) are markers of toxicity due to the susceptibility of their membranes to oxidative damage caused by effects such as aging by carbonyl derivatives, cancers and deficiencies such as anemias and syndromes (Yelinova et al., 1996). During erythropoiesis in the maturation of the red line, it is possible to see the synthesis of hemoglobin, condensation of chromatin and elimination of organelles from its initial phase generating proerythroblasts, erythroblasts and reticulocytes until the end with mature erythrocytes. During this process, the erythroblastic islands need to provide the necessary conditions for the organization of these cells in each phase, along with the generation of ATP, which is only found intracellularly, and if it is found externally, it is an indication of damage. If there is any alteration in this phase, it is possible to notice the process called oxidative stress (Yeo et al., 2019).

Integral membrane proteins maintain the morphology of erythrocytes and renew them in the event of injury, the main representative being the protein classified in band 3, responsible for ion exchange and which also mediates CL-H2O, increasing the blood ability to transport O2 more strongly throughout the body. Among this group, it is also possible to find Ankyrin, Spectrin, Glycophorin A, B and C with a similar function favoring structural changes such as in spherocytes, one of the most commonly found during the analysis, as well as in other studies (Murador; Defune, 2007;Yeo et al., 2019). The fluidity of the cell membrane depends on the phospholipids, chain length, degree of saturation, presence of glycerol and fatty acids. The main pathways of oxidative damage in RBCs act through lipid peroxidation of membranes from high oxygen tension and the formation of reactive species that bring abnormalities in their form, which are then found in intraperitoneal dosages due to the possible decrease in compounds such as cysteine, caspases and mutations in the RBC (Farag; Alagawanib, 2018). Other alterations such as Heinz corpuscles, acanthocytes and schizocytes are present due to the increased oxidative process and inflammatory induction that increases cholesterol and breaks down phospholipids (Silva; Monteiro, 2020).

As for the results of the biochemical dosages that come from the functional study of the animals serum, it was found that glucose and cholesterol were in the normal range, not predisposing to metabolic alterations such as hyperglycemia and the appearance of atherosclerosis (Melo et al., 2012; Almeida et al., 2014; Ramos et al., 2019). One of the best explanations for this effect lies in the tannins that inhibit amylase digestion and control sugar in mice, as well as having an antioxidant effect as described in other studies (Denga et al., 2019; Morais et al., 2016).

With regard to urea and creatinine, which are markers of kidney damage, these were elevated in accordance with the parameters established in studies such as Almeida et al., 2014 and Frohlich, 2014 and taken from Branco et al., 2011, and in the present study were classified as elevated in accordance with the oral control (Table 6). Based on the report of deaths at the highest doses administered intraperitoneally (500 and 1000 mg/mL), it is possible to highlight the existence of a toxic agent that was not degraded by this route, so its administration in the region of the membrane resulted in instant death that reflected effects on the doses of urea and creatinine, thus requiring further tests to identify this specific agent(s).

Table 6. Serum biochemical analysis of mice under acute oral and intraperitoneal toxicity test with the administration of ethanolic extract of *C. quercifolius* bark (CqEE).

	Groups (mg/Kg)	Glucose (mg/dL)	Total cholesterol (mg/dL)	Urea (mg/dL)	Creatinine (mg/dL)
	Control	177.97	96.4	470.3	2.14
	EWC 50	159.91	99.4	542.6	6.71
Oral administration	CqEE 200	208.81	56.0	383.0	3.71
	CqEE 500	163.87	86.7	572.1	8.43
	CqEE 1000	161.67	115.7	431.6	6.01
Intraperitoneal	Control	134.38	0.139	286.04	13.75
	CqEE 100	173.12	0.182	286.04	0.75

CqEE, Ethanolic extract of the bark of *Cnidoscolus quercifolius*

High dosages of compounds such as ammonia and urea in the blood generate toxic action and are indicative of liver and kidney function, in contrast to a normal situation in which they are reabsorbed so that no damage is done. The high mortality rate and the slight increase in urea and creatinine in the highest doses of both groups (oral and intraperitoneal) may be related to interference in anaerobic metabolism which damaged lipids, proteins and carbohydrates. After consuming high doses of the extracts, changes may have occurred in the oxidoreductase system in the role of superoxide dismutase acting as a pro-oxidant and inflammatory agent (Vannucchi et al., 1998) which led to these results, although after the end of the experiment and euthanasia of the specimens, no visible changes were observed in the main organs that were removed (Liver, Kidneys, Heart and Lungs).

IV. Conclusion

Therefore, the ethanolic extract of *Cnidoscolus quercifolius* bark seems to be toxic if consumed indiscriminately, especially if via intraperitoneal route, and the dosage is the determining agent of its toxic potential, such as when consumed orally in high concentrations, which causes changes in metabolism, organs

morphology, and is highly apparent in red blood cells. The extract can serve as an insecticidal and herbicidal compound at high dosages, requiring appropriate disposal. Complementary tests should still be carried out to better elucidate its potential, along with other avenues of therapeutic interest and concerns.

Acknowledgements

To the Coordination for the Improvement of Higher Education Personnel - CAPES (Grant 001);
To Dr. Marlene Carvalho de Alencar Barbosa for identifying the plant.

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