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Toxicological evaluation and safety of the ethanol extract from leaves of *Piptadenia stipulaceae*

Avaliação toxicológica e segurança do extrato etanólico das folhas de *Piptadenia stipulaceae*

Evolución toxicológica y seguridad del extracto etanólico de hojas de *Piptadenia stipulaceae*

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Abstract

Piptadenia stipulacea is popularly known as "Jurema Branca" and is apply in empiric therapy as anti-inflammatory, analgesic, cells regenerator, antipyretic and astringent pectoral. The present work had as objective to carry out the toxicological analysis of the ethanol extract of the leaves of *P. stipulacea*. *In vitro* toxicity was performed according to the methodologies of osmotic fragility against sheep erythrocytes and genotoxicity by *Allium cepa* and *in vivo* toxicity according to the protocol of Organization for Economic Cooperation and Development (OECD 423). Erythrocyte osmotic fragility presented low levels of hemolysis both by the qualitative evaluation of the supernatant and by the hemolytic percentage result. In cytotoxic evaluation with *Allium cepa*, no chromosomal abnormalities or differences in the cell division process (interphase, prophase, anaphase metaphase and telophase) were identified between the control group and the groups submitted to the different concentrations of the ethanolic extract of the leaves of *P. stipulaceae* (50μg/mL, 500μg/mL and 1000μg/mL). The ethanolic extract of the leaves of *P. stipulaceae* can be considered of low acute toxicity, since it did not cause the death of treated animals at the dose of 2000 mg/kg,

as recommended by OECD 423. The results obtained allow to proceed with additional tests of sub-acute and chronic toxicity, biological activities on specific organisms in order to define the low risk of this plant.
Keywords: Jurema Branca; Osmotic fragility; In vitro toxicity.

Resumo

Piptadenia stipulacea é popularmente conhecida como "Jurema Branca" e tem aplicação em terapia empírica como antiinflamatória, analgésica, regeneradora de células, antipirética e adstringente peitoral. O presente trabalho teve como objetivo realizar a análise toxicológica do extrato etanólico das folhas de *P. stipulacea*. A toxicidade in vitro foi realizada de acordo com as metodologias de fragilidade osmótica contra eritrócitos de ovelhas e genotoxicidade por *Allium cepa* e toxicidade in vivo de acordo com o protocolo da Organização para Cooperação e Desenvolvimento Econômico (OECD 423). A fragilidade osmótica eritrocitária apresentou baixos níveis de hemólise tanto pela avaliação qualitativa do sobrenadante quanto pelo resultado da porcentagem hemolítica. Na avaliação citotóxica com *Allium cepa*, não foram identificadas anormalidades cromossômicas ou diferenças no processo de divisão celular (interfase, prófase, anáfase metáfase e telófase) entre o grupo controle e os grupos submetidos às diferentes concentrações do extrato etanólico das folhas de *P . stipulaceae* (50µg / mL, 500µg / mL e 1000µg / mL). O extrato etanólico das folhas de *P. stipulaceae* pode ser considerado de baixa toxicidade aguda, uma vez que não causou a morte dos animais tratados na dose de 2.000 mg / kg, conforme recomendado pela OCDE 423. Os resultados obtidos permitem prosseguir com testes adicionais de toxicidade subaguda e crônica, atividades biológicas em organismos específicos, a fim de definir o baixo risco desta planta.
Palavras-chave: Fragilidade osmótica; Toxicidade in vitro; Toxicidade in vivo.

Resumen

Piptadenia stipulacea es conocida popularmente como "Jurema Blanca" y tiene aplicación en terapia empírica como antiinflamatorio, analgésico, regenerador celular, antipirético y astringente pectoral. El presente trabajo tuvo como objetivo realizar el análisis toxicológico del extracto etanólico de hojas de *P. stipulacea*. La toxicidad in vitro se realizó según las metodologías de fragilidad osmótica contra eritrocitos de ovino y genotoxicidad por *Allium cepa* y toxicidad in vivo según el protocolo de la Organización para la Cooperación y el Desarrollo Económicos (OCDE 423). La fragilidad osmótica de los eritrocitos mostró niveles bajos de hemólisis tanto por la evaluación cualitativa del sobrenadante como por el resultado del porcentaje hemolítico. En la evaluación citotóxica con *Allium cepa*, no se identificaron anomalías cromosómicas o diferencias en el proceso de división celular (interfase, profase, anafase, metafase y telofase) entre el grupo control y los grupos sometidos a diferentes concentraciones del extracto etanólico de hojas *P. stipulaceae* (50µg / mL, 500µg / mL y 1000µg / mL). El extracto etanólico de las hojas de *P. stipulaceae* puede considerarse de baja toxicidad aguda, ya que no provocó la muerte en los animales tratados a la dosis de 2.000 mg / kg, como recomienda la OCDE 423. Los resultados obtenidos permiten continuar. Con pruebas adicionales de toxicidad subaguda y crónica, actividades biológicas en organismos específicos, con el fin de definir el bajo riesgo de esta planta.
Palabras clave: Fragilidad osmótica; Toxicidad in vitro; Toxicidad in vivo.

1. Introduction

The genus Piptadenia Benth. is part of one of the largest groups of legumes recognized as the subfamily Mimosoideae (Sobreira *et al*., 2020). *Piptadenia stipulacea* belongs to this subfamily, being a small tree species, native to the Caatinga, widely distributed in the northeast region of Brazil with great commercial, economic and environmental interest for the region due to its multiple use characteristics (Sobreira *et al*., 2020).

According to Pereira *et al* (2020) the behavior of *P. stipulacea* clearly reflects the effects of disturbances to which a vegetation was submitted, showing itself to be quite tolerant to high levels of disturbance. The species is popularly known as white jurema (Fabricante & Andrade, 2007) and carcará (Florentino et al., 2007), being used in woodworking, civil construction, production of stakes, firewood, charcoal and in home medicine, in treatments of burns and skin problems. The species has antimicrobial, analgesic, cell regenerating, antipyretic and pectoral astringent potential (Sobreira *et al*., 2020)

Its seeds have high levels of alkaloids and therefore are important in phytochemical and pharmacological projects (Fish et al., 1955). It is widely used as food by sheep, goats and cattle, especially in the dry season when there is no pasture for food (De Melo Cavalcanti-Dantas *et al*., 2016).

In partnership with pharmacology, the potential of this species was proven through studies that showed anti-inflammatory and antinociceptive activity (De Queiroz *et al*., 2010), in addition to the non-selective spasmolytic effect in guinea pigs (Bezerra et al., 2011).

However, plants used as medicines are xenobiotics, and like all foreign bodies, their biotransformation products are potentially toxic until proven otherwise (De Queiroz *et al*., 2010). In view of this fact, this work aimed at the phytochemical investigation and the toxicological evaluation of the crude ethanol extract from the leaves of *P. stipulacea*. Taking into account the medicinal use of a species, with safety, it is necessary that it be studied from a chemical, pharmacological and toxicological point of view.

2. Methodology

2.1 Botanic Material

The species *P. stipulacea* (BENTH.) Ducke were collected in Caruaru/PE, at the croft of Malhada de Pedra in the Instituto Agronômico de Pernambuco (IPA) on 02/03/2018. An exsiccate was prepared and deposited in the Herbarium Geraldo Mariz at the Universidade Federal de Pernambuco, Centro de Biociências/ Botanic Department, with the voucher specimen number 32859.

2.2 Extract Preparation

The leaves of *P. stipulaceae* were weighed (50 g), reduced to a smaller size in a knife mill, and there was added ethyl alcohol P.A. (500 mL), leaving the mixture under extraction for 72h. After filtration and rotary evaporator concentration, a viscous material in 36.86% yield was obtained totaling 18.43g of crude extract.

2.3 Ethical Procedures

This experiment was carried out in accordance with the rules in Brazil, especially the Law 9605 - Art. 32 and decree 3,179 - Art 17 of 09/21/1999, which deals with the issue of the use of animals for scientific purposes. The project was approved by the Ethics Committee on the Use of Animals of the Federal University of Pernambuco (CEUA / UFPE) under protocol N^0 0002/2018.

2.4 Statistical Analysis

The experimental data were assessed statistically through the variance analysis (ANOVA) followed by the tests T Student e Tukey to compare averages using the software Graph Pad prism. 5.0. At where $P < 0,05$ was considered as statistically significant.

2.5 Osmotic Fragility

The osmotic fragility technique was performed according to the methodology described by Darcie and Lewis (1975). Laborclin® sheep blood samples (25µL) were exposed to the extract for 30 minutes at room temperature in different concentrations (50µg/mL, 100µg/mL, 250µg/mL, 750µg/mL e 1000µg/mL) diluted in sodium chloride solution and centrifuged at 2500 rpm for 3 minutes at 25^0C. A negative control solution of 0.9% sodium chloride and as a positive control distilled water was used as the control. The assay was performed in triplicate and the hemolytic percentage was established according to the positive control, which was designated as 100%. The supernatant was then read on the Bioplus spectrophotometer at 540nm wavelength to obtain the resulting absorbance.

2.6 Mutagenicity by *Allium cepa*

The mutagenicity analyzes of the ethanolic extract of *P. stipulacea* leaves were carried out using the *Allium cepa* test. The specimens of *A. cepa* used were of small size (with a mean weight ≈ 63.7 grams), the same origin, not germinated and healthy, acquired at the Center of Supply and Logistics of Pernambuco (CEASA / PE). The bulbs were put to germinate for 7 days at room temperature (≈ 25 ° C) in appropriate bottles, with the bottom dipped in solution containing 50 mL of distilled water (control group) and the remaining ones containing 50 mL of water distilled with the extract at the following concentrations: 50µg/mL, 500µg/mL and 1000µg/mL. On the seventh day the roots were collected, measured, washed in distilled water, hydrolyzed in HCl (1mol / mL) for 10 minutes and then the meristems were washed, then smears were made on glass slides which after dried (30 minutes) were stained with hematoxylin (Guerra & Souza, 2002). 1000 cells were analyzed by treatment by light microscopy. To analyze the toxic effects were measured the lengths of the roots, summed and performed the simple average. For the cytotoxic effects, the mitotic indices (MI) of each treatment were verified, where the cells were added at any stage of division (prophase, metaphase, anaphase and telophase), divided by total cells counted and multiplying by 100 (Sturbelle *et al*., 2010). According to the observation of any chromosomal aberration, the genotoxic effect was verified. The mutagenic effect was recorded through the occurrence of micronuclei.

2.7 Acute Toxicity

The methodology recommended by the Organization for Economic Cooperation and Development (OECD, 423) was used to evaluate the acute toxicity of the ethanolic extract of *P. stipulaceae* leaves. The test consisted of administering the 2000 mg/kg dose of the extract under study. Albino mice of the *Swiss* strain *Mus musculus* were used. Being divided into three groups each containing three animals (females), totaling nine animals for the experiment. The procedure was performed in duplicate. If there is no animal death, the extract will not be considered toxic. The ethanolic extract from the leaves of *P. stipulaceae* was solubilised in saline solution and one group received only the vehicle. After oral administration (gavage), the animals were observed continuously for two hours and then every 24 hours for 14 days for behavioral verification and their physiological functions. The animals were evaluated by the Hippocratic screening method, which verifies any impairment of their state of consciousness, disposition, activity and coordination of the motor system and muscle tone, activity of the central and autonomic nervous system. Consumption of water, feed and body weight (liver, kidney and spleen) were recorded. At the end of the experiments, all animals were anesthetized with xylazine-ketamine solution and euthanized by cervical dislocation according to “the ethical principles of animal experimentation” proposed by the Brazilian Society of Laboratory Animal Science (SBCAL / COBEA). So the abdominal and thoracic cavities were opened and all macroscopic changes have been registered. Then kidneys, liver and spleen were collected for determination of their indexes.

The organ index was calculated following the formula:

Index = organ weight (mg) / animal weight (g).

2.7.1 Assessment of Ponderal Evolution and Water and Feed Consumption

Following treatment with EEtFP.s. For the evaluation of possible effects toxic animals were weighed at the beginning and end of the experiment and evaluated daily consumption of water and feed.

2.7.2 Evaluation of Hematological and Biochemical Parameters

At the end of the experiments, after the animals were anesthetized with solution xylazine (90mg / kg) - ketamine (9mg / kg) blood samples were collected by cardiac puncture. for hematological analyzes, blood collected in a tube with EDTA and

evaluation of the red and white series. For the analysis of biochemical parameters (urea, creatinine, glutamic oxalacetic transaminase - TGO and glutamic-pyruvic transaminase - TGP) the blood was submitted to centrifugation to obtain plasma. Hematological and biochemical parameters were determined using specific kits and by analyzers automated.

2.7.3 Evaluation of Organ Indexes

After blood collection all animals were euthanized by cervical dislocation, and the organs (kidneys and liver) removed, weighed and analyzed macroscopically for the investigation of discoloration, bleeding, among others.

Organ index was calculated as:

Index = organ weight (mg) / animal weight (g).

2.7.4 Histological Evaluation

After weighing the organs, kidneys, liver and spleen were sectioned, fixed in formalin (10% formaldehyde solution) and after 24 hours histopathological processing. They went through dehydration with increasing series alcohol (70% - 100%), xylene diaphanization, impregnation and paraffin embedding. The enclosed inclusions were sectioned (3µm thick) in microtome and hematoxylin-eosin staining and observed under light microscopy (Junqueira & Carneiro, 2008).

3. Results and Discussion

3.1 Osmotic Fragility

For the osmotic fragility assay with sheep blood, the ethanolic extract of *P. stipulaceae* leaves was tested at different concentrations (50 µg/mL, 100 µg/mL, 250 µg/mL, 750 µg/mL e 1000 µg/mL) and showed no significant toxicity. There was no significant hemolysis when evaluated qualitatively by the reddish tint in the supernatant obtained after the centrifugation, with the saline solution remaining clear, characterizing the integrity of the erythrocytes that remained at the bottom of the tubes with formation of a precipitate without having undergone cellular lysis. The percentage of hemolysis was based on the following equation and the result is described in Table 1.

% Hemolysis=Ab ×100%1,32

Table 1: Percentage of hemolysis according to absorbance readings at 540nm wavelength.

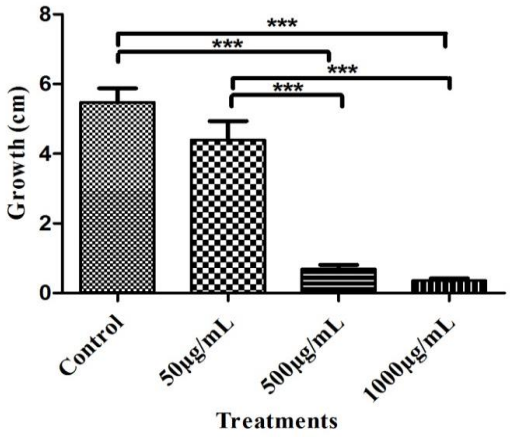
Tested Concentration	% Hemolysis
50 µg/mL	10,73%
100 µg/mL	10,46%
250 µg/mL	10,77%
750 µg/mL	10,77%
1000 µg/mL	13,14%

Source: Authors.

3.2 Mutagenicity by *Allium cepa*

The results of the test are set forth in the figures and table below. It can be seen in Figure 1, when evaluating the root length, there was a significant reduction in growth when compared to the control (distilled water). The antiproliferative activity of the ethanolic extract of the leaves of *P. stipulacea* was evidenced due to the inhibition of the cell division of *A. cepa* as the concentration of the extract increased.

Figure 1: Values of the root growth averages of *A. cepa* submitted to different concentrations of the ethanolic extract of *P. stipulaceae*. *** p<0.01.



Source: Authors.

The mitotic index (MI), presented in Table 2, corresponds to the ratio of the number of cells in division and total number of cells observed, in percentage. For cytotoxicity results, statistical analysis did not indicate any change in mitotic activity. The mitotic index did not present a significant difference between the percentages of treatments: control (18.3%) and ethanolic extract of leaves of *P. stipulaceae* at concentrations of 50 µg / mL (11.9%), 500 µg / mL (14, 8%) and 1000µg / ml (14.1%).

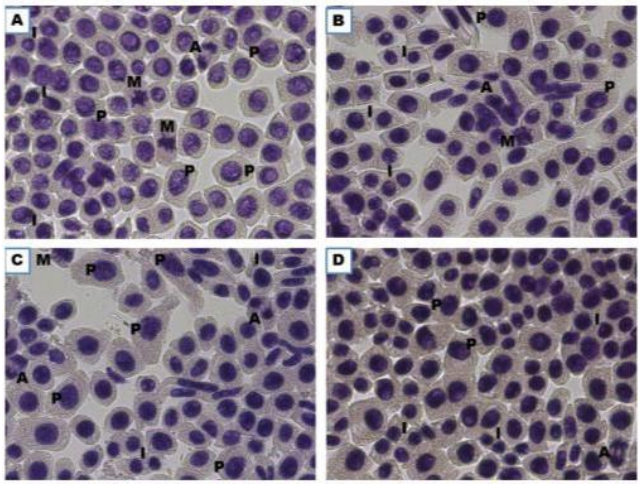
Table 2: Mitotic index of the roots of *A. cepa* submitted to the ethanolic extract of *P. stipulaceae* leaves at different concentrations.

Treatment	Nº total of cells	Nº of cells in interface	Nº of cells in division	Mitotic Index (%)
Control	1.194	1011	183	18,3%
Extract 50 µg/mL	1.104	985	119	11,9%
Extract 500 µg/mL	1.024	876	148	14,8%
Extract1000 µg/mL	1.078	937	141	14,1%

Source: Authors.

However, the morphological observations of *A. cepa* rootlets treated at the different concentrations (50µg/mL, 500µg/mL and 1000µg/mL) with ethanolic extract of the leaves of *P. stipulacea*, when compared to the control (distilled water), showed that the phases of cell division (prophase, metaphase, anaphase, telophase and interphase) in the *A. cepa* radicular showed no morphological alterations, such as chromosomal bridges and micronucleated cells. As shown in Figure 2.

Figure 2: Photomicrography of *A. cepa* rootlets at different stages of cell division. Negative control (A), submitted to the aqueous extract of leaves of *P. stipulaceae* at concentrations of 50 µg / ml (B), 500 µg / ml (C) and 1000 µg / ml (D). P-Prophase; M-Metaphase; A-Anaphase; T-Telophase; I- Interface. 10X objective. Increase – 1000X.



Source: Authors.

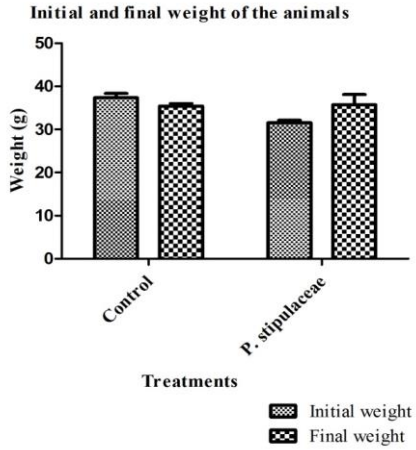
3.3 Acute Toxicity

In the present study, no signs of toxicity were observed for the ethanolic extract of the leaves of *P. stipulaceae*, since there was no mortality in the acute toxicity test according to protocol OECD 423. The alterations related to the Hippocratic screening were present until 2h after administration of the tested extract and were: agitation, irritability, stereotyped movements, tachycardia. The LD 50 estimate was higher than 2000 mg/kg and was considered to be of low toxicity according to the class method recommended by OECD 423 (OECD, 2001). The next dose to be tested would be 5000 mg / kg; however, the study of this dosage is only recommended in exceptional cases that justify its necessity.

3.3.1 Assessment of Ponderal Evolution and Water and Feed Consumption

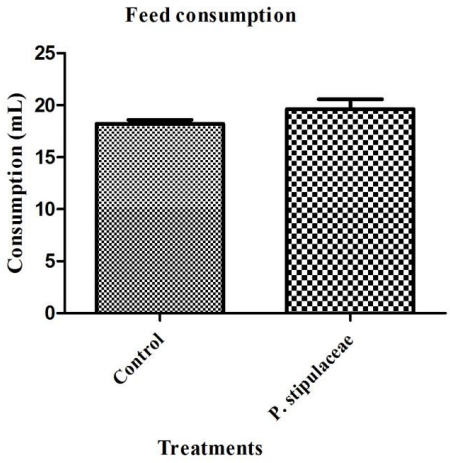
At the end of fourteen (14) days, the organs (kidneys and liver) were removed and weighed, and macroscopically analyzed for color, consistency, presence of petechiae or bleeding points. Not being observed any sign of amendment. There was no difference between the weight evolution of the animals (Figure 3) as for feed intake (Figure 4), for water consumption (Figure 5) there was a decrease in intake by the group treated with EEtFP.s.

Figure 3: Initial and final weight of the control group mice and the group treated with ethanolic extract of leaves of P. stipulaceae at the dose of 2000mg / kg.



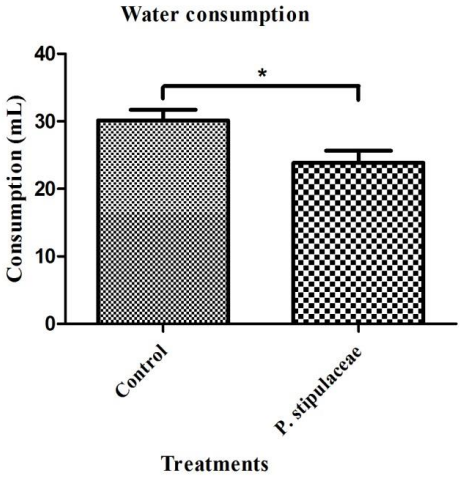
Data presented as mean ± standard error of the mean analyzed by ANOVA followed by Tukey. P < 0.05 compared to the control group. Source: Authors.

Figure 4: Feed consumption for the group of mice in the control group and the group treated with ethanolic extract of leaves of *P. stipulaceae* at the dose of 2000mg / kg.



Data presented as mean ± standard error of the mean analyzed by ANOVA followed by Tukey. P < 0.05 compared to the control group. Source: Authors.

Figure 5: Water consumption for the group of mice in the control group and the group treated with ethanolic extract of leaves of *P. stipulaceae* at the dose of 2000mg / kg. * p<0.05.



Data presented as mean ± standard error of the mean analyzed by ANOVA followed by Tukey. P < 0.05 compared to the control group. Source: Authors.

3.3.2 Evaluation of Hematological and Biochemical Parameters

The analysis of hematological parameters did not reveal significant alteration in relation to the red and white series, as we can see in Table 3. Investigation into the toxicological effects of EEtFP.s. through the analyzes the biochemical findings revealed no significant changes in the enzymatic activity of TGO (glutamic-oxalacetic transaminase) and TGP (glutamic-pyruvic transaminase) of animals of the control group and those submitted to a dose of 2000mg / kg EetFP.s. Still regarding the biochemical parameters, in the evaluation of renal function, it was possible to observe a significant increase in the serum urea concentration of EEtFP.s-treated mice. At a dose of 2000mg/kg (57.4 ± 7.33) compared to the control group (49.4 ± 9.73). No significant changes were observed in the serum concentration of creatinine at the tested dose of EetFP.s.

Table 3. Hematological parameters of mice submitted to EEtFP.s. at the dose of 2000mg/kg.

PARAMETERS	NORMAL PARAMETERS	CONTROL	EEtFP.s.
Hemácias (10^6/mm^3)	9,0 – 11,3	9,52 ± 0,506	9,82 ± 0,624
Hemoglobina (g/dL)	13,5 – 17,0	14,76 ± 1,230	15,86 ± 0,557
Hematócrito (%)	45 – 55	44,93 ± 3,700	49,7 ± 2,334
VCM (fm^3)	47 – 55	47 ± 3,286	50,66 ± 1,211
HCM (pg)	13 – 16	32,88 ± 0,746	16,15 ± 0,677
CHCM (g/dL)	29 – 34	32,88 ± 0,746	31,9 ±0,672
Leucócitos Totais (10^3/mm^3)	2 – 10	11,03 ± 1,979	6,86 ± 3,204

Data presented as mean ± standard error of the mean analyzed by ANOVA followed by Tukey. P < 0.05 compared to the control group. * Normal parameters (Source: Gad, 2007). Source: Authors.

Table 4: Biochemical values of control and treated groups with EEtFP.s. at a dose of 2000mg /kg.

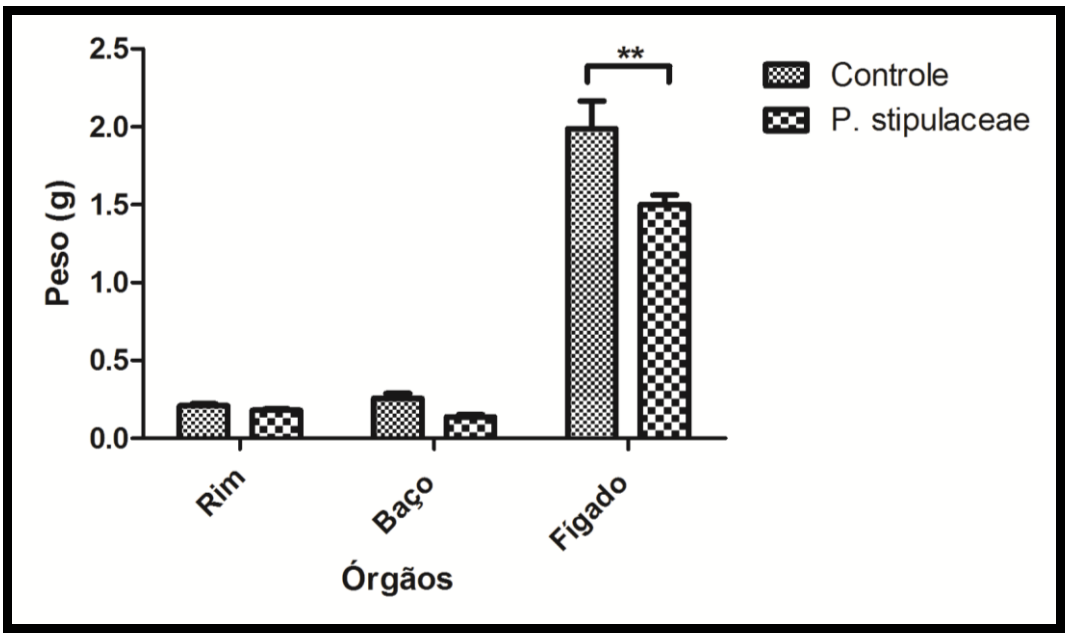
PARAMETERS	NORMAL PARAMETERS	CONTROL	EEtFP.s.
Ureia (mg/dL)	15 – 40	49,4±9,73	57,4±7,33
Creatinina (mg/dL)	0,2 – 0,6	0,41±0,03	0,38±0,06
TGO (U/L)	70 – 400	184,8±63,3	186,4±24,6
TGP (U/L)	25 – 100	45,5±9,50	46,2±23,6

* p <0.05. After two-way analysis of variance (ANOVA) followed by Bonferroni with a 95% confidence interval when compared to the control group. * Parameters normal. Source: Gad (2007).

3.3.3. Evaluation of Organ Indexes

Regarding organ indexes, no changes kidneys in mice treated with EEtFP.s. When compared to the control group, as shown in Figure 6. For the index of the liver, a significant decrease was observed.

Figure 6: Organ index for animals submitted to EEtFP.s. at a dose of 2000mg/kg and group control.

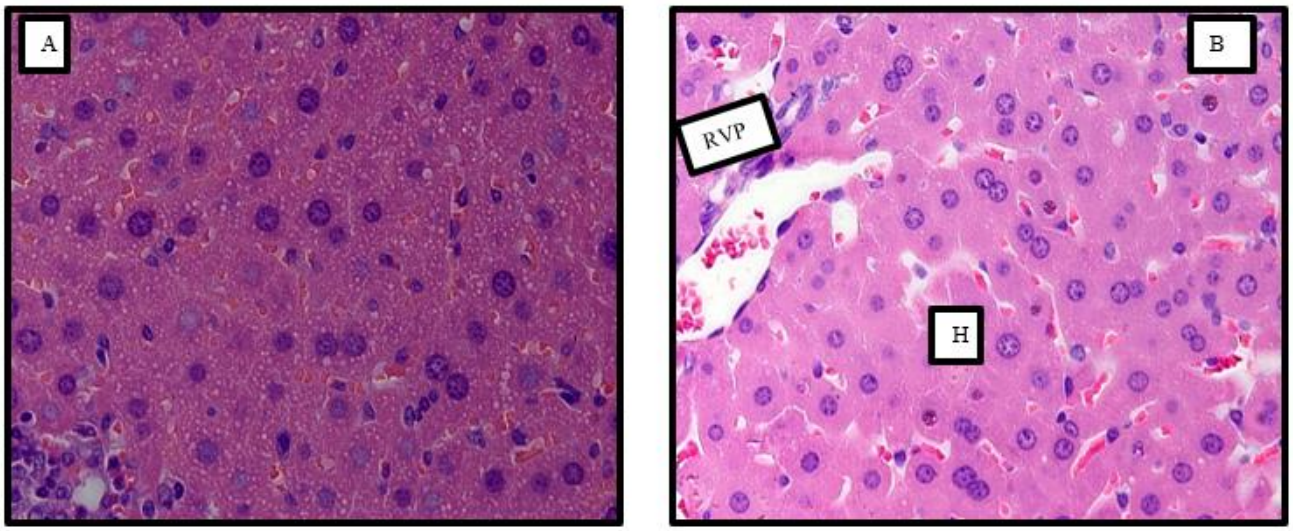


Data presented as mean ± standard error of the mean analyzed by ANOVA followed by Tukey. P < 0.05 compared to the control group. Source: Authors.

3.3.4 Histological evaluation

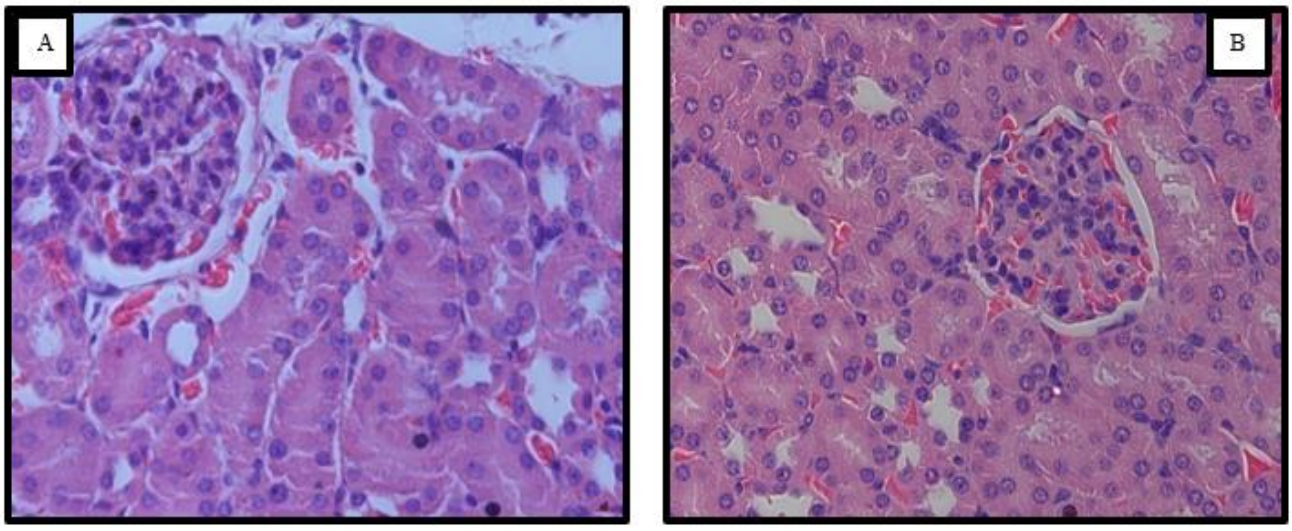
Hepatic and renal histology of animals treated with EEtFP.s. didn't reveal signific ant changes, as we can see in figures 7 and 8 respectively. In liver samples we can observe that the histological section stained with hematoxylin-eosin presents preserved tissue architecture, there is presence of portal vein, hepatocytes with visible cytoplasm and rounded nucleus organized in rows delimited by the sinusoids. In kidney slides, it is found that it is a conventional histological preparation of the renal cortex showing proximal contorted tubules (bordered cuboid cells brush), distal (smaller), and a renal corpuscle with a well-defined vascular preserved. Presence of interlobular vessel and cells with preserved architecture

Figure 7: Photomicrograph depicting liver histology of mice submitted to treatment with EEtFP.s. at a dose of 2000 mg/kg.



A - Control Group; B - Treaty group; H - Hepatocyte; RVP - Vein Branch Door. Hematoxylin-eosin (400X).

Figure 8: Photomicrograph depicting the histology of mouse kidneys submitted to treatment with EEtFP.s. at a dose of 2000 mg kg.



A - Control Group; B - Treaty group; CR - Renal Corpuscle; TCP - Proximal Contorted Tubule; DT - Distal Tubule. Source: Authors.

Whereas *P. stipulaceae* is popularly used for its potential antimicrobial, analgesic, anti-inflammatory, it is of paramount importance to assessment of its toxicity for the safety of the population using it. The hemolytic action of the different toxic compounds is attributed to several non-specific mechanisms.

For example, surfactant compounds, which 50 produce their hemolytic effect through plasma membrane solubilization erythrocyte, or by osmotic lysis, which promotes changes in the permeability of the red blood cell membrane. In contrast, xenobiotic compounds Phenolic compounds are able to promote hemolysis through oxidation of hemoglobin, forming metahemoglobulin.

The proposed study did not show hemolytic activity at the concentrations tested. However, such results do not exclude the existence of toxicity, since more concentrated samples of the extract have not been tested and pharmacological studies

about *P. stipulaceae* are scarce in the literature. The data obtained with the osmotic fragility test in this work indicated that EEtFP.s. showed no change in membrane integrity at concentrations of NaCl close to the physiological level. Similar studies have found changes in the osmotic profile in erythrocytes when incubated with plant extracts (Fechine *et al*., 2020).

It is therefore affirmed that erythrocytes incubated with natural products may suffer a disturbance in its structure and cytoskeleton caused by alteration of the membrane partition coefficient of these cells (Didelon *et al*., 2000). In recent years, the Allium cepa test has been used by its numerous advantages (reliability, cost, among others). It is a test that allows both macroscopic analysis (change in color, shape and size of the roots) microscopic (mitotic index and the occurrence of abnormalities and aberrations chromosomes (Da Silva *et al*., 2018).

The results of the cytotoxic and mutagenic capacity of EEtFP.s. rated by A. strain test by inhibiting root growth, size and nuclear form showed dose-dependent cytotoxic behavior at concentrations tested. Cytotoxicity was demonstrated by inhibition of cell division in *A. cepa* meristems, as the concentrations used increase. THE evaluation of mutagenicity obtained by observing micronuclei in cells exposed to different concentrations were low considering that it was not The presence of micronuclei was observed at the tested concentrations. So in this In this study cytotoxicity was defined by inhibition of root growth in most concentration tested (González-de-Peredo *et al*., 2021).

Subsequently, in order to evaluate the toxicity of EEtFP.s. in vivo, it was The acute toxicity test was performed according to the OECD protocol. 423. The results obtained make it clear that there was a low toxicity in the conditions evaluated. What can be confirmed by analyzing the parameters evaluated during these 14 days of observation. Significant decrease related to water consumption was observed for the group treated with EEtFP.s., however, this fact did not cause changes in the evolution weight of animals. Metabolic parameters such as weight evolution and consumption of water and are used to investigate the toxicity of a study sample on the gastrointestinal system or even the central nervous system if on more than one of the parameters in question. One of the organs of great importance to the human body is the liver, since its function is related to nutrient metabolism and biotransformation of drugs and chemicals, which gives the body protection against agents toxic substances (Al-Asmari *et al*., 2014).

Enzymes can be found in every tissue of the body and they are the responsible for several important chemical reactions in our body. Some reactions may occur in plasma and others in serum and their serum measurement may indicate a state of normality or cellular damage. For function evaluation two enzymes are of great importance, glutamicoxalacetic transaminase (TGO) and Glutamic-Pyruvic Transaminase (TGP). The action of these enzymes has been used as an indicator of hepatocellular damage (De Sousa *et al*., 2014).

TGO catalyzes the conversion of aspartate to oxaloacetate, is found in various organs and tissues including the liver, heart (myocardium), muscle among others. It is present in the cytoplasm and also in mitochondria, therefore its elevation indicates deeper cell damage. TGP catalyzes the conversion of alanine to pyruvic acid, is found in highest 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 (Mincis, 2006).

No significant change in TGO or TGP activity was observed in the animals exposed to EEtFP.s., demonstrating that it is free of toxicity under the conditions tested. Drug toxicity resulting from inadequate excretion of medicines or their metabolites may lead to a change in kidney function (De Sousa *et al*., 2014).

Drug toxicity resulting from inadequate excretion of medicines or their metabolites may lead to a change in kidney function. Of this Thus, the evaluation of blood urea and creatinine levels is an excellent indicator of renal toxicity. Particularly the accumulation of these substances nitrogen in the blood may indicate kidney failure (Henry, 2008).

Urea, the end product of protein metabolism, is excreted by the kidneys. The renal tubules reabsorb 40% of this product, so the blood levels of this product parameter are an indication of renal function and may serve as an index of glomerular filtration rate (SCHOSSLER *et al*. 2001), theoretically creatinine is best for renal function, as the amount of creatinine present in the kidneys is more constant but is not reabsorbed in the renal tubules as urea (Emanuelli *et al*., 2008).

Animals treated with EEtFP.s. presented a significant increase in the serum urea concentration, which may be indicative of decreased blood urea glomerular filtration. However, some factors not associated with renal dysfunction, such as gastrointestinal bleeding, corticosteroid therapy and high protein diet may increase urea production (Klein *et al*., 2008).

No significant clinical findings were observed in liver histopathology, not evidencing, therefore, tissue toxicity of the tested extract. In the histopathological analysis of the kidneys of animals submitted to EetFP.s. no significant changes were observed either, despite the increase in serum urea levels. This suggests that this increase caused minor damage and not was able to alter the structure of the tissue. For toxicity studies the analysis of hematological parameters is very important because the hematopoietic system is very sensitive to agent activity. toxic. Hematological analyzes are performed with direction to the erythrocytes (erythrocytes), leukocytes and thrombocytes. Exposure to EEtFP.s. led to a significant decrease in the following parameters: HCM and leukocyte number. As was observed elevation of the hematocrit and MCV, but both results are within the range of normal parameters, which does not demonstrate clinical significance. Organ indexes are important pathophysiological indicators related or not to injury caused by any substance in animals and humans (Vaghasiya; Shukla & Chanda, 2011).

4. Conclusion

The analysis of the organ index showedthat there was a significant decrease in liver index in the group treated with EEtFP.s. compared to the control group. But there was no enzymatic change specific for this organ and no histopathological findings that corroborate this result, which demonstrates absence of clinical significance. This study therefore exposes relevant information about the toxicity of the Which indicates the need for further preclinical studies in view of the scarcity of information in the literature about the studied plant.

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