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Antioxidant and Hepato-Protective Effects of *Palicourea coriacea* (Cham.) K. Schum Leaves Against Renovascular Hypertension in Rats

Efeitos Antioxidantes e Hepato-Protetores de Folhas de *Palicourea coriacea* (Cham.) K. Schum Contra Hipertensão Renovascular em Ratos

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Abstract

"Douradinha" is a plant indicated by herbalists from Campo Grande – MS for heart rhythm disorders, however it is necessary to evaluate the protective effect of *Palicourea coriacea* (Cham.) K. Schum leaves on the liver of rats in a model of renovascular hypertension and relate it to the chemical constituents and the antioxidant potential. Part of the leaves obtained at "Mercadão Central" of MS were used to obtain the aqueous and ethanolic extracts. Parts were used as indicated by the herbalists with the animals (26) randomly assigned in four groups: G1 (6) without treatment; G2 (7) with treatment; G3: Renovascular hypertension model (6) without treatment; G4: Renovascular hypertension model (7) with treatment. The treatment received a daily dose (20 mg / mL) of the plant leaves infusion by gavage. After 14 days, systolic blood pressure measurement was performed on the animal's tail by plethysmography and, following euthanasia, surgical procedure was performed to collect liver samples and peripheral blood. Hypotensive effect found during treatment was insufficient to reduce systemic blood pressure to normal levels for rats (MAP 100 mmHg). In the hypertensive group treated, the liver function enzymes were normal which may be related to the plant's antioxidant potential, attributed to polyphenols and alkaloids identified as the major constituents. It is concluded that *P. coriacea* is rich in polyphenols with antioxidant activity and the leaves concentration used was insufficient to reduce the systemic arterial pressure to normal levels, besides the infusion possibly has hepatoprotective and antioxidant effects.
Keywords: Savanna. Rubiaceae. Douradinha. Flavonoids. Phytotherapy.

Resumo

A douradinha é uma planta indicada pelos raizeiros de Campo Grande – MS para distúrbios do ritmo cardíaco, porém é necessário avaliar o efeito protetor das folhas de Palicourea coriacea (Cham.) K. Schum no fígado de ratos em um modelo de hipertensão renovascular e relaciona-o aos constituintes químicos e o potencial antioxidante. Parte das folhas obtidas no Mercadão Central de MS foram utilizadas para obter os extratos aquosos e etanólico. Parte foram utilizadas conforme indicação dos raizeiros com os animais (26), distribuídos aleatoriamente em quatro grupos: G1 (6) sem tratamento; G2 (7) com tratamento; G3: Modelo de hipertensão renovascular (6) sem tratamento; G4: Modelo de hipertensão renovascular (7) com tratamento. O tratamento recebeu dose diária (20 mg/mL) da infusão das folhas por gavagem. Após 14 dias, a medida da pressão arterial sistólica foi realizada na cauda do animal por pletismografia e, após a eutanásia, foi realizado procedimento cirúrgico para coleta de amostras de fígado e sangue periférico. O efeito hipotensivo encontrado durante o tratamento foi insuficiente para reduzir a pressão arterial sistêmica para níveis normais em ratos (PAM 100 mmHg). No grupo de hipertensos tratados, as enzimas da função hepática estavam normais e podem estar relacionadas ao potencial antioxidante da planta, atribuído aos polifenóis e alcaloides identificados como os principais constituintes. Conclui-se que P. coriacea é rica em polifenóis com atividade antioxidante e a concentração foliar utilizada foi insuficiente para reduzir a pressão arterial sistêmica para níveis normais, além da infusão possivelmente ter efeito hepatoprotetor e antioxidante.
Palavras-chave: Cerrado. Rubiaceae. Douradinha. Flavonoides. Fitoterapia.

1 Introduction

In Brazil, there are about 31.3 million individuals aged 18 years or more who receive a diagnosis of systemic arterial hypertension - SAH (Brasil, 2013) and, over the years, it is observed that heart and vascular diseases are the first cause of death in the country, representing 34% of the total number of deaths (Amodeu et al., 2016). SAH brings limitations to the patient, both in his or her labor activities and in his or her daily basic activities, which certainly reflects in the socio-economic scope of the affected person and his or her family members due to the high cost of treatment, which is largely subsidized by the Brazilian Unified Health System (SUS) (Brasil, 2006). The reflexes resulting from cardiovascular diseases are perceived in hospital admissions, their aggravations, sequels and their morbidity and mortality. These factors are cited by several studies and in vitro and in vivo studies in the search for new drugs aimed at the treatment and even the prevention of these diseases. Thus, there is the need for initial research in animals from SAH induction and its treatment (Siqueira; Siqueira-Filho; Land, 2017).

The Brazilian population also seeks alternative and complementary treatments such as the use of medicinal plants, which in some regions are the first therapeutic resources

used for health care (Brasil, 2006). In Mato Grosso do Sul State, it is common to use Cerrado (savanna biome) plants for the treatment of countless pathologies, including patients with heart rhythm disorders, among these species there is *Palicourea coriacea* (Cham.) K. Schum, of the Rubiaceae family, popularly known among others as douradinha, erva-de-rato-grande and congonha-do-campo and it is also used by popular medicine in the therapy of renal diseases acting as diuretics (Ustulin et al., 2009). The plant is a shrub of occurrence in the different phyto physiognomies of the Brazilian Cerrado, whose dry leaves are commercialized by "raizeiros" (plant collectors) at wet markets as the Mercado Municipal Adriano Valente, in Campo Grande City, capital of the State of Mato Grosso do Sul (Nunes et al., 2003; Ustulin et al., 2009).

It is evident and relevant that the problem of arterial hypertension be addressed as a public health issue and, thus, that the use of the population of undoubtedly efficient medicinal plants should be promoted as complementary treatment to diseases, but studies on the use of *P. coriacea* leaves are necessary in hypertensive animal models, to validate the therapeutic use of it and thus, to comply with the current legislation (Brasil, 2006), as a possible alternative to the SAH treatment.

The National Policy of Medicinal and Phytotherapeutic plants, approved by the Ministry of Health in 2006 (Brasil, 2006), has as priority objective the development of actions aimed at guaranteeing safe access and rational use of medicinal and phytotherapeutic plants to the development of technologies and the strengthening of productive chains and arrangements to the sustainable use of Brazilian biodiversity and the health complex development (Brasil, 2006). Considering that this Policy points out that, to guarantee the safety and efficacy of medicinal and phytotherapeutic plants, there is a need for validation of the therapeutic effects attributed to a plant, the essays with experimental models are used as subsidies for therapeutic validation in order to corroborate treatments used empirically by the population.

The objective of this study was to evaluate the antioxidant potential and protective effect of *P. coriacea* leaves in the liver of rats in renovascular hypertension and to relate the findings to the chemical constituents of the plant leaf, studying the hypotensive effect of *P. coriacea* leaves in a renovascular hypertension model in rats.

2 Material and Methods

2.1 Plant material

The dry, whole, and intact leaves of *P. coriacea* were obtained from the same batch of three stalls of "raizeiros", at the wet market "Mercado Municipal Antônio Valente", Campo Grande-MS, Brazil. The samples were catalogued, and the information contained in the labels recorded in spreadsheets. In sequence the samples were homogenized, the leaves were weighed, crushed, sieved (2.8 mm) and the powder (1000 g) stored.

2.2 Preparation of *P. coriacea* extracts and chemical analysis

Extraction was performed with ultrapure water, with zero electrical conductivity µS/cm from 400g of leaf powder. Ultrasound bath (Unidque®, 1450) was used for 60 minutes, followed by static maceration, with room temperature from 27 to 35 °C. Daily, the solution was filtered, and the solvent evaporated in rotary evaporator (Tecnal®, MA120) under reduced pressure at 45 ± 5 ºC, the procedure was repeated for seven days. The extracts were collected and dried in desiccator at reduced pressure, obtaining crude aqueous extract (ExH_2O). The same procedure was used to obtain the ethanol extract (ExEtOH), starting from 400 g of dried, ground, and sieved leaves. Extraction occurred for 10 days (Matias et al., 2020).

Part of the crude extract ExH_2O and ExEtOH were submitted to wet phytochemical analysis to detect phenolic compounds (precipitation reactions: 2% ferric chloride; 10% lead acetate and 4% copper acetate), tannins (precipitation reactions: iron salts and protein precipitation), flavonoids (cyanidin and sulfuric acid reaction), anthocyanines, anthocyanidins and flavonoids (presence of pH 2-3, 7, 8-9 and 11 staining), flavones, flavanols, flavonoids and Xanthones (pH 11), chalcones and aurones (pH 2-3 and pH 11), coumarins (ultraviolet light observation), anthraquinones (acid/base reaction), triplets and steroids (Liebermann-Burchard reaction), cardiotonic heterosides (Keller-Killiliani and Pesez test), heterocyanogenic heterosides (sulfuric acid reaction and picrosodic paper) (presence of foam and reaction of Liebermann-Burchard), foam index, iridoids (antimony chloride and anisaldehyde-H_2SO_4) and reducing sugars by the reaction of Benedict (Matos, 2009; Simões et al., 2017).

The analysis of the results was carried out based on the observation of intensity and color and/or precipitation, which is indicative of the high concentration of one of the secondary metabolite classes in the ExEtOH extract. As a standard, for samples with intense color and/or precipitation, it was called strongly positive (+++), followed by moderately positive (++), weakly positive (+), partially positive (± = with only turbidity and/or partial color change) the absence of color and/or precipitation as negative (-) (Fontoura et al., 2015).

The major chemical groups of the extracts ExH_2O and ExEtOH were confirmed by the analysis in the spectrum in the UV-visible region (Femto®, 800XI), with the wavelength determination of maximum absorbance, in the range from 200 to 800 nm, using water and ethanol as blank, the spectra were compared with the literature data (Piironen; Toivo; Lampi, 2000; Jurasekova et al., 2006; Kasal; Budesinsky; Griffiths, 2010; Silverstein et al., 2014) and confirmatory analyses were developed with three repetitions.

2.3 Dosing total phenols, flavonoids, condensed tannins and alkaloids

ExH_2O was submitted to the determination of total phenols (FT) by the Folin-Ciocalteu method, using gallic acid as standard (10 to 350 mg/mL) (Sousa et al., 2007). As a standard, gallic acid was used (Vetec®, 99%) at concentrations of 10, 50, 100, 150, 300 and 400 µg/mL to construct the calibration curve (y = 0.1326 x 0.0045, R^2 = 0.9978). The absorbance reading of the sample reaction mixture, standard and blank, occurred at 760 nm in spectrophotometer (Femto, 800XI). All reagents except the sample were prepared as blank. The analyses were developed in triplicate and the polyphenols content was determined in milligram of gallic acid/g of extract.

Flavonoid contents were determined by the aluminum chloride method (99.5%, Vetec) and, as standard, quercetin (≥ 95% Sigma), concentrations of 6.0, 8.0, 10.0, 12.0, 16.0 and 20.0 µg/mL, to construct the calibration curve (y = 0.656 x - 0.0075, R^2 = 0.9949) (Sobrinho et al., 2008).

For the determination of condensed tannins, one mg of each extract was mixed in a hydro ethanol solution (MeOH: H_2O 80:20 v:v) and added 5 mL of acid vanillin (8% of concentrated aqueous HCl and 4% of vanillin in methanol). Methanol and the standard curve with catechin were used as blank. The mixtures were incubated in a water bath for 20 minutes, at room temperature reading at 510 nm (Broadhurst; Jones, 1978). The results were expressed as catechin equivalents in mg per 100 g of extract.

The quantification of the total alkaloids extracts was developed using 40 mL of each extract separately in the concentration and 1000 µg/mL. The extracts were acidified to pH between 2.0 - 2.5 with 1 mol/L HCl and 4 mL of Dragendorff's reagent and centrifuged at 2400 rpm 30 $minutes^{-1}$. The supernatant was discarded, and the residue was treated with a solution containing 1 mL of ethyl alcohol; 2 mL of sodium sulfite (1%) and centrifuged again (2400 rpm 30^{-1} minutes). The supernatant was discarded, and the residue treated with concentrated nitric acid (2 mL). The solution was transferred to a 50 mL volumetric flask and the volume was filled with distilled water. From this solution, an aliquot (1 mL) was used and added in 5 mL of thiourea at 3% (w/v), homogenized and the reading in a spectrophotometer at 435 nm. Nitric acid and thiourea solution were used as white and as a standard berberine linearity was obtained between 40 and 200 µg/mL. The alkaloids content was expressed in mg per 100 g of dry extract weight.

2.4 Antioxidant potential through free radicals sequestration method (DPPH)

The antioxidant activity potential was determined based on the free radical scavenger activity of 2,2-diphenyl-1-picril-hydrazil (DPPH). Part of the ExH_2O and ExEtOH extracts were diluted at concentrations of 250, 200, 150, 100, 50 and 25 µg/mL. These solutions received 2 mL of a DPPH solution in methanol (24 mg/100 mL of methanol). After 30 minutes, absorbance was determined in a UV-VIS spectrophotometer at a wavelength of 515 nm. For DPPH solution in methanol, BHT (butylhydroxytoluene) was used as a negative control in the same concentrations as those used in the samples (Thaipong et al., 2006).

$$\%AA = \left(\frac{A0-A}{A0}\right) x100$$

The percentage of antioxidant and residual DPPH activity in the reactional medium (%AA) was calculated by the following formula: where A0 is the absorbance of DPPH (negative control) and A is the absorbance of the sample with DPPH (Sousa et al., 2007). The EC_{50} determination (efficient concentration), i.e., the sample or the pattern concentration that causes 50% inhibition of the initial DPPH concentration, was obtained by linear regression.

2.5 Preparation of administered solution

The powder of *P. coriacea* leaves was submitted to infusion and the concentration of the solution was based on the popular use consisting of 5g of leaves powder for 250 mL of water (Nunes et al., 2003; Ustulin et al., 2009).

2.6 The animals

Male Wistar rats (*Rattus norvegicus* albinus) were used, weighing approximately 200-220 g, originated from the vivarium of Federal University of Mato Grosso do Sul, Campo Grande, MS, Brazil. The animals were kept under controlled conditions of luminosity (12 hours of light/12 hours of darkness) and temperature (mean of 23 °C), drinking water and feeding commercial ration Socil® ad libitum, at the vivarium of Anhanguera-Uniderp university. The cages hygiene was performed three times a week. The project was approved by the Committee on Ethics in Animal use (CEUA) of Anhanguera-Uniderp University under the number 3081.

The experimental design with the 26 rats was randomly organized into four groups: G1: SHAM (false surgery) (n=6), without treatment; G2: SHAM + treatment with *P. coriacea* infusion (n=7); G3: Renovascular hypertension model (2R-1C) (n = 6), without treatment; G4: Renovascular hypertension model (2R-1C) treatment with *P. coriacea* infusion (n=7).

To obtain rats with renal hypertension (2R-1C) (Goldblatt et al., 1934), the animals were anesthetized with Ketamine and Xylazine hydrochloride solution (70:30, dose of 0.1 mL/100g of animal, i.p.) to perform median laparotomy and exposure of the left renal artery pedicle, where the silver clip with an opening of 0.2 mm was implanted. SHAM animals were submitted to the same conditions, but without the placement of a silver clip in the renal artery (Schaffenburg, 1959). After surgery, a period of six weeks was expected for the correct induction of renovascular hypertension in the animals.

2.7 Systolic pressure measurement

Systolic blood pressure (SBP) was determined by the tail plethysmography technique. The mean of three measurements was used for each animal. For greater fidelity of the values obtained, the animals spent 5 days of adaptation, aiming at reducing stress and a possible change in the pressure value. SBP was measured only in groups 3 (SHAM with treatment) and 4 (2R-1C with treatment) before treatment was started and at the end of treatment. The animals in group 4 that presented systolic pressure greater than or equal to 160 mmHg, after six weeks, were used in the experiments.

2.8 Treatment

The treatment was performed by the gavage method. The concentration used was 20mg/kg of aqueous infusion of *P. coriacea* per animal, for 14 uninterrupted days, always at the same time.

At the end of the experimental period, euthanasia was performed by intraperitoneal administration of a lethal dose of the anesthetic pharmacologic association of Ketamine hydrochloride + Xylazine (70:30 - 0.4 mL/100 g of animal) (Massone, 2003), according to the Normative Resolution n° 37 of 2018 of CONCEA (Nacional Council of Animal Experimentation Control) (Brasil, 2018).

After euthanasia and blood collection by cardiac puncture, the animals were first perfused with 20 mL of saline solution (0.9%) and then with 20 mL of 10% buffered formalin, and then the liver was collected. After the procedures, the animal carcasses were frozen in freezer at -20ºC and disposed of in a container for wastes disposal located at Anhanguera-Uniderp University, Agrarian Campus.

2.9 Biochemical Analyses

Biochemical liver function analyzes were performed using commercial kits for Alanine aminotransferase (ALT) and aspartate aminotransferase (AST). Using the BioPlus 200® semi-automatic analyzer instrument (Bioplus Produtos para Laboratórios Ltda). The results of the biochemical measurements were statistically evaluated using the Student's "t" test or, for more than two comparisons, by ANOVA ($P < 0,05$) followed by Newman-Keuls post-test.

2.10 Histological analysis

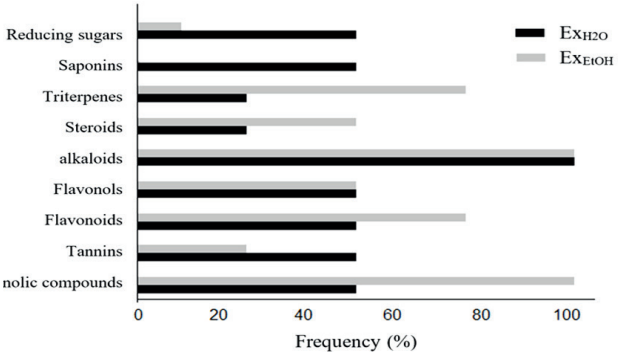
The liver cut-off samples of the studied groups were kept in buffered formalin 10% for 24 hours and subsequently processed by standard technique and included in histological paraffin. The histological slides were stained using the hematoxylin-eosin (H&E) technique. All slides were analyzed by light optical microscopy (Nikon Eclipse E-600, optical microscope, Tokyo, Japan). The images were obtained using a video microscopy system, connected to the microscope, and the captured images were transmitted to a computer equipped with the program Imagelab® for PC Version 6.1 (SOFT-LIT-170-9690-ILSPC-V-6-1 (Bio-Rad Laboratories, Inc.).

3 Results and Discussion

3.1 Phytochemical results

The phytochemical screening of ExH_2O (9 classes) and ExEtOH (8 classes) extracts of *P. coriacea* leaves indicated that the solvent extractor influenced the diversity and quantity of the phytoconstituents. The two extracts present the same classes of secondary metabolites, with the exception of saponins detected only in the ExH2O extract. (Figure 1).

Figure 1 - Frequency (%) of secondary metabolites found in aqueous extract (ExH_2O) and ethanolic extract (ExEtOH) of *P. coriacea* leaves, Campo Grande, Mato Grosso do Sul



Source: research data.

However, in ExEtOH the triterpenes, alkaloids, phenolic compounds, flavonoids and steroids were higher than those found in ExH_2O, whereas the tannins and reducing sugars were higher than ExEtOH, solvent with higher polarity (Figure 1).

The results of the phenolic and flavonoid compound contents of the extracts associated with the highest antioxidant activity of ExEtOH extract confirmed the phytochemical findings (Table 1). The predominance of phenols and flavonoids justifies the ability of these classes to act in the free radicals sequestration.

Table 1 - Phytochemical profile (UV visible), quantification of total phenols, flavonoids and antioxidant potential of the $ExEH_2O$ and ExtEtOH leaves of *P. coreacea* extracts, obtained at "Mercadão Central Adriano Valente", Campo Grande-MS, Brazil

Extrat.	Total phenols (EAG mg/g)	Flavonoids (Querct. mg/g)	Alkaloids (mg/g)	λ_{max} (nm)	IC_{50} (µ/mL)
Ex_{EH2O}	$590.6 \pm 1.6_b$	$495.0 \pm 3.3_b$	$78.5 \pm 3.8_b$	347; 372; 401	$85.3 \pm 5.8_b$
Ext_{EtOH}	$745.9 \pm 2.8_a$	$729.4 \pm 2.9_a$	$130.8 \pm 1.5_a$	326; 335; 368	$33.5 \pm 0.4_a$

Extrat: Extracts. Total phenols (mg of EAG/g ± SD). Flavonoids (mg of Quercetin/g ± SD). IC50 = EC50 (µg/mL) = Concentration sufficient to obtain 50% of the maximum capacity to sequester free radicals. *Mean ± SD. ($p < 0.01$). Similar letters do not differ statistically.
Source: research data.

When it comes to the genus *Palicourea*, phytochemical studies revealed the presence of triplets, phenolic acids, coumarins, cyclic peptide, indole monoterpene, alkaloids, and pseudoalkaloids. Whereas for *P. coriacea*, studies have shown that extracts of *P. coriacea* leaves have phenolic compounds, flavonoids, steroids, saponins, tannins, triterpenes (ursolic acid), anthraquinones (2-hydroxy-3-methylantraccinone) and sugars (Cragg; Newman; Yang, 2006; Düsman et al., 2004; Formagio et al., 2019; Nascimento et al., 2006; Rosa et al., 2010), as found in the extracts investigated in this study, in addition, coumarins and anthraquinones were also recorded less frequently.

An interesting characteristic of douradinha is the presence of ursolic acid, a triterpenoid, pointed out as the main phytoconstituent found in the plant that is associated with diuretic property (Nascimento et al., 2006; Somova et al., 2003ab), however, the triterpenes were not major in this study.

The alkaloids were mostly found in the two extracts, these, described in different organs of *P. coriacea*, besides being rich source of allantoin and calycanthine which are nitrogenous compounds, with typical characteristics of pyrrolizidine alkaloids. Allantoin favors cell proliferation, accelerating the regeneration of injured skin, and calycanthine is considered a potent convulsive, like strychnine, a potent stimulant of the central nervous system (Laube et al., 2002; Lynch, 2004; Rajendra; Lynch; Schofield, 1997).

The effects on the nervous system are common for the different alkaloid classes, but the use of plants in popular medicine for the treatment of hypertension, in general, are not related to alkaloids, except for some species of the genus Erythrina which, since antiquity, according to ethnobotanic reports, they are used in the treatment of arterial hypertension until the present days (Cabral; Maciel, 2011). Although "douradinha" is mentioned by "raizeiros" of Campo Grande, MS, for the treatment of heart rhythm disorders (Nunes et al., 2003; Ustulin et al., 2009), this effect was not observed in this study.

The antioxidant activity of the ExH_2O and ExEtOH extracts in relation to DPPH (stable free radical 2,2-diphenyl-1-picril-hydrazyl) reported in Table 1 showed that ExEtOH presented better performance against DPPH than ExH_2O. The results with both extracts of *P. coriacea* leaves were higher when compared with leaves' extract of another Palicourea: P. rigida (Rosa et al., 2010), of specimens from Goiás State also in Midwest Brazilian region, where the authors related the antioxidant potential to the phenolic compound contents.

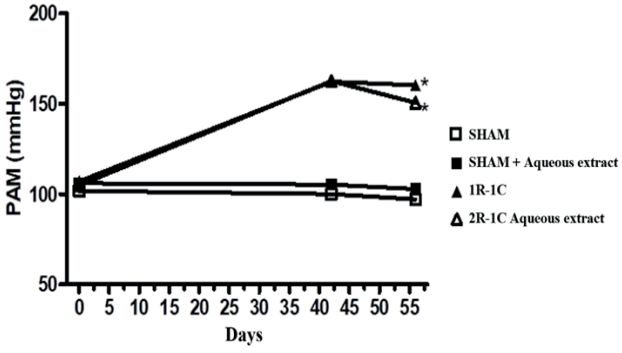
On the other hand, another study (Formagio et al., 2019), performed with the methanolic extract of leaves and stems of *P. coriacea*, collected in Dourados City, Southern region of the State of Mato Grosso do Sul, the antioxidant potential was CE_{50} > 250 µg/mL, considered inactive. Whereas the phenolic compounds (800.35 ± 9.45 mg GAE/g), flavonoids (719.40 ± 5.66 mg QE/g), flavonols (240.80 ± 12.39 mg QE/g) and condensate tannins (94.10 ± 15.20 mg CE/g) found in the methanolic extract were superior to the findings of the present study.

The variability of secondary metabolite classes, phenolic and flavonoid compounds and antioxidant potential may be observed due to several factors, such as climatic, geographical and maturation stage of the plants collected, and may affect the composition of the bioactive products of medicinal plants and their biological properties (Gobbo-Neto; Lopes, 2007). However, in this study it is possible to infer that regardless of the extracting solvent, $ExEH_2O$ extract and ExtEtOH extract, alkaloids and polyphenols are the majority in the plant, in the case of Rubiaceae, polyphenols and antioxidant action and anti-inflammatory potential are common (Claro et al., 2023, 2024).

3.2 Mean blood pressure measurement

Mean blood pressure (MAP) increased significantly in group 2R-1C (MBP = 167 mm Hg ± 10.6) when compared to the other groups studied (MBP = 100 mm Hg ± 8.0). However, after treatment with aqueous extract of *P. coriacea* (20mg/kg animal^{-1}) in group 2R-1C, MBP reduction was observed, but not significantly (P > 0.05), that is, it did not return to SHAM levels (MBP =100 mmHg), and there was no significant difference (P > 0.05) between the different hypertensive groups (Figure 2).

Figure 2 - Results of mean blood pressure evaluation (MAP) evaluated in SHAM and 2R-1C groups that received or not the treatment with *P. coriacea* aqueous extract



Source: research data.

The results indicate that the leaves infusion of *P. coriacea* commercialized at wet market Adriano Valente, used in the concentration tested, during 14-day period, despite the use in traditional medicine in the treatment of cardiovascular diseases such as hypertension, and chronic renal diseases (Ustulin et al., 2009), was not effective in controlling blood pressure indices.

Biochemical analyzes, after 14 days of treatment, of the hepatic parameters of ALT and AST demonstrated that, despite hypertension, the liver enzyme function was not altered.

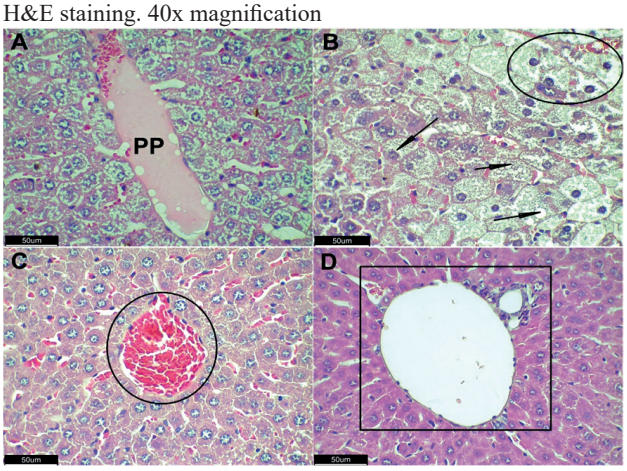
3.3 Histological analysis

In the liver, microscopic morphological evaluation

revealed mild proteinaceous deposition inside blood vessels, vascular congestion, and presence of spaces among the hepatocytes in the SHAM without treatment (Figure 3-A) and SHAM with treatment.

In the SHAM group with treatment, it was still possible to show areas of hydropic degeneration (cell tumefaction) (Figure 3-B). In the hypertensive group (2R-1C) without treatment (Figure 3-C), the same morphological findings of the SHAM group without treatment were evidenced. Whereas the hypertensive group (2R-1C) treated with the infusion of *P. coriacea* leaves, the liver (Figure 3-D) presented normal morphological aspect of the hepatic tissue.

Figure 3 - Light photomicrography (A) SHAM group without treatment with proteinaceous deposition inside the vessels (PP). (B) SHAM group with treatment, showing hepatocytes with areas of hydropic degeneration (circle and arrows). (C) Hypertension model group without treatment, showing vascular congestion in terminal venula (centrilobular vein). (D) Hypertension model group with treatment showing portal triad with usual morphology. H&E staining. 40x magnification



Source: the authors.

In the liver the plant's protective action may be related to phenolic and flavonoids compounds, chemical groups with proven antioxidant action. Therefore, it is possible to infer that these natural antioxidants had a protective effect against hepatotoxicity. This protection may have occurred because these substances act as attenuators of reactive oxygen species and consequently maintain normal enzymatic levels and because they are leukotriene-forming inhibitors, prostaglandin-forming enhancers (Babu et al., 2008; Gladine et al., 2007).

4 Conclusion

The solvent extractor influenced the diversity and quantity of secondary metabolites.

The infusion of *P. coriacea* leaves at concentration with 20mg/kg was not sufficient to obtain the reduction of systemic blood pressure in animals with renovascular hypertension at normal levels (MBP 100 mmHg).

The infusion of *P. coriacea* leaves possibly protected hepatic damage in animals with renovascular hypertension confirmed by biochemical and histopathology analysis.

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