

***Partamona cupira* geopropolis attenuates liver fibrosis and preserves hepatic architecture in experimental cirrhosis¹**

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ABSTRACT.- Costa KMFM, Moura CEB, Salatino A, Negri G, Jara CEP, Paiva KAR, Coelho WAC, Costa WP, Araújo Júnior HN, Góis RCS, Silva JB, Teófilo TS, Batista JS. ***Partamona cupira* geopropolis attenuates liver fibrosis and preserves hepatic architecture in experimental cirrhosis.** *Pesquisa Veterinária Brasileira* 46:e07865, 2026. Universidade Federal Rural do Semi-Árido, Rua Francisco Mota 572, Presidente Costa e Silva, Mossoró, RN 59625-900, Brazil. E-mail: helio.noberto@uece.br

Liver cirrhosis are progressive chronic disorders characterized by excessive extracellular matrix deposition, architectural distortion, and loss of hepatic function. This study investigated the chemical composition, antioxidant activity, and hepatoprotective effects of geopropolis produced by the stingless bee *Partamona cupira* in a rat model of thioacetamide-induced cirrhosis. Hydroethanolic extracts of geopropolis were chemically characterized by HPLC-DAD-ESI-MS/MS and evaluated for antioxidant activity using the DPPH assay. Forty male Wistar rats were randomly allocated into four experimental groups: control, geopropolis-treated, geopropolis plus thioacetamide, and thioacetamide-only. After 14 weeks, serum biochemical parameters, macroscopic and histopathological liver changes, stereological parameters, and collagen deposition were assessed. Seventeen phenolic compounds were identified in the geopropolis extract, which exhibited marked antioxidant activity. Animals receiving geopropolis during cirrhosis induction showed significantly lower serum alanine aminotransferase and aspartate aminotransferase activities compared with untreated cirrhotic animals. Histologically, geopropolis administration reduced the severity of cirrhosis, attenuated collagen accumulation, decreased type I collagen deposition, and preserved hepatic parenchymal architecture. Stereological analysis confirmed a lower volumetric density of collagen and improved preservation of hepatocytes and hepatic sinusoids in treated animals. These findings demonstrate that *P. cupira* geopropolis

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exerts antioxidant, hepatoprotective, and antifibrotic effects, attenuating liver fibrosis and preserving hepatic architecture in experimental cirrhosis.

INDEX TERMS: antioxidant activity, hepatoprotection, liver damage, phenolic compounds, propolis.

RESUMO.- [O geoprópolis de *Partamona cupira* atenua a fibrose hepática e preserva a arquitetura do fígado na cirrose experimental.] A cirrose hepática é uma doença crônica progressiva caracterizada pela deposição excessiva de matriz extracelular, distorção da arquitetura e perda da função hepática. Este estudo investigou a composição química, a atividade antioxidante e os efeitos hepatoprotetores da geoprópolis produzida pela abelha sem ferrão *Partamona cupira* em um modelo de cirrose induzida por tioacetamida em ratos. Extratos hidroetanólicos de geoprópolis foram caracterizados quimicamente por HPLC-DAD-ESI-MS/MS e avaliados quanto à atividade antioxidante pelo ensaio de DPPH. Quarenta ratos Wistar machos foram distribuídos aleatoriamente em quatro grupos experimentais: controle, tratado com geoprópolis, geoprópolis associada à tioacetamida e somente tioacetamida. Após 14 semanas, foram avaliados parâmetros bioquímicos séricos, alterações macroscópicas e histopatológicas hepáticas, parâmetros estereológicos e deposição de colágeno. Foram identificados 17 compostos fenólicos no extrato de geoprópolis, que apresentou acentuada atividade antioxidante. Os animais que receberam geoprópolis durante a indução da cirrose apresentaram atividades séricas de alanina aminotransferase e aspartato aminotransferase significativamente menores em comparação aos animais cirróticos não tratados. Histologicamente, a administração de geoprópolis reduziu a gravidade da cirrose, atenuou o acúmulo de colágeno, diminuiu a deposição de colágeno tipo I e preservou a arquitetura do parênquima hepático. A análise estereológica confirmou menor densidade volumétrica de colágeno e maior preservação dos hepatócitos e sinusoides hepáticos nos animais tratados. Esses achados demonstram que a geoprópolis de *P. cupira* exerce efeitos antioxidante, hepatoprotetor e antifibrótico, atenuando a fibrose hepática e preservando a arquitetura hepática na cirrose experimental.

TERMOS DE INDEXAÇÃO: atividade antioxidante, hepatoproteção, lesão hepática, compostos fenólicos, própolis.

INTRODUCTION

Chronic liver diseases remain a major cause of morbidity and mortality worldwide and frequently progress to liver cirrhosis. These conditions are characterized by excessive deposition of extracellular matrix components, particularly collagen fibers, resulting in distortion of the hepatic architecture, impairment of liver function, and ultimately liver failure (Parola and Pinzani, 2019). Although advances in medical treatment have improved the management of chronic liver diseases, effective therapeutic strategies capable of preventing or reversing hepatic fibrogenesis remain limited (Shan et al., 2019). Consequently, the search for natural compounds with antioxidant, anti-inflammatory, and antifibrotic properties has gained increasing attention.

Oxidative stress plays a central role in the pathogenesis and progression of chronic liver injury. The excessive production of reactive oxygen species promotes hepatocellular damage, activation of hepatic stellate cells, and deposition of extracellular matrix, contributing directly to the development of cirrhosis (Allameh et al., 2023; Che et al., 2023). In this context, natural products rich in phenolic compounds have emerged as promising alternatives due to their ability to modulate oxidative damage and inflammatory pathways involved in hepatic fibrogenesis (Niu et al., 2024; Wenbo et al., 2024).

Among these natural products, propolis and geopropolis produced by bees have attracted considerable scientific interest. Numerous studies have demonstrated that these products possess antioxidant, antimicrobial, anti-inflammatory, antiproliferative, and tissue-protective activities (Cunha et al., 2013; Silva et al., 2016). Such biological properties are largely attributed to their complex chemical composition, particularly their high content of phenolic compounds and flavonoids. Experimental evidence has also suggested hepatoprotective effects of propolis from different bee species, including attenuation of oxidative stress and reduction of liver injury (Silva et al., 2013a; Sousa et al., 2015).

Geopropolis produced by stingless bees (Meliponinae) differs from conventional propolis because it contains plant resins mixed with wax, pollen, and soil particles, resulting in a distinct chemical composition and biological activity. Among the stingless bees native to northeastern Brazil, *Partamona cupira* is of particular interest due to its ecological importance and the traditional use of its geopropolis by rural communities. Previous studies have demonstrated that geopropolis from native Brazilian stingless bees is rich in phenolic compounds and exhibits significant antioxidant activity (Silva et al., 2016; Cisiolotto et al., 2018). However, information regarding the biological activity of geopropolis produced by *P. cupira* remains scarce, particularly concerning its potential effects on cirrhosis and tissue remodeling during chronic liver injury.

Therefore, this study aimed to characterize the chemical composition and antioxidant activity of geopropolis produced by *P. cupira* and to investigate its hepatoprotective and antifibrotic effects in a rat model

of thioacetamide-induced cirrhosis. We hypothesized that the phenolic-rich geopropolis would attenuate cirrhosis, reduce collagen deposition, and preserve hepatic architecture during chronic liver injury.

MATERIALS AND METHODS

Ethical approval. The experimental protocol was submitted to the Animal Ethics Committee of the University of the State of Rio Grande do Norte (protocol number: 006/15).

Animals and housing conditions. Forty male *Rattus norvegicus* animals of the Wistar strain were used, which were aged 60 days and weighed approximately 240 g, were used in this study. The animals were kept in polypropylene boxes (four animals per box) lined with wood shavings, under controlled environmental conditions (temperature: 21–23 °C, 12 h light/dark cycle). Animals had *ad libitum* access to water and a standard commercial diet (23% crude protein, 4% total lipids, 5% fiber, and 12% minerals). Body weight was recorded at the beginning and at the end of the experimental period.

Geopropolis samples and extract preparation. Geopropolis samples produced by *Partamona cupira* bees were collected and used in this study. Taxonomic identification of the bees was performed at the Zoology Laboratory of the Federal Rural University of the Semi-Arid (Mossoró, RN, Brazil), according to Camargo (1980). The geopropolis samples were collected in April 2018 from apiaries located in Mossoró, Rio Grande do Norte, Brazil (5°11'S, 37°20'W), a region characterized by a tropical semi-arid climate, with an average annual temperature of 27 °C, relative humidity of 50%, and mean annual rainfall of 500 mm.

For extract preparation, 40 g of raw geopropolis was crushed in a blender (Walita®, 500 W) and transferred to a flat-bottom flask. Subsequently, 70% ethanol was added at a ratio of 5:1 (5 mL of solvent per gram of geopropolis), and the mixture was maintained at room temperature. The supernatant was then filtered, and the extract was concentrated by rotary evaporation at 40 °C until complete solvent removal. The dry extract was weighed, and the yield was calculated as a percentage of the initial mass.

Chemical characterization of hydroethanolic extract of geopropolis. The chemical composition of the hydroethanolic extract of geopropolis (HEG) was determined using high-performance liquid chromatography coupled to diode-array detection and electrospray ionization tandem mass spectrometry (HPLC-DAD-ESI-MS/MS). Analyses were performed using a Shimadzu DADSPD-M10AVP system (Kyoto, Japan) equipped with a Zorbax Eclipse Plus C18 column (4.6 × 150 mm, 3.5 µm), coupled to an Esquire 3000 Plus mass spectrometer (Bruker Daltonics, Billerica, MA, USA) with a quadrupole ion trap analyzer.

The column temperature was maintained at 45 °C, with a flow rate of 1 mL/min. Detection wavelengths ranged from 240 to 400 nm, with chromatograms recorded at 280 and 330 nm. The mobile phase consisted of 0.1% aqueous acetic acid (A) and methanol (B), using a gradient elution program. Mass spectrometry analyses were performed in positive and negative ion modes under previously described conditions (Hatami et al., 2014). Compound identification was based on UV spectra, mass spectral data, comparison with databases (Phenol-Explorer, ChemSpider, Metlin, and HMDB), literature data (Kranic et al. 2016, Salatino et al. 2019), and authentic standards.

In vitro antioxidant activity using the DPPH assay. The antioxidant activity of HEG was evaluated using the DPPH (2,2-diphenyl-1-picryl-hydrazil) free radical scavenging method (Mensor et al., 2015). A methanolic solution of the extract (20 µg/mL) was mixed with a DPPH solution (220 µg/mL). After 20 min of reaction, absorbance was measured at 518 nm using a Shimadzu UV-1601 spectrophotometer. All analyses were performed in triplicate. Antioxidant activity (%) was calculated using the formula: $AA = 100 - [(Aa - Ab) \times 100] / Ac$, where Aa is the sample absorbance, Ab is the blank absorbance, and Ac is the control absorbance.

Experimental design and induction of cirrhosis. A sample of HEG was selected for the in vivo hepatoprotective evaluation based on predefined chemical and antioxidant criteria, including total phenolic content, total flavonoid content, and in vitro antioxidant activity, as described above. The animals were randomly distributed into four experimental groups (n = 10 per group) and monitored over a 14-week period. Throughout the experiment, all animals received daily treatment by oral gavage according to their respective group protocols, with HEG prepared in distilled water immediately prior to administration and homogenized by manual agitation to ensure adequate dispersion.

The HEG was prepared in distilled water immediately before administration and homogenized by manual agitation to ensure adequate dispersion. The animals were divided into four groups: Group I (control) received 0.5 mL of distilled water once daily by gavage for 14 weeks; Group II received HEG at a dose of 500 mg/kg once daily for 14 weeks; Group III received HEG (500 mg/kg) once daily by gavage and underwent concomitant cirrhosis induction; and Group IV underwent cirrhosis induction and received 0.5 mL of distilled water daily by gavage. Cirrhosis was induced by intraperitoneal administration of TAA at a dose of 0.2 g/kg, three times per week for 14 weeks, as previously described (Amin et al., 2012). In Group III, HEG administration was continued throughout the experimental period, and on days when HEG administration coincided with TAA injection, HEG was administered 1 hour before TAA. The oral administration route and HEG dose were selected based on previous studies (Silva et al., 2019).

Blood collection and serum biochemical analysis. At the end of the experimental period, animals were anesthetized with ketamine (100 mg/kg, i.p.) and xylazine (100 mg/kg, i.p.). Following anesthesia, a celiotomy was performed to access the hepatic vein, and approximately 3 mL of blood was collected from each animal using sterile syringes. Blood samples were transferred to tubes without anticoagulant and processed for serum biochemical analysis.

Serum levels of alanine aminotransferase (ALT), aspartate aminotransferase (AST), albumin, total protein, glucose, and triglycerides were determined using commercial kits (VIDA Biotecnologia®) and analyzed by spectrophotometry at the Clinical Pathology Laboratory of the Federal Rural University of the Semi-Arid.

Macroscopic and histopathological analysis. After euthanasia by exsanguination under deep anesthesia, all animals were subjected to necropsy. The livers were removed, weighed, and examined macroscopically for alterations in size, color, consistency, presence of nodules, and other features suggestive of cirrhosis. Relative liver weight was calculated as a percentage of body weight.

Liver samples were washed in 0.9% saline solution, fixed in 10% buffered formalin, processed routinely, embedded in paraffin, sectioned at 5 μ m, and stained with hematoxylin and eosin (HE). Histopathological analysis was conducted to characterize hepatic architecture and quantify lesion severity. Liver alterations were classified according to a four-tiered scoring system: Score 0, absence of morphological abnormalities, indicating preserved hepatic parenchyma; Score 1, discrete lesions characterized by mild inflammatory infiltrates, limited hepatocellular necrosis, focal bile duct hyperplasia, and isolated regenerative nodules; Score 2, moderate structural compromise, evidenced by more pronounced inflammatory infiltrates, extensive hepatocellular necrosis, marked bile duct hyperplasia, and multifocal regenerative nodules; and Score 3, severe hepatic injury, defined by intense inflammatory infiltrates, extensive portal fibrosis, diffuse bile duct hyperplasia, and the presence of large regenerative nodules. Picrosirius red staining was also performed for the identification and quantification of collagen fibers (types I and III).

Stereological analysis. Liver samples were processed following a systematic uniform random sampling protocol (Marcos et al., 2012). At least two fragments per animal were analyzed. From each fragment, two sections (5 μ m thick) were obtained, and five random microscopic fields per section were captured under 20 $\times$ magnification.

A test grid containing 50 points was superimposed on each image using Image Pro-Plus software (version 4.5.0.29), and the volumetric density (Vv) of hepatocytes, sinusoids, and collagen was calculated using the formula $Vv = PP/TPt$ (%), where PP represents the number of points over the structure of interest and TPt is the total number of test points.

Collagen morphometry. Collagen quantification was performed on picrosirius red-stained sections using polarized light microscopy (Nikon Eclipse MA100). Images were captured and analyzed using Image Pro-Plus software. Collagen fibers were quantified by automated color segmentation, with standardized thresholds: 0–169 for parenchyma, 170–190 for type I collagen (red), and 191–255 for type III collagen (green).

Statistical analysis. Data were expressed as mean $\pm$ standard deviation, along with minimum and maximum values. Normality and homogeneity of variances were assessed using the Kolmogorov–Smirnov and Levene tests, respectively. Parametric data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's post hoc test. Non-parametric data were analyzed using the Kruskal–Wallis test, followed by Dunn's test. Histological findings were compared using the chi-square test.

Comparisons between initial and final body weight, weight gain, and liver weight were performed using paired t-tests or non-parametric equivalents when appropriate. Statistical analyses were performed using SPSS software (version 23.0), and differences were considered statistically significant at $p < 0.05$.

RESULTS

The yields of extracts A and B were 45.3% and 48.1%, respectively. A total of 17 compounds were identified in the hydroethanolic extracts of geopropolis produced by *Partamona cupira* (Supplementary Table). These compounds were mainly distributed into the following chemical classes: flavonols, flavones, and chalcones, in addition to phenolic derivatives. The values of IC₅₀ regarding antioxidant activity were 50.0 μ g/mL for geopropolis samples.

The mean serum levels of AST, ALT, and TP did not differ significantly between GI, GII, and GIII. The levels of ALT and AST were higher in G4 than in other groups. In GIV, there was also a significant reduction in the mean values of TP compared with those of GI, GII, and GIII. The average levels of GLUC and serum TRIGs did not vary between treatments (Table 1).

Animals in GI and GII showed a significant increase in weight. The parameter related to liver weight (absolute and relative) was statistically similar between GI and GII. There was a significant increase in liver weight in GIII and GIV and significant difference compared to the other groups (Table 2).

In the macroscopic examination of the livers, it was possible to verify that the animals of the GI and GII had a morphological aspect within the normality observed for the species, without anatomical alterations (Fig. 1-2). The GIII animals had cirrhotic livers with small regenerative nodules, less thickened capsules, and a relatively

soft consistency and color, closer to the pattern (Fig. 3). All GIV animals developed cirrhosis, as evidenced by the presence of opaque-colored livers with multiple regenerative nodules of variable size on the surface, thickened capsule, and hardened consistency (Fig. 4).

Histopathological evaluation of the slides stained with H&E revealed that the hepatic tissue of GI and GII animals did not present changes, showing a normal histopathological appearance (Fig. 5-6). Animals in groups GIII and GIV showed an increase in lesions in the liver parenchyma ($p=0.02$), and the injuries were mainly regenerative and fibrotic, confirming cirrhosis observed in the macroscopic examination (Fig. 7-8).

The intensity of the injuries was higher in GIV ($p=0.03$) than in other groups. In these animals (GIV), the histological examination showed portal fibrosis, in addition to the presence of large regenerative nodules of hepatocytes surrounded by thick fibrous septa and bile duct hyperplasia (Fig. 8). In the liver of GIII animals, mild fibrosis was observed in the portal space; additionally, small regenerative nodules surrounded by fine connective tissue septa were noted, and mild bile duct hyperplasia occurred (Fig. 7).

The analysis of liver tissues in the different experimental groups by stereology showed that there was no significant difference between the percentage of hepatocytes in GI and GII. The percentage of GIII hepatocytes did not differ significantly from that of GII. However, the percentage of hepatocytes in the GIV was significantly reduced compared with that in other groups. The percentage of sinusoids did not differ significantly between GI, GII, and GIII. However, similar to hepatocytes, there was a significant reduction in the percentage of sinusoids in GIV.

With regard to the percentage of collagen present in the liver tissue, stereological analysis also showed that there was no significant difference in the percentage of collagen in GI and GII. However, a significant increase in the percentage of collagen in GIII and GIV was observed; when comparing these groups, the percentage of collagen present in GIII was lower than that in GIV (Table 3).

In the slides stained with picrosirius red, collagen was quantified in the hepatic tissue of the animals in the different experimental groups. Using this staining method, it was possible to quantify and calculate the percentage of collagen type I (red color), type III (green color), and hepatic parenchyma in relation to the total area of the image, which is equivalent to the volumetric proportion of the referred quantified parameters in the liver. The hepatic parenchyma was the main component of the total imaging area in all experimental groups (Fig. 9-12).

Data analysis showed that there was no significant difference in type I collagen, type III collagen, and hepatic parenchyma between GI and GII (Fig. 9-10). However, there was a significant increase in the percentage of type I collagen between GIII and GIV when compared with that in the other groups (Fig. 11-12); the percentage of this type of collagen was higher in GIV than in GIII. The percentage of collagen type III also increased in groups GIII and GIV compared with that in the other groups. A significant reduction in the parenchyma in the GIII and GIV groups was also noted when compared with that in the other groups (Table 4).

DISCUSSION

This is the first study to report the characterization of chemical constituents and antioxidant activity of geopropolis produced by the stingless bee *P. cupira*. The yield of the extract is higher than that recommended by the current Brazilian legislation for the yield of propolis extract minimum: 35% (Brasil, 2001).

The chemical profile of *P. cupira* geopropolis identified in the present study provides a distinctive basis for interpreting its biological potential. Seventeen phenolic compounds were detected, with a predominance of chalcones (1, 2, 7, and 15), methylated flavones (9, 11, 12, 13, and 16), and flavonols (3, 4, 5, 8, and 12), indicating a chemically diverse extract rather than a profile dominated by a single class of bioactive constituents. Of particular interest, this composition showed similarities to geopropolis and propolis previously associated with *Mimosa tenuiflora* as a resin source in northeastern Brazil, suggesting that the semiarid flora may contribute substantially to the chemical identity of *P. cupira* geopropolis. The coexistence of these compounds may be particularly relevant to the hepatoprotective and antifibrotic effects observed *in vivo*, since their combined antioxidant potential could contribute to reduce oxidative injury during chronic TAA exposure.

The diversity of flavonoids, as verified in the geopropolis of the present study, may indicate that the product is of high quality; the presence and concentration of these compounds are used as a qualification index for propolis samples (Lu et al., 2004). The antioxidant activity is directly proportional to the total phenol and flavonoid contents (Silva et al., 2013b; Cabral et al., 2009). Phenolic compounds with significant amounts of hydroxyls are able to intercept the free radical oxidation chain through the hydrogen donation of their hydroxyls, promoting greater antioxidant activity, which acts by reducing oxidative stress and can promote protection against DNA damage and hepatoprotective activity (Kataoka and Cardoso, 2013).

The sequestering capacity of the free radical DPPH can be represented by the IC_{50} values, which is a parameter indicative of the inhibitory concentration needed to decrease the DPPH by 50% (Nascimento et al., 2010). In general, the lower the IC_{50} value (%), the greater the sequestering capacity of the radical and, therefore, the higher the antioxidant activity. An IC_{50} value less than 50 $\mu\text{g/mL}$ is considered very active; 50–100 $\mu\text{g/mL}$,

moderately active; 100–200 µg/mL, slightly active; and above 200 µg/mL, inactive (Reynertson et al., 2005). Based on this classification, it is concluded that the samples in this study have very active antioxidant.

Among the experimental models used to induce cirrhosis, TAA is particularly suitable because it produces macroscopic and histological changes resembling those observed in human cirrhosis, while being associated with relatively low mortality (Laleman et al., 2006). In this context, circulating biochemical markers of liver function are routinely analyzed to evaluate the extent of hepatic damage induced by TAA and the therapeutic efficacy of potential protective agents. Increase in the levels of AST and ALT enzymes may reflect liver function: when injury or destruction of liver cells occurs, these enzymes are released into the bloodstream. These data corroborate the findings of a previous report on the hepatoprotective effect of Brazilian propolis in rats with hepatotoxicity induced by carbon tetrachloride (Paulino et al., 2014) and with an investigation that involved pretreatment of rats with asiaticoside (Jesus et al., 2000). The significant reduction in serum TP levels in GIV clearly reflects the morphological and functional changes in the liver of TAA-treated animals. Low levels of TP in serum may reflect severe hepatocellular lesions (Schuppan and Afdhal, 2008; Ramadan et al., 2015).

The lower weight gain in animals from groups GIII and GIV is likely related to impaired metabolism and utilization of nutrients, resulting from the cirrhotic condition. The increase in liver weight in GIII and GIV is likely related to the pathological aspects characteristic of cirrhotic liver tissue. The main pathological processes that occur in cirrhosis are fibrosis, cell regeneration, or proliferation, thus liver hypertrophy often occurs, as observed in the livers of animals in GIII and GIV, these results being in accordance with the literature, where the increased liver proportion in animals with cirrhosis is related to the development of tissue repair processes (Ramadori and Saile, 2004).

It is worth noting that although no statistical difference in liver weight was found in GIII and GIV, stereological analysis showed a significant difference in the percentage of hepatocytes and collagen between these groups. The percentage of hepatocytes was 62.6% and 48.5%, respectively, for GIII and GIV, while the percentage of collagen was 19.5% and 37.6%, respectively, for GIII and GIV, indicating an improvement in the morphological aspect of liver tissue, which probably contributes to the functional improvement of the organ, as can be seen in the analysis of serum biochemistry.

Macroscopic and histological evaluations of the livers of GII animals showed that the oral administration of geopropolis hydroethanolic extract did not induce hepatotoxic effects, since the livers of the animals in this group did not present changes, showing normal histopathological aspect. In addition, a hepatoprotective effect on the severity of cirrhosis was observed because the livers of GIII animals had smaller regenerative nodules than the livers of GIV animals. The organ capsule presented less thickening, in addition to a relatively soft consistency and color closer to the normal standards. In the histological examination of the livers of the animals in this group, it was also possible to verify that the deposition of connective tissue was less intense, and the septa involving the regenerative nodules were thin. Similarly, the Brazilian green propolis extract preserved the structural integrity of the hepatocellular membrane, showing hepatoprotective activity (Paulino et al., 2014; Ramadan et al., 2015).

The changes in the percentages of sinusoids also reflect the histological changes that occurred in the hepatic tissue of the rats subjected to different experimental protocols. Hepatic sinusoids are capillaries or channels located in the space between the hepatic cords, where the blood flows until the terminal hepatic vein. The walls of the veins are covered with fenestrated endothelial cells that expose the hepatocytes directly to a series of hormones, growth factors, and nutrients, with hepatotropic action (Paulino et al., 2014). The significant reduction in the percentage of sinusoids in GIV can be explained by the establishment of cirrhosis that occurred with greater intensity in this group. Hepatocyte death leads to fibrosis repair, which involves the deposition of fibrous tissue around the sinusoid vessels and causes obliterate fibrosis, resulting in impairments in the intrahepatic blood flow and biliary intrahepatic flow (Bedossa and Paradis, 2003).

The changes observed in the percentages of collagen are related to the characteristic pathological aspects of liver tissue with cirrhosis. Cirrhosis is defined as the final phase of chronic liver injury, which is characterized by degeneration, hepatocyte death, and deposition of large amounts of fibrous tissue, mainly collagen fibers (Jiang et al., 2016). Thus, the significant increase in collagen in GIV clearly reflects this condition. The significant reduction in the percentage of collagen in the GIII indicates that geopropolis impeded the advancement and severity of TAA-induced liver damage. The changes observed in the percentages of collagen types I and III confirm the dynamic and remodeling state that occurs in collagen during the process of liver damage repair. Type I collagen is characterized by long, thick fibers. This type of collagen is present in the liver in patients with advanced cirrhosis, while type III collagen is characterized by thinner fibers (Aguar et al., 2009). With regard to the results obtained in this study, it is necessary to emphasize that the significant reduction in the percentage of collagen type I in GIII may reflect a morphological and functional improvement in the liver, once again confirming the hepatoprotective effect of geopropolis.

CONCLUSION

Partamona cupira geopropolis attenuated liver fibrosis and preserved hepatic architecture in rats with thioacetamide-induced cirrhosis. These effects were associated with its phenolic-rich composition, antioxidant activity, reduced collagen deposition, and improved histological preservation of the liver parenchyma. The findings provide experimental evidence supporting the hepatoprotective and antifibrotic potential of geopropolis and encourage further investigation of its therapeutic applications in chronic liver disease.

Conflict of interest statement.- The authors declare that they have no conflicts of interest.

Credit author statement.- All authors contributed to the study conception and design. Costa KMFM, Moura CEB, Araújo Júnior HN, Teófilo TS: conceptualization, investigation, supervision, visualization and writing. Salatino A, Negri G, Jara CEP: methodology, investigation, supervision and validation. Coelho WAC: data curation and formal analysis. Paiva KAR, Costa WP, Góis RCS, Silva JB: investigation, methodology. Batista JS: project administration, supervision, validation and writing. All authors reviewed and approved the final version of the manuscript.

Data availability statement.- The research data are available upon request.

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Figures

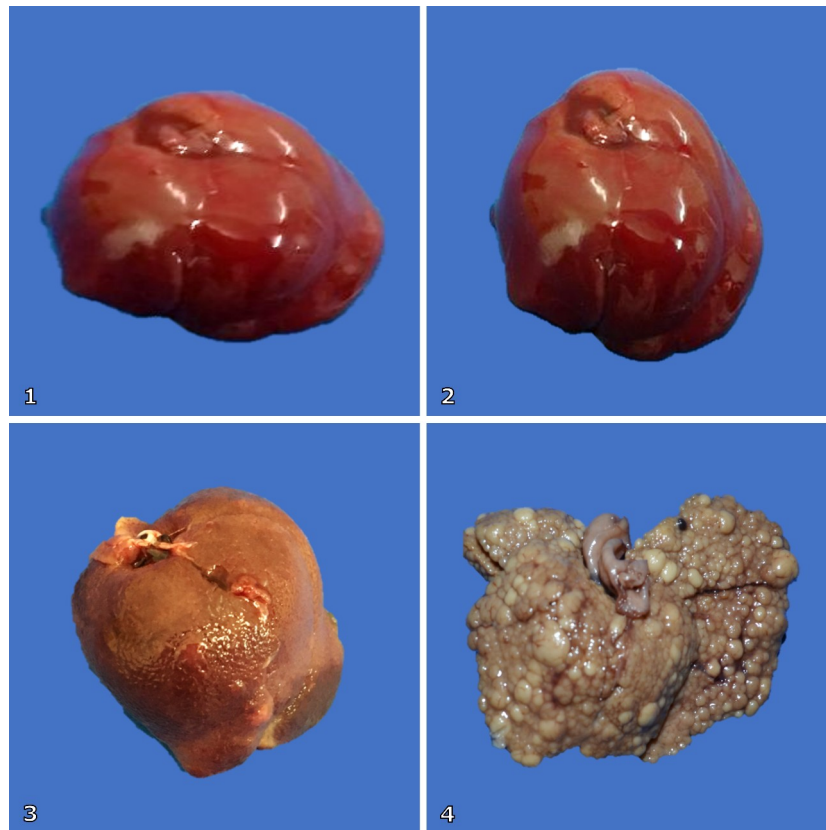


Fig. 1-4. Effect of geopropolis on the macroscopic appearance of the livers of *Rattus norvegicus* in the experimental groups. **(1)** GI: control. **(2)** GII: 500 mg/kg HEG. **(3)** GIII: 500 mg/kg HEG and TAA-induced cirrhosis. **(4)** GIV: TAA-induced cirrhosis. GI and GII show livers with normal macroscopic morphology. GIV shows large regenerative nodules on the liver surface, whereas and GIII shows smaller regenerative nodules.

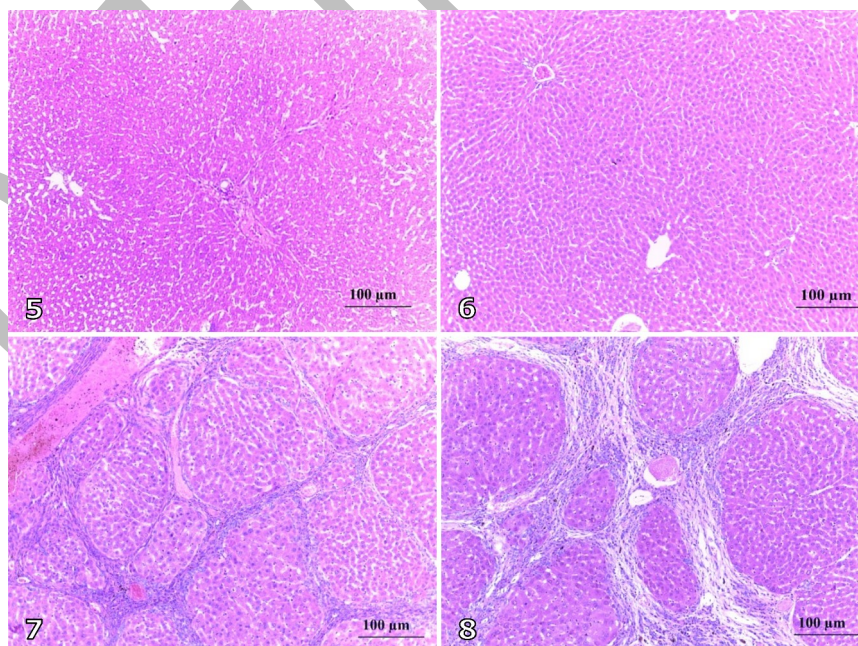


Fig. 5-8. Effect of geopropolis on the histological aspects of the livers of *Rattus norvegicus* in the experimental groups. **(5)** GI: control. **(6)** GII: 500 mg/kg HEG. **(7)** GIII: 500 mg/kg HEG and TAA-induced cirrhosis. **(8)** GIV: TAA-induced cirrhosis. GI and GII show preserved hepatic architecture. GIV shows large regenerative nodules

surrounded by thick fibrous connective tissue septa, whereas GIII shows smaller regenerative nodules surrounded by thinner connective tissue septa. HE.

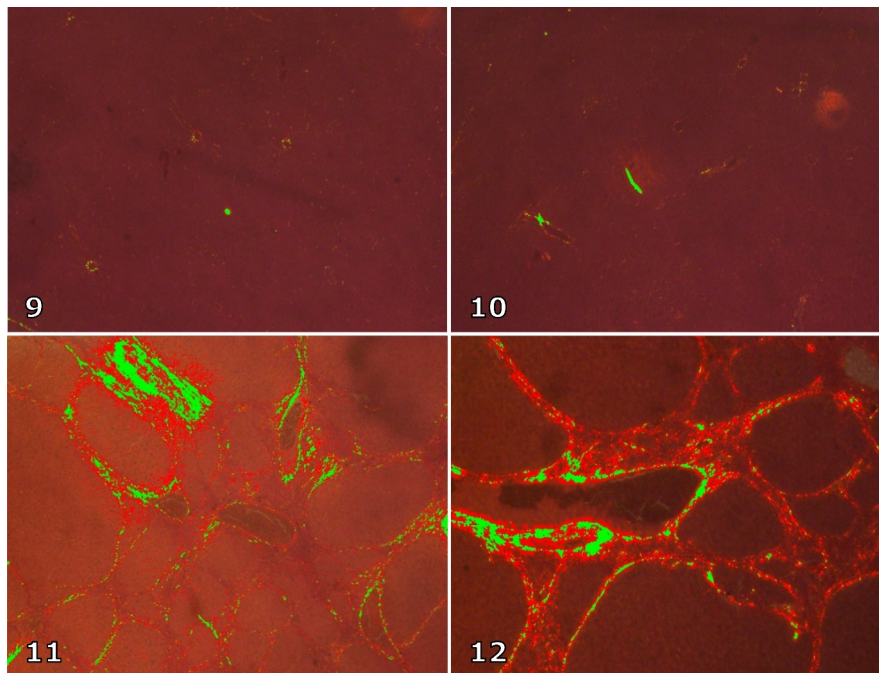


Fig. 9-12. Effect of geopropolis on collagen deposition in the liver of *Rattus norvegicus* in the experimental groups. **(9)** GI: control. **(10)** GII: 500 mg/kg HEG. **(11)** GIII: 500 mg/kg HEG and TAA-induced cirrhosis. **(12)** GIV: TAA-induced cirrhosis. Picrosirius Red staining shows type I collagen in red and type III collagen in green. Obj. 20x.

Tables

Table 1. Hepatoprotective effect of the geopropolis produced by *Partamona cupira* on the serum biochemical parameters of *Rattus norvegicus* in the experimental groups: GI (control), GII (500 mg/kg HPE), GIII (500 mg/kg HEG, and subjected to induction of cirrhosis by TAA), and GIV (submitted to induction of cirrhosis by TAA).

Variables	Experimental groups*			
	GI	II	III	GIV
AST (U/L)	87.67 ± 12.23 ^b	90.67 ± 7.20 ^b	92.80 ± 14.92 ^b	169.58 ± 27.22 ^a
ALT (U/L)	74.33 ± 8.64 ^b	74.83 ± 9.66 ^b	76.91 ± 16.11 ^b	155.50 ± 46.68 ^a
GLUC (mg/dL)	127.50 ± 4.59 ^a	145.17 ± 34.98 ^a	152.67 ± 31.42 ^a	156.83 ± 27.82 ^a
TRIG (mg/dL)	41.67 ± 12.95 ^a	49.50 ± 14.32 ^a	41.20 ± 10.36 ^a	45.58 ± 6.93 ^a
TP (g/dL)	7.93 ± 0.65 ^a	7.03 ± 0.41 ^a	6.71 ± 0.40 ^a	3.85 ± 0.66 ^b

* Values are expressed as mean values ± standard deviation, n = 10. ^{a,b,c} Mean values followed by different lowercase letters on the line indicate a significant difference (p<0.05), as calculated using Tukey's test. AST: aspartate transaminase; ALT: enzyme alanine transaminase; GLUC: glucose; TRIG: triglycerides; TP: total protein

Table 2. Effect of geopropolis produced by *Partamona cupira* on the body and liver weights of *Rattus norvegicus* in the experimental groups: GI (control), GII (500 mg/kg HEG), GIII (500 mg/kg HEG and subjected to induction of cirrhosis by TAA), and GIV (submitted to induction of cirrhosis by TAA).

Variables	Experimental groups*			
	GI	II	III	GIV
Initial body weights	233.00 ± 41.00 ^b	220.40 ± 28.90 ^b	249.70 ± 13.20 ^{ab}	277.80 ± 42.50 ^a
Final body weights	296.80 ± 64.60 ^a	286.80 ± 21.30 ^a	277.20 ± 38.80 ^a	294.80 ± 35.80 ^a
Weights gain	63.70 ± 28.10 ^{**}	66.40 ± 37.10 ^{**}	27.50 ± 31.70	17.10 ± 50.80
Liver weights absolute	10.10 ± 2.70 ^b	8.40 ± 0.90 ^b	20.20 ± 7.40 ^a	17.00 ± 0.90 ^a
Liver weights relative	3.30 ± 0.40 ^b	3.00 ± 0.30 ^b	6.80 ± 2.40 ^a	6.00 ± 0.50 ^a

*Values are expressed as mean values ± standard deviation. ^{a,b} Mean values followed by different lowercase letters on rows indicate significant difference (p<0.05) as calculated using Kruskal-Wallis' test. ** Statistical difference (p<0.05 – Wilcoxon).

Online First

Table 3. Effect of the geopropolis produced by *Partamona cupira* on the volume density of hepatocytes, sinusoids, and collagen of *Rattus norvegicus* in the experimental groups: GI (control), GII (500 mg/kg HPE), GIII (500 mg/kg HEG and subjected to induction of cirrhosis by TAA), and GIV (submitted to induction of cirrhosis by TAA).

Variables	Experimental groups*			
	GI	II	III	GIV
Hepatocytes	78.56 + 6.12 ^a	70.44 + 1.33 ^{ab}	65.64 + 7.49 ^b	48.56 + 3.76 ^c
Sinusoids	21.08 + 5.19 ^a	23.00 + 1.83 ^a	19.00 + 3.09 ^{ab}	15.76 + 4.11 ^b
Collagen	6.04 + 1.54 ^c	5.56 + 2.34 ^c	19.56 + 6.45 ^b	37.68 + 6.70 ^a

* Values expressed as mean values ± standard deviation, n = 10. ^{a,b,c} Mean values followed by different lowercase letters on rows indicate a significant difference (p<0.05), as calculated using Tukey's test.

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Table 4. Effect of geopropolis produced by *Partamona cupira* on the collagen quantification of *Rattus norvegicus* in the experimental groups: GI (control), GII (500 mg/kg HPE), GIII (500 mg/kg HEG and subjected to induction of cirrhosis by TAA), and GIV (submitted to induction of cirrhosis by TAA).

Variables	Experimental groups*			
	GI	II	III	GIV
Type I	1.83 ± 0.00 ^a	1.86 ± 0.00 ^a	6.04 ± 4.36 ^b	16.14 ± 2.26 ^c
Type III	2.90 ± 0.03 ^a	2.27 ± 0.04 ^a	6.05 ± 1.96 ^b	5.20 ± 2.02 ^b
Parenchyma	95.27 ± 0.03 ^a	95.88 ± 0.04 ^a	80.75 ± 2.36 ^b	80.78 ± 3.94 ^b

* Values are expressed as mean values ± standard deviation. ^{a,b,c} Mean values followed by different lowercase letters on rows indicate significant difference (p<0.05) as calculated using Tukey's test.