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EVALUATION OF THE HEPATOPROTECTIVE ACTIVITY OF THE AQUEOUS EXTRACT OF CHRYSOBALANUS ICACO LEAVES

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ABSTRACT

The metabolization of substances in the human body is carried out mainly by the liver, but some substances when in excess, such as Paracetamol, can lead to liver toxicity. *Chrysobalanus icaco* L., a plant with potent antioxidant metabolites, shows promise in combating antioxidant imbalance and hepatoprotection action. The objective of this study was to evaluate the protective action of the aqueous extract of *C. icaco* leaves (EAFCi) against paracetamol-induced hepatotoxicity. For this, the plant material was collected and the leaf extract was prepared through decoction in distilled water (5:100 w/v). For the *in vivo* evaluation of the hepatoprotective activity, Wistar rats were divided into 3 groups of 5 animals each, which were treated with saline, silymarin and EAFCi at a dose of 100 mg/kg

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(groups I, II and III, respectively). On the seventh day of treatment, Paracetamol was administered and, after twelve days, euthanasia was performed to remove the liver. Reduced glutathione levels, lipid peroxidation, and histopathological examination were then evaluated. With the methodology used, a reduction in MDA levels and an increase in GSH levels were observed in the groups that were administered silymarin and the 100mg/kg dose of EAFCi, when compared to the control group. In addition, in the histological analysis of the liver, it was observed that the animals treated with silymarin and with the dose of 100mg/kg of EAFCi had normal liver morphology, unlike the negative control group, which showed significant changes. In this sense, it is concluded that the administration of EAFCi at a dose of 100mg/kg in Wistar rats was able to inhibit the hepatotoxicity induced by Paracetamol by decreasing MDA, increasing GSH and preserving liver morphology.

Keywords: Bioprospecting. Oxidative stress. Hepatoprotection. Plant extracts.

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INTRODUCTION

The liver is the main human organ with the function of metabolizing endogenous and xenobiotic substances (Thompson *et al*., 2017); in addition, it is also involved with protein synthesis and fatty acid metabolization (Mohamad *et al*., 2015). Disturbances in this process of metabolization can lead to hepatotoxicity. Some substances can cause severe damage to hepatocytes, such as antibiotics, non-steroidal anti-inflammatory drugs (NSAIDs), and excessive consumption of Paracetamol, one of the most popular analgesics in the world (Aslmarz *et al*., 2019).

The high consumption of Paracetamol (N-acetyl-p-aminophenol; 4-hydroxyacetanilide; 4-acetamidophenol or N-(4-hydroxyphenyl) acetamide) causes hepatic necrosis, inflammation, and production of reactive oxygen species (Mohamad *et al*., 2015) through a biotransformation reaction via cytochrome P-450 that leads to the formation of N-acetyl-p-benzoquinone-imine (NAPQI), a reactive metabolite, which in high concentrations cannot be conjugated with reduced glutathione (GSH) because of the high consumption. Elevated levels of NAPQI and decreased levels of GSH lead to mitochondrial disturbance, with elevated production of reactive oxygen species, as well as other alterations leading to inflammation and hepatic necrosis, which elevates serum markers of hepatotoxicity such as alanine (AST) and aspartate aminotransferase (ALT), alkaline phosphatase (ALP), and gamma-glutamyl transferase (γ-GT) (Freitag, 2014).

The high concentration of reactive oxygen species leads to oxidative damage with consequent lipid peroxidation, enzyme inhibition, protein oxidation, and DNA and RNA damage. Thus, its balance is vital for the control of oxidative stress, with antioxidants being the main sources of protection against this damage. Among them is glutathione (GSH), which at higher levels indicates a greater response to oxidative stress, while its decreased levels indicate a lower capacity to destroy free radicals and ROS (Pedrete, 2020).

Herbal medicines are considered effective and safe alternative treatments to treat various diseases. Silymarin, a plant popularly known in Brazil as cardomarian, milk thistle, thistle, thistle, thistle or thistle, is a herbal medicine already well marketed in Brazil for the treatment and/or prevention of hepatobiliary diseases, mainly due to its hepatoprotective effect, being the standard drug used in research. This property is due to the flavolignan complex, with silybin as the main compound (Freitag, 2014). In recent decades, they have received increasing attention as potential therapeutic agents to prevent and treat liver diseases (Yoon *et al*., 2016). Flavonoids, a type of polyphenol, are oxidized by free radicals,

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generating more stable radicals with less activity. They help protect the body from damage mediated by reactive oxygen species (Aslmarz *et al*., 2019; Mohamad *et al*., 2015).

Many plants are rich in antioxidant components such as *Chrysobalanus icaco* Linnaeus, popularly known as guajerú, ajuru, baijirú, bajuru, guajuru, abajeru. Its chemical composition includes flavonoids derived from myricetin and quercetin (Ribeiro *et al*., 2020). The consumption of *C. icaco* tea has been widely used in folk medicine for the control of various diseases (Ferreira-Machado *et al*., 2004). Pharmacological studies have reported that the aqueous extract of *C. icaco* leaves has analgesic, anti-inflammatory, and hypoglycemic properties (White *et al*., 2016). Its anti-inflammatory property is due to the inhibition of the release of pro-inflammatory mediators such as nitric oxide, histamine and prostaglandins; through the inhibition of pro-inflammatory enzymes such as cyclooxygenase-2, nitric oxide synthase and lipoxygenase. In addition, some research has shown that myricetin as the main phytochemical of *C. icaco* leaves, being important for its antioxidant property, in addition to other flavonoids and terpenes (Onilude; Kazeem; Adu, 2021).

Due to the wide medicinal use of *C. icaco* and its photochemical characteristics, the primary objective of this work is to investigate the protective activity of the aqueous extract of *C. icaco* leaves (EAFCi) against paracetamol-induced hepatotoxicity.

METHODOLOGY

BOTANICAL MATERIAL

The botanical material used was collected in the municipality of Itamaracá, in Pernambuco, in October 2020. The leaves were dried in an oven at 40ºC and crushed. Subsequently, 50g of the powder was subjected to extraction by infusion in 1000 ml of distilled water at 100ºC for 15 min. Then, the extract was filtered, concentrated in a rotary evaporator under reduced pressure and dried in a freeze dryer. The extract was stored at 4ºC and solubilized in saline solution at the desired concentrations minutes before the experiments.

EXPERIMENTAL DESIGN

The experimental animals were divided into three groups of five rats each. Each group received the following treatments orally for seven days: in group I (negative control), the rats received vehicle (saline solution). In group II (positive control), the animals were

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treated with a reference drug, silymarin (50 mg/kg). In group III, the animals were pretreated with the aqueous extract of *C. icaco* at a dose of 100 mg/kg. After this period, the animals were fasted for 8 hours and then received paracetamol orally at a dose of 250 mg/kg. After 12 hours, the animals were anesthetized with ketamine and xylazine (8:2; v/v), and their livers were collected for determination of antioxidant activity and histological analysis.

HISTOLOGICAL ANALYSIS

After laparotomy, the liver was removed, macroscopically analyzed, and weighed. It was then fixed in formalin (10% formaldehyde) for processing in a routine histopathological technique. Subsequently, the tissue fragments were sectioned at a thickness of 5.0 µm and stained with hematoxylin-eosin for analysis.

ANTIOXIDANT ACTIVITY

The livers were homogenized in 1.15% KCl buffer solution with 3 mM EDTA (5 mL/g of tissue) to evaluate the antioxidant activities through the quantification of lipid peroxidation. The measurement of the levels of thiobarbituric acid reactive substances (TBARS) was carried out using the methodology described by Ohkawa *et al*. (1979). The absorbance of the organic phase was measured at 535 nm and the result was corrected by the protein concentration of the homogenate. On the other hand, the evaluation of Reduced Glutathione Levels (GSH) was carried out by the measurement of non-protein sulfhydryl groups was carried out by the method of Sedlak and Lindsay (1968), and the results were corrected by the protein concentration of the homogenate and expressed in µg GSH/mg of protein. In both cases, protein measurement was performed using the Folin method (Lowry *et al*., 1951), using 0.1% bovine serum albumin (BSA) as standard.

STATISTICAL ANALYSIS

The values were expressed as mean ± standard deviation of the mean (SMD). Statistical analyses were performed using the Prism 8.0® program. The homogeneity of the variances was tested by the Bartlett method. If the variance was homogeneous, the data will be analyzed by ANOVA, followed, when necessary, by Dunnett's test. When the data do not assume a normal distribution, the non-parametric Kruskall-Wallis tests will be used, followed by the Dunn test. The significance level for rejecting the nullity hypothesis was set at 5% ($p<0.05$).

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RESULTS AND DISCUSSION

Liver diseases are a worldwide public health problem, triggered mainly by viruses, metabolic diseases or chemical components. In our study, the hepatoprotective activity of the aqueous extract of *Chrysobalanus icaco* leaves was verified through the acetaminophen-induced liver poisoning model in Wistar rats, a reliable model for the study of hepatoprotective factors, which has been used in several intraperitoneal and oral doses to induce hepatotoxicity (Yao *et al*., 2015).

Lipid peroxidation is a process in which free radicals, such as reactive oxygen species (ROS) and reactive nitrogen species (RNAs), attack the double bonds between carbons in lipids. This mechanism involves removing a hydrogen atom from a carbon and adding an oxygen molecule. The process results in a diversity of complex products, including peroxyl lipid radicals and hydroperoxides as initial byproducts, as well as predominant secondary compounds such as malondialdehyde (MDA) and 4-hydroxynanone (Tsikas *et al*., 2017). MDA has been widely used in biomedical research as a marker of lipid peroxidation due to its easy reaction with thiobarbituric acid (TBA), and the TBARS (Thiobarbituric Acid Reactive Substances) assay is widely accepted as an effective method to assess overall oxidative stress levels through lipid oxidation in a biological sample.

Regarding the potential to inhibit the formation of thiobarbituric acid reactive substances (TBARS) in the aqueous extract of *C. icaco* leaves (EAFCi), the results show that treatment with silymarin and EAFCi at a dose of 100mg/kg was able to reduce the levels of malonialdehyde (MDA) in the liver, when compared to the negative control group (Graph 1).

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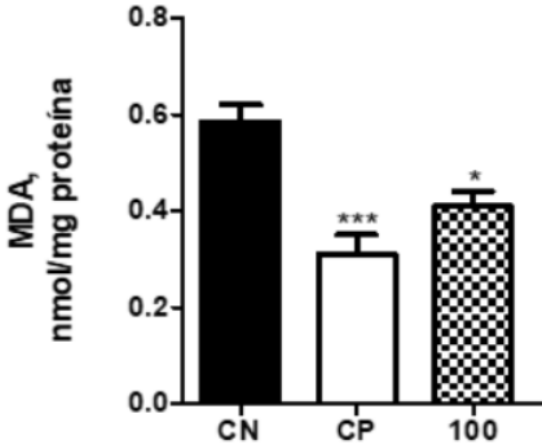
Graph 1: Measurement of the levels of thiobarbituric acid reactive substances (TBARS) in the negative control (NC), positive control (PC) groups and in the groups that received the 100 mg/kg doses of EAFCi.



Source: The author (2024). Legend The values represent the means ± d.p.m. (n= 5/group). Statistically different from the control group (ANOVA followed by Tukey, *p <0.05; *p<0.01; ***p<0.00).

Reduced glutathione, also known as GSH, is an endogenous component of cellular metabolism, a tripeptide composed of glycine, cysteine, and glutamic acid. It is an integral part of the biotransformation of xenobiotic substances and serves to protect the body from oxidizing agents. The conjugation of glutathione (facilitated by a family of glutathione transferase enzymes) helps contribute to detoxification by binding electrophiles that could bind to proteins or nucleic acids, resulting in cell damage and genetic mutations. The greater its quantity, the more protected the body is (Pizzorno, 2014). Treatment with EAFCi increased the GSH level in the groups treated with silymarin and EAFCi at a dose of 100 mg/kg, when compared to the negative control. With this, we can affirm that EAFCi has an antioxidant activity at the concentration tested in this study.

A previous study developed by Cavalcanti et al. (2023) evaluated the antioxidant activity of the aqueous extract of *C. icaco leaves* against the diphenylpicrylhydrazil (DPPH) and 2,2'-azinobis-(3-ethylbenzothiazoline-6-sulfonic acid) radical assays It was verified that the dose of 62.5 µg/mL of the extract was able to overcome the IC50 parameter with 50.60 ± 0.26 % in the DPPH assay, as well as the ABTS assay showed sequestration of 49.31 ± 3.45 % of the radical at the concentration of 500 µg/mL and 65.65 ± 6.04 % at the concentration of 1000 µg/mL, evidencing the good antioxidant potential of the extract.

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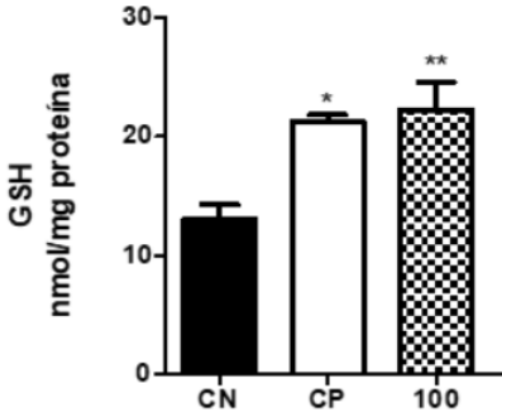
Graph 2: Measurement of glutathione reductase (GSH) levels in the negative control (CN), positive control (CP) groups and in the groups that received doses of 100 mg/kg of EAFCi.



Source: The author (2024). Legend: The values represent the means ± d.p.m. (n= 5/group). Statistically different from the control group (ANOVA followed by Tukey, *p <0.05; **p<0.01)

Paracetamol-induced hepatic giving is already well established in the literature (Aslmarz *et al*., 2019). In this work, it was evidenced that the use of Paracetamol increased the oxidant content with the increase in MDA levels and decreased the levels of reduced glutathione, an antioxidant agent, in the animals of the negative control group. It is noticed, however, that the treatment with EAFCi at a dose of 100 mg/kg was able to prevent the damage caused by paracetamol in the livers of the animals, a result that could be proven in the histopathological examination, in which the liver of the animals of the group treated with silymarin (positive control) and the one treated with 100 mg/kg of EAFCi presented hepatocytes of polygonal morphology, with preservation of tissue architecture. On the other hand, in the microscopic analysis of the liver tissue of the animals in the negative control group, hemorrhagic focus, presence of inflammatory infiltrate of polymorphonuclear cells, hepatocyte with vacuolated cytoplasm, macronucleolus and chromatin condensation (Figure 1) were found.

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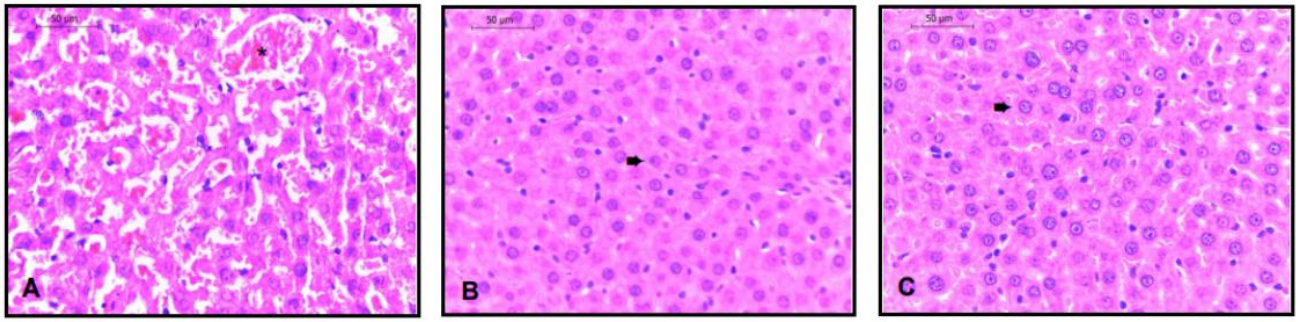
Figure 1: Photomicrograph of the livers of the experimental groups. (A) negative control, (B) positive control, (C) 100mg/kg.



Source: The author (2024). Legend: Asterisk – Vein with congestion; short arrow – polyhedral hepatocytes. H.E.

Regarding the potential for hepatoprotection, it was observed that there is an association with the presence of bioactive compounds in plants, such as flavonoids, phenolic compounds, lignins and others, which play a fundamental role in the hepatoprotective effects attributed to plant species. It is known that hepatotoxic agents act as precursors of highly reactive molecules, with the ability to cause significant damage to biological structures and that inflammation resulting from injury can substantially intensify oxidative stress, which makes bioactive compounds, especially those with antioxidant activity, an important focus in scientific research. In this context, plants gain prominence due to their phytochemical composition (Pereira *et al*., 2016). Previous studies have shown that fractions rich in phenolic compounds and flavonoids, from various fruits or medicinal herbs, as well as proanthocyanidin extracts, have the ability to prevent liver injury, necrosis, and apoptosis in mouse models subjected to hepatotoxic agents (Liu *et al*., 2016; Sun *et al*., 2021; Zhou *et al*., 2018).

In the present study, silymarin and *Chrysobalanus icaco* extract demonstrated similar hepatoprotective effects. The potential hepatic protection mechanism attributed to silymarin is related to its high content of phenolic compounds, known for their significant contribution to antioxidant activity (Dash, 2007). The results obtained for the extract are consistent with previous studies on the chemical composition of *C. icaco* leaves, in which the main chemical constituents analyzed by UPLC-DAD-ESI-QTOF-MS/MS predominantly included terpenes, aglycone, and glycosylated flavonoids (Ribeiro *et al*., 2020).

Thus, it is plausible that the mechanism of action of the hepatoprotective effect of the extract is associated with its antioxidant activity, due to its high concentration of flavonoids, recognized for their potent ability to eliminate free radicals and strengthen the antioxidant

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defense system. In addition, the content of steroids, with anti-inflammatory properties, may also play a relevant role in the hepatoprotection conferred by the extract.

CONCLUSION

It was concluded that the aqueous extract of the leaves of *Chrysobalanus icaco* at a dose of 100mg/kg has hepatoprotective properties against Paracetamol-induced liver damage, through the increase in the levels of GSH, which has an antioxidant character, and the decrease in the levels of MDA, which shows that the EAFCi at the dose of 100mg/kg was able to reduce the amount of substances resulting from oxidation. These results corroborate the histopathological analysis of the liver, which did not show significant alteration of the normal morphology in the group with the administration of the dose of 100mg/kg of the EAFCi. This hepatoprotective character is probably conferred by the presence of flavonoids in the EAFCi.

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