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ORIGINAL ARTICLE

Investigation of antioxidant activity of ethanolic extract of the bark of *Caesalpinia ferrea* in Swiss mice exposed to paracetamol

Investigação da atividade antioxidante do extrato etanólico da casca de Caesalpinia ferrea em camundongos Swiss expostos ao paracetamol

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Abstract

Aims: it was evaluated the antioxidant effect of the ethanolic extract of *Caesalpinia ferrea* bark in a model of oxidative stress induced by paracetamol (PCM).

Methods: male *Swiss* mice were subdivided into four groups (control; PCM; PCM+extract; extract; n=8) in which a dose of paracetamol (250 mg.kg^{-1}) was administered and after 3 hours the treatment with the extract (100 mg.kg^{-1}/ day) was administered for seven days, via gavage. Oxidative stress biomarkers were determined, such as catalase, glutathione-S-transferase, reduced glutathione, ascorbic acid, thiobarbituric acid reactive substances and carbonylated proteins of liver, kidneys and brain and plasma parameters through the dosage of glucose, cholesterol, triglycerides, aspartate aminotransferase and alanine aminotransferase.

Results: the *Caesalpinia ferrea* extract was able to reverse the lipid and protein damage caused by the drug in the liver tissue and caused the same effect in the renal and brain tissues in the carbonylated proteins. The extract alone decreased liver glutathione-S-transferase and increased catalase and brain glutathione-S--transferase activity, in addition to lowering glucose and cholesterol, but without altering the triglycerides.

Conclusions: it was possible to conclude that the ethanolic extract of the bark of *Caesalpinia ferrea* has a good antioxidant activity, probably due to the presence of tannins, in view of the damage caused by the high dose of paracetamol in the samples investigated. However, more studies are needed for a better understanding of the effects of this extract compared to the effects found in this research.

Keywords: *Caesalpinia ferrea*, biochemistry, ethanolic extract, oxidative stress, paracetamol, acetaminophen.

Resumo

Objetivos: foi avaliado o efeito antioxidante do extrato etanólico da casca de *Caesalpinia ferrea* em modelo de estresse oxidativo induzido por paracetamol (acetaminofeno, PCM).

Métodos: camundongos *Swiss* machos foram subdivididos em quatro grupos (controle; PCM; PCM+extrato; extrato; n=8) nos quais foi administrada uma dose de paracetamol (250 mg.kg-^{1}) e após três horas foi administrado o tratamento com o extrato (100 mg.kg-^{1}/ dia) por sete dias, via gavagem. Foram determinados biomarcadores de estresse oxidativo, como catalase, glutationa-S-transferase, glutationa reduzida, ácido ascórbico, substâncias reativas ao ácido tiobarbitúrico e proteínas carboniladas do fígado, rins e cérebro, além de parâmetros plasmáticos através da dosagem de glicose, colesterol, triglicerídeos, aspartato aminotransferase e alanina aminotransferase.

Resultados: o extrato de *Caesalpinia ferrea* foi capaz de reverter os danos lipídicos e proteicos causados pela droga no tecido hepático, e também causou o

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mesmo efeito nos tecidos renal e cerebral nas proteínas carboniladas. O extrato sozinho diminuiu a atividade da glutationa-S-transferase hepática e aumentou a da catalase e glutationa-S-transferase cerebral, além de diminuir a glicose e o colesterol, mas sem alterar os triglicerídeos.

Conclusões: foi possível concluir que o extrato etanólico da casca de *Caesalpinia ferrea* apresenta uma boa atividade antioxidante, provavelmente devido à presença de taninos, tendo em vista os danos causados pela alta dose de paracetamol nas amostras investigadas. Entretanto, mais estudos são necessários para um melhor entendimento dos efeitos deste extrato frente aos efeitos encontrados nesta pesquisa.

Palavras-chave: *Caesalpinia ferrea*, bioquímica, extrato etanólico, estresse oxidativo, paracetamol, acetaminofeno.

Introduction

Brazil has had a long history of using medicinal plants to treat the health problems of the population, its use is built on experience and transmitted orally (1), with knowledge about medicinal plants often representing the only therapeutic option for many communities (2). According to the World Health Organization, a medicinal plant is defined as "any plant that has, in one or more organs, substances that can be used for therapeutic purposes or that are precursors of semi-synthetic drugs" (3).

Caesalpinia ferrea Mart (*C. ferrea*) is a legume widely distributed in the North and Northeast regions of Brazil (4), popularly known as pau-ferro or jucá, belonging to the Leguminosae-Caesalpinoidae family (5). Its bark is widely used in folk medicine because it has antiinflammatory (6), analgesic (7), antimutagenic (8) and chemopreventive properties towards cancer (9), besides hypoglycemic activity and demonstrated molecular mechanisms of *C. ferrea* bark extracts in streptozotocin-induced diabetes in *Wistar* rats (10). According to Araújo et al. (11), the presence of phenolic compounds, such as tannins and flavonoids present in the skin of *C. ferrea*, can positively interfere in the effects associated with wound healing and antiinflammatory activity. In the phytochemical analyses, several compounds in the genus *Caesalpinia* were isolated, the main ones being flavonoids, diterpenes and steroids (12). In studies carried out with the bark of the stem of *C. ferrea* revealed the presence of flavonoids (catechins and epicatechins), coumarins, saponins, steroids, hydrolyzable tannins (gallic acid and ellagic acid) and other phenolic compounds (10).

Oxidative stress is defined as a state of imbalance between oxidizing radicals, free radicals, and antioxidant systems as the body's internal defense mechanism (13, 14). The imbalance occurs because of the excessive level of reactive oxygen species (ROS) or the inadequate functioning of the antioxidant system (15). ROS are molecules with an unpaired electron, including peroxides, superoxide, hydroxyl radical and singlet oxygen, therefore being very reactive and toxic to cells, as they affect different cellular components in various ways (16).

Paracetamol (PCM; N-acetyl-p-aminophenol or acetaminophen) is a widely used antipyretic and analgesic medication, which can induce severe acute drug-induced liver damage when taken in overdose (17). PCM is oxidized by cytochrome P450, which leads to the formation of the toxic electrophilic metabolite, N-acetyl-p-benzoquinone imine (NAPQI) (18). Therefore, the overdosing, such as 1 $g.kg^{-1}$ of PCM, leads to the accumulation of this metabolite, which is a highly reactive compound that acts as a precursor to the toxic side effects observed in the body (19). This fact is triggered because there is saturation of the glucuronidation and sulfation pathways, producing more NAPQI, which depletes reduced glutathione (GSH) reserves and GSH-dependent enzymes and forms protein adducts by binding to the cysteine groups in cellular proteins and forming *N*-acetyl-*p*-aminophenol-cysteine adducts (20). With the increase in this protein adducts formed by NAPQI, oxidative stress, mitochondrial damage and centrilobular necrosis occurs, and eventually leads to liver damage (21).

Considering the antiinflammatory properties already described by *C. ferrea* and studies done by Wyrepkowski et al (8) who identified antioxidant compounds such as ellagic acid and gallic acid found in the ethanolic extract of the stem bark of *C. ferrea*, in addition to that overdosage with PCM can deplete glutathione and release proinflammatory agents, for instance, inflammatory

cytokines (22), we hypothesized that the stem bark extract from *C. ferrea* can exhibit antioxidant activity against PCM-induced oxidative stress in mice. For this, the animals will be intoxicated with a high dose of PCM and treated for seven days with the ethanolic extract of the plant.

Methods

Extract preparation

The bark of the stem of *C. ferrea* was collected in the city of Juína / MT, Brazil (11 ° 22'40 "S and 58 ° 44'27" W) and the botanical identification was performed at the Universidade Federal de Mato Grosso (UFMT), Sinop / MT campus, Brazil. An exsiccata No. 3021 was deposited at the Herbarium Centro Norte Mato-Grossense.

According to the methodology of Wyrepkowski et al. (8) for the preparation of the extract, the stem bark was dried and ground to powder (1610.0 g) of *C. ferrea*. Hereafter it was mashed at room temperature with ethanol (4 × 48 h). The solution was evaporated under vacuum resulting in 260.09 g (16.2%) of crude ethanolic extract (EXT) from the stem bark. From the EXT, chemical studies were also carried out to quantify total phenols and antioxidant potential with 2,2-Diphenyl-1-picrylhydrazyl (DPPH).

Determination of Total Phenols Content

The concentration of total phenols from EXT was determined colorimetrically according to the standard procedure of Folin-Ciocauteau (23). For the same, 0.625 mg of EXT was used and dissolved in 10 mL of MeOH (62.5 µg.mL^{-1}), 10 mL of Folin-Ciocauteau solution obtained by diluting 0.667 mL of commercial Folin Ciocauteau with distilled water, 5 mL of saturated Na_2CO_3 solution (200 g.L^{-1}); 2.5 mg of Gallic Acid (Merck®, Darmstadt, Germany), used as a standard, in 10 mL of MeOH (250 µg.mL-^{1}). The calibration curve with gallic acid was performed at concentrations of 7.81, 15.62, 31.25, 62.5, 125.0 and 250.0 µg. mL^{-1}. Next, 150 µL of the Folin-Ciocauteau solution and 50 µL of sample in standard dilutions were added to a 96-well plate, after three minutes, 50 µL of saturated Na_2CO_3 solution was added and 2 h were left until the reaction was completed. After the reaction, reading was performed in a UV/Visible spectrophotometer at 750 nm. The entire procedure was performed in triplicate. The principle of the procedure for evaluating total phenols is the reaction between the Folin--Ciocalteau reagent and phenolic compounds, with subsequent oxidation of the phenols and formation of a blue complex (24). In the first reaction step, deprotonation of phenolic compounds (for example, the standard gallic acid) occurs in a basic medium, generating phenolate anions. From there, an oxidation-reduction reaction occurs between the phenolate anion and the Folin reagent, in which the molybdenum, component of the Folin reagent, is reduced and the reaction medium changes color from yellow to blue (25).

Assessment of Antiradical Potential

The EXT of *C. ferrea* stem bark was also analyzed for its ability to capture the free radical DPPH. The antiradical potential was evaluated by means of a spectrophotometric assay, according to the methodology described by Pauletti et al. (26) with modifications. A solution of 0.004% DPPH in methanol was used which was mixed with the EXT solution. Stock solutions were prepared from 2.5 mg of the test sample (EXT and standards) in 10 mL of methanol (250 mg.mL-^{1}). Then, dilutions were performed up to concentrations of 6.25; 12.5; 25.0; 50.0; 100.0 and 200.0 µg.mL^{-1}. To each 20 µL of the sample, 200 µL of the DPPH solution was added. After 30 minutes of reaction, the absorbance of the solutions was measured at 517 nm. The reference solution was made with 20 µL of methanol plus 200 µL of the DPPH solution. Gallic acid (Merck®, Darmstadt, Germany) and quercetin (Sigma-Aldrich®, St. Louis, USA) standards were used, which were submitted to the same experimental procedure as the EXT. The entire procedure was performed in triplicate. Antiradical activity was determined by the equation:

Antiradical activity (%) = ((Negative control absorbance - sample absorbance)/ Negative control

absorbance x 100)

The efficient concentration of EXT, that is, the amount of antioxidant needed to decrease the initial concentration of DPPH by 50% (EC_{50}), was determined using the Microsoft Office Excel Program, from an exponential curve, obtained by plotting on the abscissa the sample concentrations ($\mu g.mL^{-1}$) and in the ordinate, the percentage of remaining DPPH (% DPPHREM).

This test evaluates the ability of a substance to scavenge the stable free radical DPPH and is based on the reduction of the DPPH solution. DPPH is violet in color, has absorption at 517 nm and, when reduced, turns yellow. This reduction occurs when there are substances that can cede a radical species (radical hydrogen) to DPPH, giving rise to another stable radical, which is associated with the antiradical property (27).

Animals and In Vivo Treatment

This study was approved by the Comitê de Ética no Uso de Animais under protocol number 23108.781869/12-0. Male *Swiss* mice with an average weight of 30 ± 5 g were obtained from the Vivarium of Universidade Federal de Mato Grosso, Campus Cuiabá. First, the animals were acclimated for 14 days with food and water *ad libitum*, room temperature (25 ± 1 ˚C), relative humidity (51 ± 2%) and with a 12-hour light / dark period.

After acclimatization the animals were divided into 4 groups (n = 8) according to the following groups: Control (filtered water + Tween 80), PCM (250 $mg.kg^{-1}$ PCM in a single dose + Tween 80), PCM + EXT (250 $mg.kg^{-1}$ of PCM in a single dose + 100 $mg.kg^{-1}$ of EXT) and EXT (filtered water + 100 $mg.kg^{-1}$ of EXT). Subsequently, oxidative stress was induced through PCM acute intoxication at a dose of 250 $mg.kg^{-1}$, which was based on a study by Olaleye and Rocha (28). The administration of the treatment started after three hours of the administration of the PCM, in which it occurred with filtered water or with the EXT prepared in an aqueous solution containing 0.1% of Tween 80 (vehicle) at the dosage of 100 $mg.kg^{-1}$. This dose was established according to the Malone Hippocratic test (29). The animals were treated orally by gavage (0.3 mL/day/per animal) for a period of 7 days.

After 24 hours of the last dose and after 8 hours of fasting, the animals were anesthetized (ketamine 50 mg.kg-1, xylazine 2 $mg.kg^{-1}$ and acepromazine 2 $mg.kg^{-1}$), blood was collected (with heparinized syringes) by cardiac puncture and centrifuged at 1000 g for 10 minutes to obtain plasma. Subsequently, the animals were euthanized by cervical dislocation, the liver, kidneys and brain were removed and washed with saline solution (0.15 M NaCl) and then frozen at -85 ˚C.

Biochemical Analysis

Enzymatic antioxidant activities were measured in liver, kidney and brain tissues. Catalase activity (CAT) was determined according to Nelson and Kiesow (30). The principle is based on the decomposition of H_2O_2 and measured spectrophotometrically at 240 nm and expressed in µmol H_2O_2 min^{-1} mg $protein^{-1}$. The activity of glutathione-S-transferase (GST) was determined according to Habig et al. (31) and the enzymatic activity was measured according to the formation of the GS-DNB adduct. The result was expressed in µmol GS-DNB min^{-1} mg $protein^{-1}$.

The non-enzymatic antioxidant GSH was measured by the colorimetric method that consists of a reaction of the sulfhydryl groups developed by Sedlak and Lindsay (32) in the liver, kidneys and brain and quantified at 412 nm. The result was expressed in µmol GSH mg $protein^{-1}$ and compared to a standard GSH curve. The levels of ascorbic acid (ASA) in the liver and brain were determined according to Roe (33), using the colorimetric method and read at 520 nm absorbance. The result was expressed in µmol ASA g $tissue^{-1}$ and compared with a standard curve of ascorbic acid.

The oxidative damage to lipids in the liver was determined by spectrophotometric identification of the levels of thiobarbituric acid reactive substances (TBARS) and presented as malondialdehyde (MDA) levels, according to Buege and Aust (34). The amount of lipid peroxidation was

expressed in nmol MDA mg protein^{-1}. Oxidative damage to proteins in the liver, kidneys and brain was measured by determining carbonyl groups according to Yan et al. (35). The amount of carbonylated proteins (carbonyl) was expressed in nmol of carbonyl mg protein^{-1}.

The protein content of the sample (except ASA) was determined during the analysis by the method of Bradford (36) at 595 nm, using bovine serum albumin as a standard for the construction of the calibration curve.

The enzymatic activities of alanine aminotransferase (ALT) and aspartate aminotransferase (AST), and the quantification of plasma glucose, total cholesterol and triglycerides in plasma were performed using commercial kits (Labtest®, Diagnostic S.A, Minas Gerais, Brazil).

Statistical Analysis

Results were presented as mean and standard deviation, analyzed according to one-way ANOVA and followed by Tukey's post hoc test. The homogeneity of the variances was verified using the Bartlett's test. The level of significance established for rejection of the null hypothesis was 5%.

Results

Total Phenols

The total phenol content was determined by interpolating the absorbance of the samples from the analytical curve constructed with the gallic acid standard (7.81 to 250 µg.mL^{-1}) and expressed as mg of gallic acid equivalent per g of EXT. The analytical curve was constructed from the absorbance values at 750 nm and having methanol as "blank". The equation of the analytical curve for gallic acid was C = 93.118A – 41.834, where C is the concentration of gallic acid and A is the absorbance at 750 nm and the correlation coefficient R = 0.9956 (**Figure 1).** The result obtained from the total phenols content was 480.00 mg gallic acid equivalent for each g of EXT from the stem bark of *C. ferrea*.

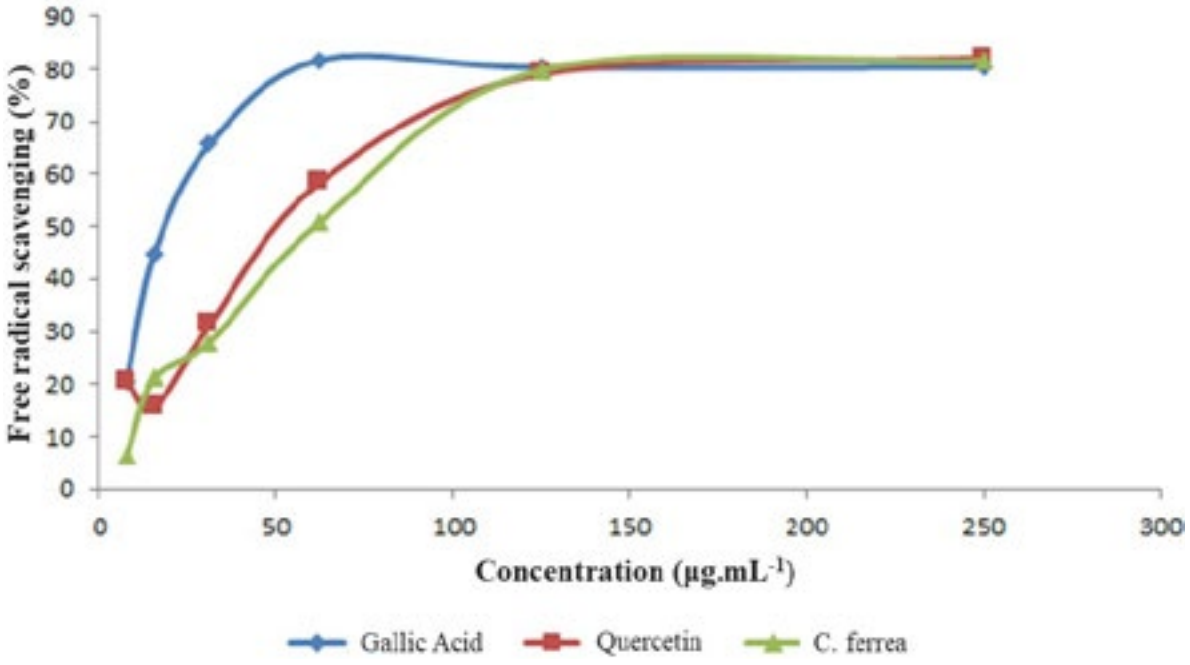

Figure 1. Analytical curve of total phenols at 750 nm. Standard - Gallic Acid.

Assessment of Antiradical Potential

The results of the anti-radical activity of the ethanolic extract of *C. ferrea* and of the gallic acid and quercetin standards, obtained with the antiradical assay by DPPH, are presented in the **Figure 2**. The amount of EXT necessary to decrease the initial concentration of DPPH by 50% (EC_{50}), revealed values of EC_{50} = 55.43±0.34 µg.mL^{-1} for the EXT and EC_{50} = 48.80±0.82 µg.mL^{-1} and EC_{50} = 21.80±1.23 µg.mL^{-1} for quercetin and gallic acid standards, respectively.

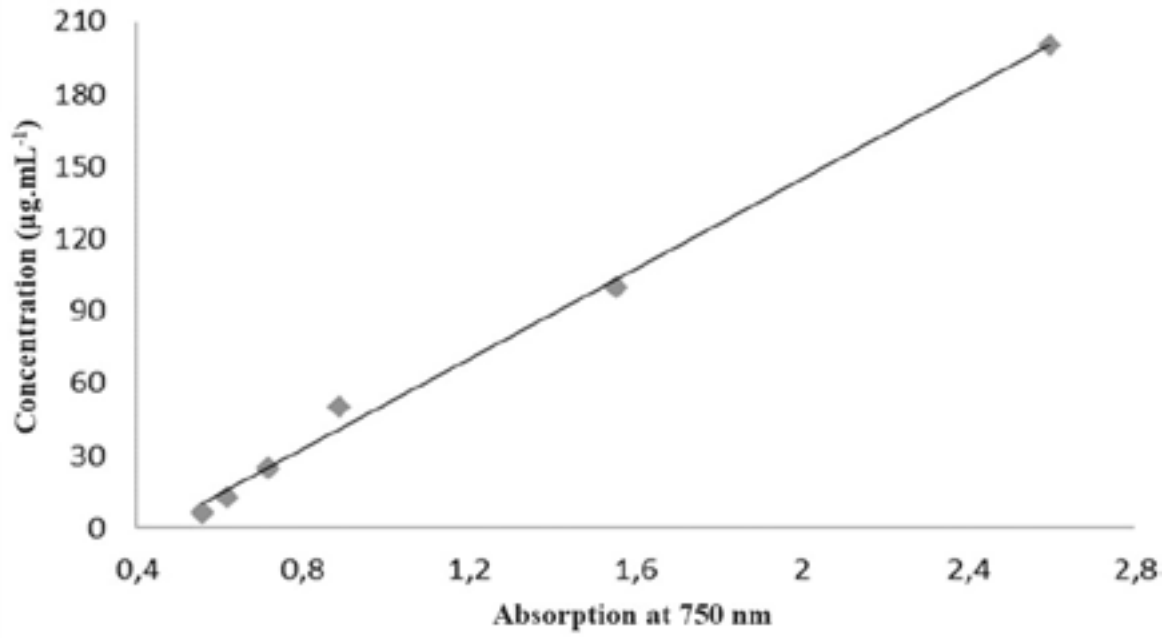

Figure 2. Percentage of DPPH inhibition by the ethanolic extract from *C. ferrea* and by the standards quercetin and gallic acid. DPPH, 1,1-diphenyl-2-picryl hydrazil.

Biochemical Analysis

The results of **Figure 3** are presented in sequence. CAT activity decreased in the PCM group and EXT didn't recover this effect. We observed that all the treatments were able to reduce the hepatic GST activity. For the hepatic GSH levels, there was a decrease after the administration of the oxidative stress inducer (PCM) and the extract was not able to reverse this damage (PCM+EXT *versus* PCM), but for ASA levels, the same effect didn't occur. On the other hand, the EXT reduced the increase of TBARS and protein carbonylation levels in the PCM group, although the EXT alone increased TBARS levels. In addition, such as observed in **Table 1**, the same pattern of result was obtained for kidney and brain once EXT reduced protein carbonylation increased by PCM. Besides, CAT and GST activities in the brain tissue increased in the EXT group, but this effect didn't occur in renal tissue. For plasma analyses, EXT reduced the increase ALT activity in the PCM group returning to control values but not to AST activity, and diminished glucose and cholesterol levels *per se* without altering triglycerides.

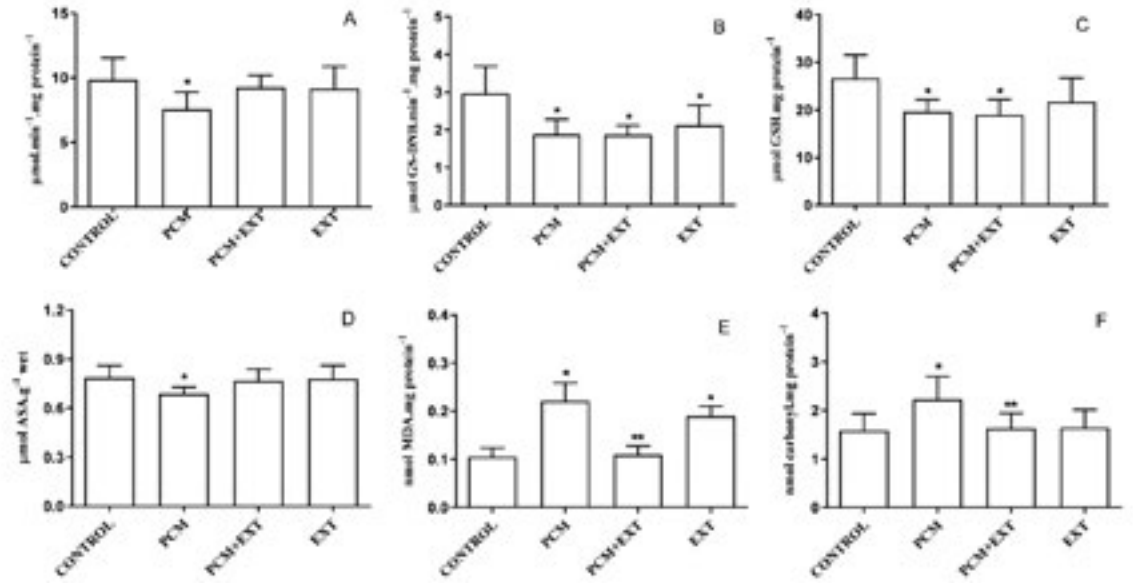

Figure 3. Effect of ethanolic extract from *C. ferrea* under oxidative stress induced by PCM on liver tissue, graphs of: (A) CAT, (B) GST, (C) GSH, (D) ASA, (E) TBARS and (F) Carbonyl (n = 8). ASA, ascorbic acid; ALT, alanine aminotransferase; Carbonyl, carbonylated proteins; CAT, catalase; EXT, crude ethanolic extract; GSH, reduced glutathione; GST, glutathione-S-transferase; PCM, Paracetamol, TBARS, thiobarbituric acid reactive substances. *p <0.05 compared to Control group; **p <0.05 compared to PCM group. 1-way ANOVA followed by Tukey test.

TABLE 1 – Effect of ethanolic extract from *C. ferrea* under PCM-induced oxidative stress on renal and brain tissue and plasmatic parameters.

Group	CONTROL	PCM	PCM + EXT	EXT
Renal Tissue				
CAT	13.4±2.1	14.2±2.7	14.2±1.6	15.1±1.3
GST	1.3±0.19	1.0±0.23*	1.1±0.15	1.2±0.17
Carbonyl	6.3±1.4	15.0±3.1*	7.7±1.9ǂ	8.8±1.8
Brain Tissue				
CAT	0.74±0.16	0.79±0.18	0.68±0.15	1.29±0.19*
GST	0.12±0.02	0.14±0.02	0.12±0.02	0.18±0.02*
GSH	12.7±1.96	15.2±3.43	12.8±1.31	14.5±2.43
ASA	0.41±0.04	0.45±0.03	0.39±0.03	0.40±0.02
Carbonyl	4.5±0.8	7.2±1.8*	4.7±1.1ǂ	5.5±1.3
Plasma				
Glucose	208.6±31	188.9±37	132.5±24*	91.3±19*
Cholesterol	79.0±9	70.3±14	46.2±9*	61.1±14*
Triglycerides	88.4±17	76.4±16	87.2±20	82.8±14
ALT	27.1±6	47.0±10*	32.9±5ǂ	32.6±7
AST	85.3±21	169.0±39*	155.2±23*	362.6±28*

ASA, ascorbic acid; ALT, alanine aminotransferase; AST, aspartate aminotransferase; Carbonyl, carbonylated proteins; CAT, catalase; EXT, crude ethanolic extract; GST, glutathione-S-transferase; reduced glutathione, GSH, reduced glutathione; PCM, Paracetamol. The results are expressed as mean ± SD; n = 8 animals. *p <0.0001 compared to Control group, ǂp <0.0001 compared to PCM group. 1-way ANOVA followed by Tukey test.

Discussion

Plants produce a variety of antioxidant substances, among which phenolic compounds stand out. The extract evaluated in this study showed high levels of phenolic compounds when compared to thyme (*Thymus vulgaris* L.), sage (*Salvia officinalis* L.), and marjoram (*Origanum majorana* L.) extracts (37). The antioxidant activity of phenolic compounds is mainly due to their reducing properties and, thus, they play an important role in the neutralization or scavenging of free radicals and chelation of transition metals, acting both in the initiation stage and in the propagation of the oxidative process (38). Therefore, the anti-radical effect of EXT observed in this study is possibly due to the high content of total phenols since the anti-radical activity is normally correlated with the presence of phenols (38). In general, polyphenols and in particular tannins have a structure for the scavenging of radicals, indicating antioxidant capacity (39). Fruits and plant extract rich in ellagic acid, gallic acid and hydrolysable tannins are reported in the literature to exert strong antioxidant effects (40, 41).

In this line, the use of medicinal plants for the treatment of diseases in humans has increased considerably worldwide (42). This is due to the difficulty in accessing health care by the population, who do not have their demands and needs met and therefore, are partially provided by the use of alternative therapies and also by a personal alternative (43). One of the major problems faced is that the use of phytotherapies and medicinal plants currently used by self-medication or by medical prescription has no known toxic (44). Thus, in this study, we decided to investigate the possible effect of the EXT as an antioxidant in the model of oxidative stress induced by paracetamol, whose hepatotoxicity in high doses generates ROS and inflammatory processes (28).

There is a great diversity of secondary metabolites present in plants, making them a source of biomolecules for therapeutic purposes, such as phenolic compounds that contain antioxidant properties due to their chemical structure (45). The EXT used in this experiment was studied and the phytochemical parts were analyzed by Wyrepkowski et al. (8) who characterized and quantified the compounds, identifying twenty-six molecules, in which hydrolysable tannins derived from gallic acid and ellagic acid predominated. On the other hand, Souza et al. (45), studying the crude ethanolic extract and ethyl acetate extract from leaves of the same plant, observed different compounds, such as the flavonoids rutin, amentoflavone, quercetin-3--D-glycoside, taxifolin, apigenin, quercitrin and luteolin which were found in the crude ethanolic extract and all of these, except quercitrin were identified in the ethyl acetate extract, which demonstrates that different parts of the plant have different bioactive compounds and that can result in different effects.

Tannins are polyphenolic compounds extracted from plants and are composed of different chemical structures (46), in which hydrolysable tannins can be found that include gallotannins and ellagitannins (47). They consist of gallic acid esters and ellagic acid glycosides that are formed from chiquimic acid (48) or condensed tannins that are polymers of proanthocyanidins (46). They are secondary metabolites whose characteristic are the ability to complex with macro and micro molecules and can act in the sequestration of free radicals, allowing for a series of pharmacological applications (49). Gallic acid can eliminate free radicals, acting as an antioxidant and is responsible for some biological activities (50) as an antioxidant and anti-inflammatory agent (51), as well as a bactericidal and bacteriostatic agent (52), along with anticancer and antiangiogenic properties (53). Ellagic acid, on the other hand, has antioxidant properties and the ability to capture free radicals that result in the prevention or reduction of oxidative stress, a condition involved in several disorders, in addition to having a neuroprotective and anti-carcinogenic effect (54). On the same note, flavonoids contain antioxidant, anti-cancer, antimicrobial, antiviral and anti-aging properties, in which they can promote various biological effects in different types of cells (55). As an antioxidant, flavonoids can suppress the

formation of ROS by inhibiting enzymes that generate these compounds (such as microsomal monooxygenase and NADH oxidase), scavenging, chelating metals involved in the generation of these molecules, or by increasing the activities of antioxidant and detoxifying enzymes (such as glutathione peroxidase, glutathione reductase and CAT) (56). Catalase is involved in blocking the chain of free radicals produced by living beings together with superoxide dismutase and glutathione peroxidase, in which they prevent oxidative modifications of DNA, proteins and lipids (57). Its action occurs in catalyzing the conversion of hydrogen peroxide (H_2O_2) into water and molecular oxygen (58). In our study, the EXT was not able to recover the inhibition of this enzyme that was caused by the medication.

PCM is a widely studied drug, as it has a hepatotoxic potential after an overdose in experimental animals and in humans (59). In this context, Blieden et al. (60) observed that the PCM hepatotoxicity remains the leading cause of acute liver failure in the USA and Europe, with over 300,000 hospitalizations annually in the USA and up to 42 % of all cases of acute liver failure attributable to acetaminophen overdose. The conventional dose of PCM is metabolized to non-toxic compounds that are excreted in the urine, while a small remaining part is metabolized by CYPs to a highly reactive intermediate metabolite, NAPQI (61). NAPQI binds to glutathione (GSH) and leads to the rapid depletion of hepatic GSH, consequently after GSH depletion; NAPQI binds to other cellular thiol proteins (especially mitochondrial proteins) and disrupts the mitochondrial respiratory chain, which causes the formation of mitochondrial ROS (62). In our studies, we observed a decrease in hepatic GSH levels after the administration of the oxidative stress inducer (PCM) and the EXT was not able to recover this non-enzymatic antioxidant. However, in pharmacological doses of PCM, NAPQI is rapidly conjugated with GSH spontaneously and by GST (63), which in this case, in high doses, is depleted and therefore is not conjugated. Consequently, there is a decrease in GST activity, as occurred in our study in which GST in the liver decreased in the PCM groups and the EXT was not able to restore this enzymatic antioxidant.

The depletion of GSH induced by NAPQI can aggravate oxidative stress and lipid peroxidation, which ends up leading to the acceleration of necrosis and apoptosis in the liver tissue (64). According to Hasanein and Sharifi (65), oxidative stress induced by a high dose of PCM significantly increased the concentration of MDA in the liver of *Wistar* rats. This is in line with our study, in which there was an increase in TBARS in the liver of the PCM group and the EXT was able to reverse the damage caused by the drug, decreasing liver lipid peroxidation, however, the EXT *per se* presented increased TBARS levels. Lipid peroxidation, under toxic conditions as in the case with this drug, can induce cells to necrosis or apoptosis because the extent of oxidative damage exceeds their repair capacity, and therefore, causes damage to the molecular components of cells, which can facilitate the development of various diseases and premature aging (66).

Protein carbonylation is irreversible and generally results in impairment or even loss of protein function (67). Its oxidative modification can be initiated by the reaction with hydroxyl radicals, but it is even more dependent on the concentration of the superoxide radical and hydrogen peroxide in the medium (68). In our studies, protein damage increased in the PCM group, and the plant extract was able to reverse this damage. This fact may have happened due to ellagic acid, which is considered a polyphenolic compound, with protective effects that can be attributed to some factors, which include binding to DNA, inhibition of ROS production and its elimination, protection of DNA against injuries from alkylating agents, thereby ellagic acid neutralizes free radicals and inhibits lipid peroxidation and protein carbonylation (69). For the levels of ASA, a good antioxidant, soluble in water and considered essential to the organism through its action as a redox agent in biological systems (70), it was found that this non-enzymatic antioxidant decreased in PCM group, and the EXT did not interfere in this parameter. The same

results occurred in the assay by Magalhães et al. (71), in which they used the same oxidative stress model, but used the ethanolic extract and the ethyl acetate fraction extract from the leaves of *Trattinnickia rhoifolia*.

Drug-induced liver damage, in this work using PCM as a model, can lead to increased concentrations of serum enzymes such as AST, ALT, alkaline phosphatase, in addition to increased serum bilirubin, glucose, triglycerides, urea and creatinine (72). The permeability of hepatocyte cell membranes is impaired after cell damage, which in this case was caused by PCM. Because of this, the liver enzymes AST and ALT are released into the circulation, which causes a significant increase in their levels (73, 74). In our study, there was an increase in the enzymes ALT and AST activities, however, for ALT, the extract was able to restore the values at the control levels. These increases in the enzyme's activities (PCM group) are in line with the model used in the studies by Olaleye and Rocha (28). On the other hand, although the extract had a good effect on ALT, the same cannot be seen in the mitochondrial enzyme AST. According to Krithika and Verna (75), flavonoids can reduce ALT and AST activity in the serum of the animal exposed to the liver injury model. In our study, this EXT presents several compounds, and we didn't know the extent of effect each one can cause. Interestingly, GST reduction and an increase in TBARS, which measures lipid peroxidation, were observed. So, new studies are required to purify and identify the active compounds in this extract. On the other hand, in our research, there was a decrease in glucose and cholesterol.

These data demonstrate that the extract had a beneficial action, as it decreased serum glucose and cholesterol, demonstrating that the extract has a hypoglycemic and hypocholesterolemic action. Studies have shown that different hydrolysable tannins contain biological properties such as antitumor, antimutagenic, antidiabetic and antibiotic (76), and in the EXT some compounds were found, consisting of hydrolysable tannins such as gallic acid and ellagic acid that were quantified according to Wyrepkowski et al. (8). Still, the results by Souza et al. (45) demonstrated that the extract of the leaves of *C. ferrea* has a hypoglycemic action and decreased triglyceride levels, which contradicts our findings regarding triglycerides, but demonstrates that to some extent this plant interferes with the metabolism of different lipid molecules. It is suggested that tannins may have an insulin-like effect on tissues sensitive to their action, this demonstrates that they can act on cells by modifying or interacting with certain specific proteins found in important intracellular signaling pathways and, therefore, affect their role in the improvement of hyperglycemia (40, 77). Furthermore, tannins are associated with the inhibition of cholesterol biosynthesis, in which it inhibits the enzyme hydroxymethylglutaryl-CoA reductase, so that its absorption can decrease this parameter (78).

However, paracetamol overdose can cause hepatotoxicity and nephrotoxicity (79), through ROS, which are one of the main mechanisms of renal pathogenesis and which can lead to apoptosis and senescence of these cells, in addition to fibrosis in the kidney (80, 81). In our studies, there was a depletion in GST and an increase in protein damage in the PCM group and the extract was able to reverse this renal protein damage. A similar result was found by Souza et al. (45) who used the same oxidative stress model. We can suggest that this beneficial effect against protein carbonylation may be related to gallic acid that has antioxidant and anti-inflammatory effects (82), and its protective action consists of the ability to inhibit cell damage induced by ROS, in addition to regulating positively the expression of glutathione peroxidase, although it was not investigated in this study, and attenuate the presence of free radicals (83). This damage was also seen by Pereira et al. (84), in which they used the same experimental model, but it was reversed by the ethanolic and ethyl acetate stem bark extracts of Copaifera multijuga at a concentration of 250 mg kg^{-1}.

In brain tissue, oxidative stress occurs because this structure is more susceptible, it has high metabolic activity, a high density of oxidizable substrates and because it has a relatively low

antioxidant defense (85). Through this, the metabolism of PCM the cytochrome P450-dependent pathway is associated with the development of harmful substances, such as NAPQI in brain astrocytes and neurons (86, 87). Our findings showed that the EXT increased the activity of antioxidant enzymes CAT and GST in brain tissue and was able to decrease the damage caused by the drug to protein carbonylation. This action of the extract may be due to the fact that gallic acid has the ability to permeate the blood-brain barrier and act in a neuroprotective way (88) through its antioxidant and free radical scavenging properties, inactivating the enzymes responsible for production of ROS or by the positive regulation of antioxidant enzymes (89), which in this way, increased CAT and GST.

It is known through the literature that paracetamol overdose causes several damages to the organism, however, with the administration of the ethanolic extract of *C. ferrea* it was possible to reduce liver damage through the reversal of lipid peroxidation (TBARS) and protein damage (carbonyl) caused in the three tissues (liver, kidney and brain). The extract was shown to have a hypoglycemic and hypocholesterolemic effect. The antioxidant effect of the extract can be demonstrated through its constituents, mainly tannins such as gallic acid and ellagic acid. The study with this part of the plant is unprecedented and, therefore, it is necessary to investigate further to better elucidate the possible benefits of the stem bark of *C. ferrea*.

Notes

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Conflicts of interest disclosure

The authors declare no competing interests relevant to the content of this study.

Authors' contributions

All the authors declare to have made substantial contributions to the conception, or design, or acquisition, or analysis, or interpretation of data; and drafting the work or revising it critically for important intellectual content; and to approve the version to be published.

Availability of data and responsibility for the results

All the authors declare to have had full access to the available data and they assume full responsibility for the integrity of these results.

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