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GASTROPROTECTIVE AND CICATRIZING ACTIVITY OF THE *ZIZIPHUS JOAZEIRO* MART. LEAF HYDROALCOHOLIC EXTRACT

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Ziziphus joazeiro Mart., popularly known as 'juazeiro', is a species used in popular medicine for the treatment of bronchitis, gastric ulcers, skin wounds, and in the manufacture of cosmetic and food products. The objective of this study is to evaluate the gastroprotective and cicatrizing activity of the *Z. joazeiro* Mart. leaf hydroalcoholic extract (EHFZJ). The acute pre-clinical toxicity was determined by the single administration of the EHFZJ (2000 mg/kg/p.o.) and by assessing clinical signs of toxicity, according to established criteria by Malone, or mortality. Gastroprotective activity was identified through classical models of acute gastric lesions induced by indomethacin, absolute and acidified ethanol (100, 200 and 400 mg/kg/*per os*) and the physical barrier mechanism (400 mg/kg/*per os* or intraperitoneally). The cicatrizing activity of the EHFZJ was investigated by measuring the speed of wound closure and the percentage of contraction. The acute pre-clinical toxicity of EHFZJ showed no signs of toxicity and mortality. The EHFZJ demonstrated a gastroprotective effect at the 400 mg/kg dose in the classical models of acute gastric injury induced by indomethacin, absolute and acidified ethanol. The EHFZJ administration (orally) demonstrated significant inhibition, suggesting a possible physical barrier mechanism exists. The EHFZJ showed no significant differences in terms of percentage of contraction or the speed of wound closure during the observation times (0, 3, 7, 11 and 14 days). The results obtained in this study provide evidence of a potential gastroprotective activity for the *Ziziphus joazeiro* Mart. leaf hydroalcoholic extract.

Key words: *gastroprotection, Ziziphus joazeiro, cicatrizing properties, gastric lesions, indomethacin, quercetin, catechins, wounds*

INTRODUCTION

Gastric ulcer is a widely distributed disease in the world which can be caused by several factors, such as: stress, use of non-steroidal anti-inflammatory drugs (NSAIDs), alcohol ingestion and infection by the *Helicobacter pylori* bacterium (1). Ulceration occurs due to an imbalance between the defense factors, which include nitric oxide (NO), mucus and bicarbonate secretion, blood flow and prostaglandins, and mucosal aggressors, such as free radicals, increased hydrochloric acid and pepsin secretions, and reactive oxygen species (ROS) (2, 3). Treatment for gastric ulcers consists of using antacid medications, histamine 2 receptor antagonist (H2-Ras) and proton pump inhibitors, which although effective, their long-term use promotes the presentation of adverse effects, including hypersensitivity, arrhythmia, impotence and hematopoietic disorders (4, 5).

Wounds can be defined as injuries of a physical, chemical or thermal character, which as a consequence lead to the opening or rupturing of the skin's integrity. Wounds are also conceptualized as the rupture of the anatomical and functional integrity of living tissue (6). Cicatrizing consists of an orderly and punctual process, occurring in three phases: inflammation, proliferation and remodeling. However, flaws in some aspects of the remodeling process exist in chronic wounds, resulting in inadequate wound healing (7). The types of wounds, their etiologies and sites of involvement are as diverse as possible and therefore presents as another serious public health problem. Although great advances have been invested in research and development of resources and technologies in this area and great advances have been verified in understanding the phenomena involved in the various phases of tissue repair, this is still a problem that has repercussions on high financial costs and profound social consequences on the patients with these injuries, since they often develop sequences that can lead to the loss of limbs and their functions, with consequent departure from work and their activities of daily living (8).

In the therapeutic context, medicinal plants continue to be used worldwide to prevent and treat various pathologies. Given

this, natural products are gaining more and more attention as sources for the development of new molecules with bioactive potential (9), since these are known to be potent therapeutic agents (10).

The *Ziziphus joazeiro* Mart. species belongs to the Rhamnaceae family and the *Ziziphus* genus, which encompasses 100 species distributed worldwide (11). This genus has nine species in Brazil, five of which are endemic, including *Z. joazeiro* that is a tree typical of the Brazilian Northeast. In popular medicine, the different parts are used for food (fruit), as well as extract preparations with the bark were used as bucal antiseptic (12), leaves and roots, which are used for the treatment of fever, pain, infections, gingivitis, respiratory problems (13, 14), wounds, liver and heart disease, as well as a diuretic, including others (15). Studies using different *Z. joazeiro* parts present biological activities that have been proven in the literature such as: antimutagenic (bark) (16), antimicrobial (fruit, leaves and bark of the stem) (17, 18), antifungal (stem and leaf) (19), gastroprotective (scrapings) (20), antiparasitic (leaf and stem bark) (21), as well as bacterial and fungal biofilm eradication (leaf and stem bark) (22).

In view of this new paradigm, several medicinal plants have been the target of studies for the formulation of new drugs for the treatment of gastrointestinal disorders and skin lesions. Given the popular use of *Ziziphus joazeiro* for such purposes, this study aims to evaluate the possible gastroprotective and cicatrizing activity of the *Ziziphus joazeiro* Mart. leaf hydroalcoholic extract (EHFZJ).

MATERIALS AND METHODS

The hydroalcoholic extract from the leaves of Ziziphus joazeiro Mart. (EHFZJ)

The methodology the preparation of hydroalcoholic extract the leaves of *Ziziphus joazeiro* Mart. (EHFZJ) was obtained and described in a study previously conducted by Brito *et al*. (15). *Z. joazeiro* Mart. leaves were collected in Chapada do Araripe (Crato city, Ceara, Brazil). The exemplar of this specie was identified by Dr. Maria Arlene Pessoa da Silva, deposited in the Herbarium Dardano de Andrade Lima of Universidade Regional do Cariri (URCA Crato-CE, Brazil) with voucher of the identification (no 30.2013). The collected leaves were washed, dried at room temperature, and pulverized. The powder material was extracted by maceration using a 1:1 ethanol and water mixture for 72 hours at room temperature. The mixture then was filtered, concentrated under vacuum in a rotary evaporator at 45°C (27 – 30 rpm and 760 mmHg) and dried in a water bath under a nitrogen atmosphere. The extract was conditioned under refrigeration until the time of the assays.

Animals used and ethical aspects of the research

Swiss (*Mus musculus*) mice of both sexes, weighing between 15 and 30 grams or rat (Wistar albino) of both sexes, weighing between 200 and 250 grams, from the Regional University of Cariri (Universidade Regional do Cariri - URCA) bioterium were used for the pharmacological tests. The animals were kept in the experimentation room for two days before the tests at 25°C, following the 12-hour light and dark cycle with water and ration *ad libitum*. The animals were then fasted for 15 hours on the day of the experiment.

The study was carried out in accordance with the Bioethics rules recognized by law: 11.794/08, which regulates the use of animals for scientific procedures, and was authorized by the Committee for Experimentation and Use of Animals of the Regional University of Cariri (CEUA/URCA), under the approved protocol number no. 21/2012.2.

Acute preclinical toxicity

The acute preclinical toxicity investigation of the hydroalcoholic extract the leaves of *Ziziphus joazeiro* Mart., (EHFZJ) followed the OECD (Organization for Economic Co-operation and Development) guidelines for the acute toxic dose class test (Acute Toxic Class Method - OECD 425 - 2001) (23). To determine the acute preclinical oral toxicity, the animals (n = 5) were treated orally (v.o.), with the extract being administered with a dose of 2000 mg/kg/*per os* (p.o.) In the case of mortality, the lowest doses were calculated by the regression factor of the 3.2, when no information on the slope of the dose-response curve for the substance to be tested exists (23).

The animals were observed at 30, 60, 120, 180 and 240 minutes after treatment and, daily, for 14 days for signs of toxicity, time of onset, intensity, duration and progression, with these, as well as systematic behavioral changes identified, according to established criteria by Malone (1977), through a hypocratic screening being recorded, the intensity of which were scored from zero to four, corresponding, respectively to: absent, rare, little, moderate and intense (24).

Gastric lesion induced by absolute ethanol (96%) or acidified ethanol

The animals (Swiss mice/n = 6) were divided into a negative control (0.9% saline, 0.1 mL/10 g/p.o.), positive control (omeprazole 30 mg/kg/p.o.) and EHFZJ (100, 200 and 400 mg/kg/p.o.). One hour after treatment, the animals were administered $ethanol_{abs}$ (0.2 mL/animal p.o.) or $ethanol_{acid}$ (0.2 mL of a 0.3 M HCL solution in 60% ethanol, oral administration (p.o.). One hour after lesion induction, the animals were anesthetized and sacrificed, having their stomachs removed and opened along the curvature, washed with 0.9% saline and compressed between two slides. The images were scanned and digitalized according to the groups, with subsequent analysis using the Image J software, with the lesioned area being expressed as a percentage of ulcered area by the total gastric body area (%U/cm^2) (25,26) using the equation:

$$\%U = \sum_{i=0}^{n} T_u / T.$$

T_u = corresponds to the total number of pixels per cm^2 of the ulcerated area and T = match a total gastric body in pixels per cm^2.

Gastric lesion induction by indomethacin

The animals (Swiss mice/n = 6) were divided into a negative control (0.9% saline, 0.1 mL/10 g/p.o.), positive control (omeprazole 30 mg/kg/p.o.) and EHFZJ (100, 200 and 400 mg/kg/p.o.). One hour after pre-treatment, lesions were induced using indomethacin (10 mg/kg, p.o). After 3 hours of administering the inducing agent, the pre-treatments were repeated. After 6 hours of indomethacin administration, the animals were anesthetized and sacrificed, having their stomachs removed and opened along the curvature, washed with 0.9% saline and compressed between two slides.

The images were scanned and digitalized according to the groups, with the injured area being quantified and qualified using scores previously established by the methodology, in which points are assigned to different observations such as: mucosal color, loss of mucosal folds, petechiae, edema, hemorrhage, loss of mucus and ulcer lesions, with scores being given according to the severity of the present signs, where 0 indicates absent or normal,

1 is mild, 2 is moderate and 3 is serious. As for the degree of lesion, a percentage was attributed, with up to 25% (injured area), equal to 50% (moderate) and greater than 50% (intense) (27). The ulcerations were represented by somatory all score points (NP).

Physical barrier test

The animals were treated with 0.9% saline (0.1 mL/10 g/p.o. - negative control) or the EHFZJ (400 mg/kg) by oral (p.o.) or intraperitoneal administration (i.p.). One hour after the oral treatments or after 30 minutes after intraperitoneal administration of EHFZJ (400 mg/kg), the animals received acidified ethanol (0.2 mL/animal p.o.). After 1 h of lesion induction by acidified ethanol, according to their respective times, the animals were anesthetized and sacrificed, having their stomachs removed and opened along the curvature, washed with 0.9% saline and compressed between two slides. The images were scanned and digitalized according to the groups, with subsequent analysis using the Image J software, with the injured area expressed as a percentage of the total gastric body area.

Cicatrizing activity

The animals (Wistar rats/n = 6) weighing between 200 – 250 g were divided into groups, being previously identified, weighed and anesthetized (xylazine: 10 mg/kg/i.p. and ketamine hydrochloride: 60 mg/kg/i.p.). Subsequently, the animals underwent a trichotomy in the dorsal region, and four 8 mm cutaneous excisions were performed with the aid of a stainless steel metallic punch, removing a circular segment of skin, exposing the muscular fasciae, with each excision being for a treatment: base cream (negative control), EHFZJ cream (100 mg/g, 200 mg/g and 400 mg/g).

After the surgical procedure, the animals were accommodated in properly disinfected boxes. For 14 days, the animals were topically treated (1 g/animal) according to the respective groups, with skin lesions being evaluated every three days for the presence of hyperemia, edema, bleeding, exudate and type of tissue formed. Images of the lesions were emitted on days 0°, 7°, 11° and 14°, which were digitalized, followed by analysis using the Image J software, with the total lesion area being expressed in cm^2, according to their groups.

The percentage of wound contraction wes calculed using the equation: %C = 100 * (Ad0 – Ada)/Ad0 (Ad0 = wound area on day 0, and Ada = wound area on the day of analysis), while, the cutaneous wound closure speed, expressed in cm^2/day, was calculated according to the equation: VFf = %C/DAC (DAC is the day of wound cicatrizing analysis and %C is the percentage of the cicatrized wound (28).

Statistical analysis

The results were presented as mean ± standard error of the mean (S.E.M), evaluated by a one-way analysis of variance (ANOVA) and the Newman-Keuls and Tukey multiple comparison tests (when necessary), with calculations being made using the GraphPad Prism statistical software, according to the values obtained in the tests.

RESULTS

Acute preclinical toxicity

Acute oral treatment of the *Ziziphus joazeiro* leaf hydroalcoholic extract presented low toxicity, according to the protocol established by OECD, 2001 (23), since animals treated with the EHFZJ showed no signs of mortality and toxicity, such as depression, excitement, seizure, salivation, piloerection and tearing, during the 14 day evaluation period. Thus, it was decided to use concentrations lower than 10% of the acute preclinical toxicity of the EHFZJ to make up the doses used in the *in vivo* assays: 100, 200 and 400 mg/kg, thus maintaining the safety of the tests.

Gastric lesion induced by absolute ethanol, acidified ethanol and indomethacin

In the absolute ethanol-induced gastric lesion model, the negative control group had a significant percentage of lesioned area with an average of 27.01 ± 3.26 (%U/cm^2). Oral treatment with the EHFZJ at 100, 200 and 400 mg/kg doses presented the following respective averages: 23.37 ± 2.14 (%U/cm^2); 22.86 ± 2.53 (%U/cm^2) and 14.27 ± 2.26 (%U/cm^2) showed a significant protective effect. However, the dose of 400 mg/kg dose and omeprazole (30 mg/kg) no presented a significant difference in reducing lesions by 60.7% ($P < 0.001$) and 57.11% ($P < 0.001$), respectively, compared to the negative control group, thus showing a gastroprotective activity (*Fig. 1A*).

In the acidified ethanol-induced gastric lesion model, the group negative control presented a higher percentage of lesioned area with an average of 30.32 ± 2.28 (%U/cm^2), which demonstrates the effectiveness of the harmful agent. All groups treated with the EHFJZ (100, 200 and 400 mg/kg doses) presented a significative protective effect an average of 10.61 ± 0.50 (%U/cm^2); 18.32 ± 2.07 (%U/cm^2); 12.26 ± 0.65 (%U/cm^2) when compared to the negative control. The percentual of protection of the 200, and 400 mg/kg dose no presented a significant difference in reducing lesions when compared with and omeprazole (30 mg/kg) presenting values of reduction of 59.56% ($P < 0.001$) and 71.18% ($P < 0.001$), and 65.01% ($P < 0.001$), respectively, (*Fig. 1B*).

In the indomethacin-induced gastric lesion assay, the EHFZJ at 100, 200 and 400 mg/kg doses obtained averages of 5.85 ± 0.40 NP ($P < 0.05$), 4.28 ± 0.60 NP ($P < 0.001$) and 2.85 ± 0.40 NP ($P < 0.0001$), respectively, while the reference drug omeprazole (30 mg/kg) presented an average of 2.42 ± 0.42 NP ($P < 0.0001$), when compared to the negative control group with an average of 8.14 ± 0.70 NP, demonstrating the effectiveness of the harmful agent, given its higher lesioned area score. All the groups treated with the EHFZJ presented significance when compared to the negative control, thus showing an effective gastroprotective action. The EHFZJ at a 400 mg/kg dose demonstrated an effectiveness in terms of gastric mucosa negative control protection in all the gastric lesion models (*Fig. 1C*).

Physical barrier test

Stomachs in the negative control group showed a large lesioned area due to the action of the acidified ethanol, with an average of 22.62 ± 1.37 (%U/cm^2). The EHFZJ at a 400 mg/kg dose showed an average of 9.28 ± 1.14 (%U/cm^2) when administered orally, with a significance of $P < 0.05$ compared to the negative control, however, when administered intraperitoneally, an average of 33.39 ± 4.87 (%U/cm^2) was observed, with a significance of $P < 0.01$ compared to the control group (*Fig. 2*).

Cicatrizing activity

The macroscopic analysis of the cutaneous wounds on the back of animals treated with the EHFZJ demonstrated a positive evolution of tissue repair, since hemorrhages and

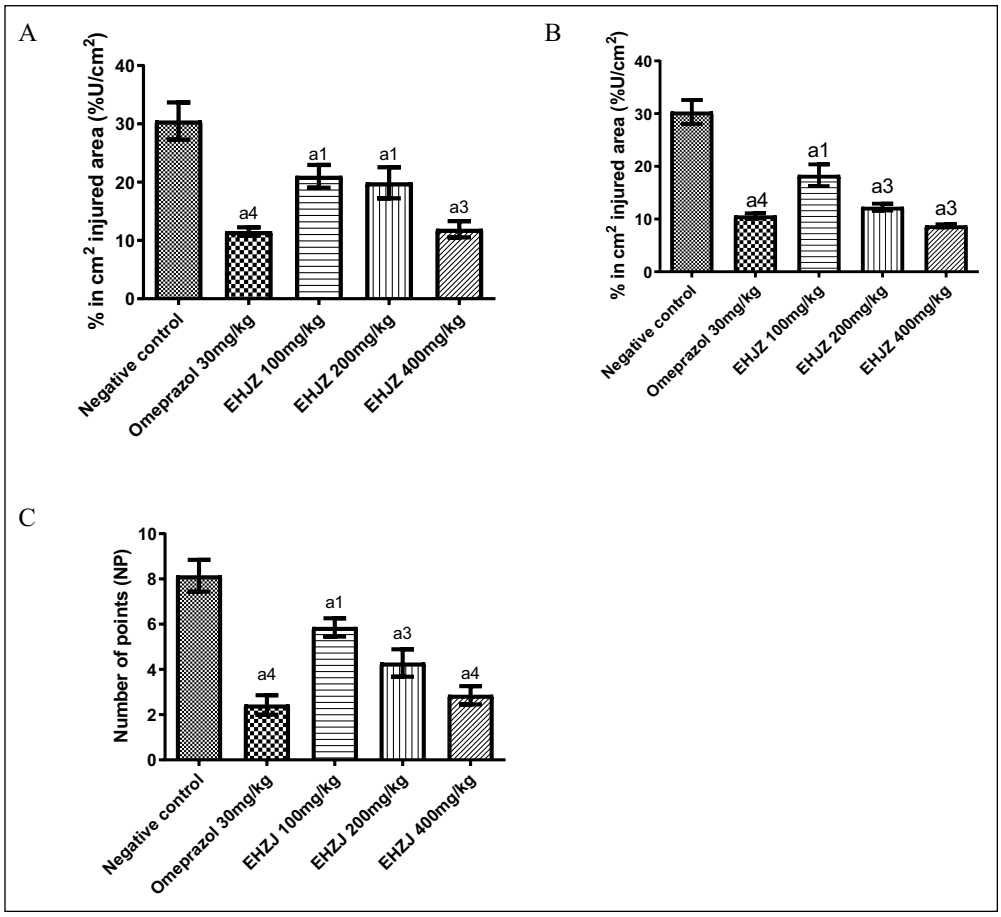

Fig. 1. Effect of the oral administration of the *Ziziphus joazeiro* Mart. leaf hydroalcoholic extract (EHFZJ) on gastric lesions induced by absolute ethanol (A), acidified ethanol (B) and indomethacin (C) in mice.
The results are expressed as the mean ± standard error of the mean for 6 animals/group using an analysis of variance (ANOVA) and Student Newman-Keuls as a *post hoc* test. $^{a1}P < 0.05$ versus control; $^{a2}P < 0.01$ versus control; $^{a3}P < 0.001$ versus control; $^{a4}P < 0.0001$ versus control.

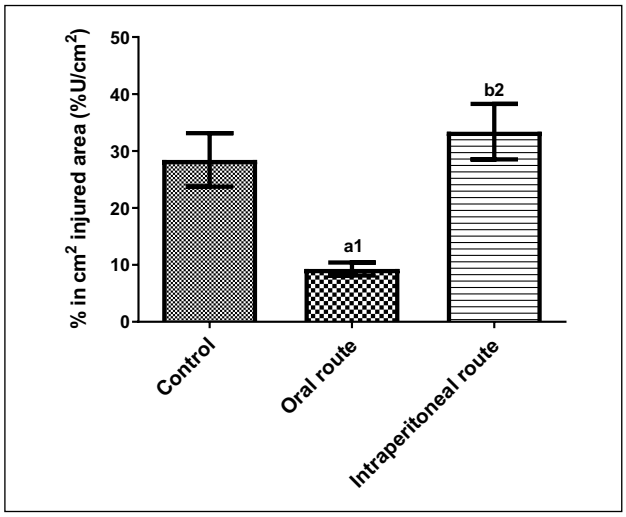

Fig. 2. Physical barrier test effect, comparing the intraperitoneal administration of the *Ziziphus joazeiro* Mart. leaf hydroalcoholic extract (EHFZJ) versus its oral administration on gastric lesions induced by acidified ethanol in mice.
The results are expressed as the mean ± standard error of the mean for 6 animals/group by analysis of variance (ANOVA) and Student Newman-Keuls test as a *post hoc* test. a1: $P < 0.05$ versus negative control; $^{b2}P < 0.01$ versus *via* oral.

purulent secretions were absent in all wounds during the different observation times (0, 3, 7, 11 and 14 days) and with the different concentrations (base cream, EHFZJ at 100, 200 and 400 mg/g). In terms of the healing process, crusts and exudates were evidenced from the third day of observation onwards in all treatment groups. The epithelialization phase was observed on the 11th day of analysis, and on the 14th day all wounds had evolved into maturation tissue with hair growth surrounding all surgical incisions. Clinical observations during daily treatments showed adequate recovery, good general conditions, presence of physical activity and adequate intake of food and water in all groups (*Table 1*).

In terms of the speed of wound closure of the skin lesions on the animals' backs, as a function of time in days (cm²/day), the data shows the speed of wound closure increases at all observation days (0, 3, 7, 11 and 14) for all groups (base cream, EHFZJ 100, 200 and 400 mg/g). Lesions treated with the EHFZJ cream at 100 and 200 mg/g concentrations had a faster speed of wound closure than lesions treated with the base cream and the 400 mg/g EHFZJ cream, which may justify its popular use for this purpose, however, differences between the groups were not statistically significant, showing that its use has no proven scientific efficacy, thus new corroborative tests are needed (*Fig. 3*).

The percentage of wound contraction comparisons (*Table 1*) corroborate with the speed of wound closure data (*Fig. 3*), demonstrating a positive evolution for all groups. However, it is noteworthy that contraction patterns were similar for all groups, with no statistically significant differences between them, and therefore, the use of the *Ziziphus joazeiro* extract did not interfere with the natural cicatrizing process, thus the absence of an EHFZJ cicatrizing activity may be attributed to the small quantities of these substances being identified in the present extract.

Table 1. The percentage of wound contraction on days 0, 3, 7, 11 and 14 following treatment with the base cream (vehicle), 100 mg/g of *Ziziphus joazeiro* Mart. leaf hydroalcoholic extract (EHFZJ) cream, 200 mg/g EHFZJ cream and 400 mg/g EHFZJ cream.

Day	Basis (vehicle)	EHFZJ 100 mg/g	EHFZJ 200 mg/g	EHFZJ 400 mg/g
0	0%	0%	0%	0%
3	8.39 ± 5.86	22.69 ± 1.44	20.04 ± 2.71	18.54 ± 3.45
7	33.74 ± 8.14	40.26 ± 1.77	42.68 ± 1.63	25.70 ± 2.61
11	80.40 ± 1.23	86.64 ± 1.06	78.16 ± 1.28	66.90 ± 1.45
14	90.50 ± 1.11	95.04 ± 1.02	93.87 ± 1.03	84.86 ± 1.08

The results are presented as the mean ± standard error of the mean, as evaluated by an analysis of variance (ANOVA), with the calculations being performed using the GraphPad Prism statistical software, according to the values obtained in the tests. The results are expressed as the mean ± standard deviation (SD).

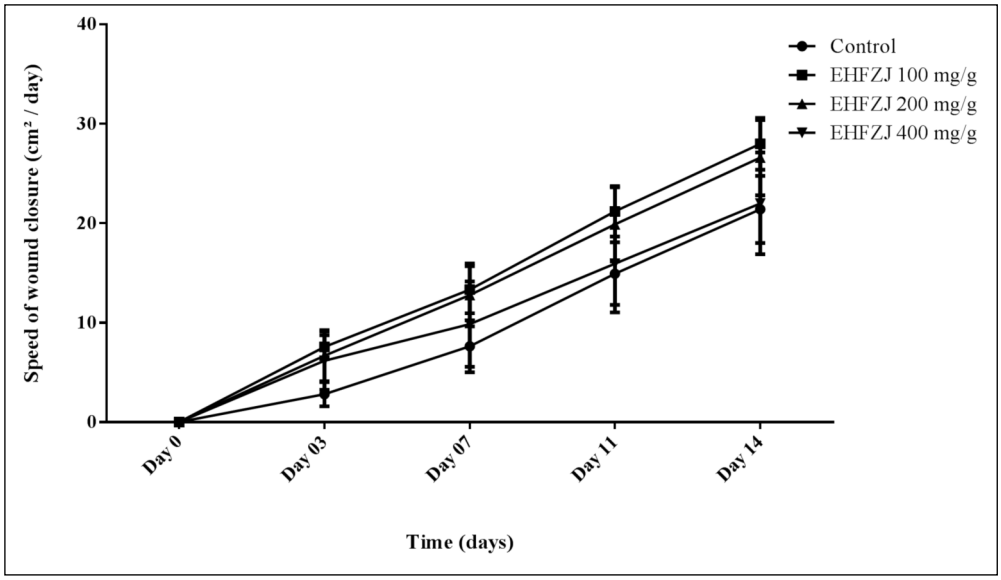

Fig. 3. Speed of wound closure (cm²/day) of animals submitted to dorsal skin lesion on days 0, 3, 7, 11 and 14 after daily treatment with liquid crystal cream and the *Ziziphus joazeiro* Mart. leaf hydroalcoholic extract (EHFZJ) cream at 100, 200 and 400 mg/g concentrations.
The results are expressed as the mean ± standard error of the mean for 6 animals/ group by an analysis of variance (ANOVA) and Student Newman-Keuls test as a *post hoc* test.

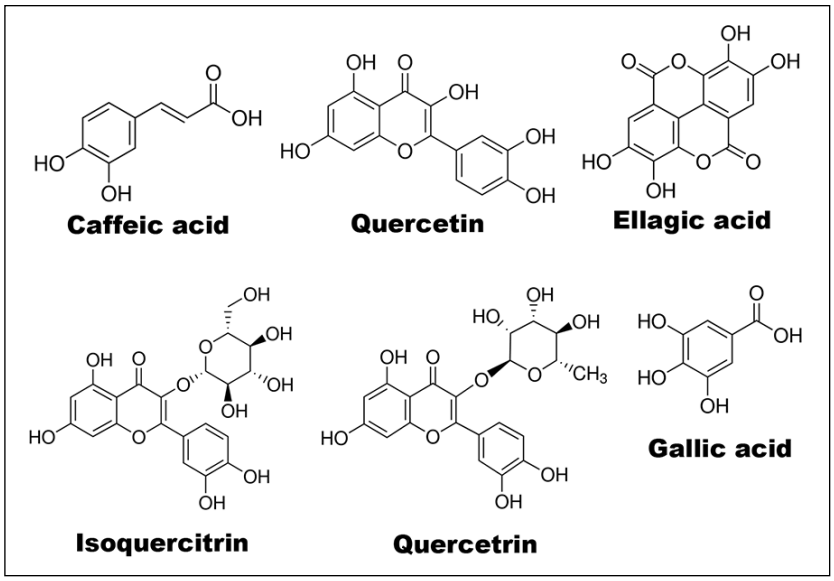

Fig. 4. Chemical structure of major compounds present in the hydroalcoholic extract the leaves of *Ziziphus joazeiro* Mart., (EHFZJ).

DISCUSSION

In the study previously published, using the identic extract, the same group of authors demonstrated that the fingerprint of flavonoids class was compounded by caffeic acid, gallic acid, ellagic acid, isoquercitrin, quercitine, and quercetin (*Fig. 4*) and the polyphenols class with presence of catechin and epicathena and phenolic acids such as gallic acid and coffee (15). Flavonoids possess activities that have been proven in the literature such as: antioxidant (29-31), antiallergic, anti-tumor,

anti-hepatotoxic, antiulcerogenic, antiplatelet, antimicrobial and antiviral (32).

Catechin and epicatechin belong to another class of secondary metabolites known as tannins, which can be found in different plant parts such as leaves, barks, inner bark, fruits and seeds, and which have polyphenols in their constitution, being chemically classified into hydrolyzables and condensates (33). For tannins, studies report activities such as: antibacterial (34), anti-inflammatory, antiparasitic, cicatrizing and gastroprotective (35). Among the phenolic acids identified in the EHFZJ, gallic acid (1.36%) and caffeic acid (2.96%) represent the major constituent, where in the literature, both present important pro and antioxidant activities (36-38).

According to Larini (39), orally administered substances are classified based on their average lethal dose (LD_{50}) and/or acute preclinical toxicity and its toxic potential as: extreme (LD_{50} equal to or less than 25 mg/kg), high (LD_{50} between 100 and 500 mg/kg), medium (LD_{50} between 500 and 2000 mg/kg) and low (LD_{50} above 2000 mg/kg). Therefore, in this study, concentrations lower than 10% of the EHFZJ acute preclinical toxicity were chosen to compose the doses (100, 200 and 400 mg/kg) used in *in vivo* tests, thus maintaining safety.

The EHFZJ demonstrated a gastroprotective action (400 mg/kg) in gastric lesion models induced by absolute ethanol, indomethacin and acidified ethanol. Absolute ethanol promotes necrotic lesions in the gastric mucosa due to reduced bicarbonate secretion and mucus production, which are triggered by decreased blood flow, ischemia, free radical production, endothelin release, inhibition of prostaglandin production and mast cell degranulation (40, 41), with the addition of hydrochloric acid (HCl) speeding up and making this mechanism more severe, since tissue damage and necrosis are augmented (26, 40).

Indomethacin is an NSAID, which is directly involved in gastric toxicity due to its inhibitory action over cyclooxygenases (COX), blocking the synthesis of prostaglandins which mediate the production of mucus, increase blood flow, tissue repair and maintenance of the mucosal immunity. In addition, indomethacin induces the release of reactive oxygen species and the consequent reduction in antioxidant factors (41, 42).

Therefore, the gastroprotective action of the EHFZJ may be associated with the presence of its major constituents, such as caffeic acid (43) and quercetin (44), which possess an antioxidant action, leading to a reduction in free radicals. A study with extract from the flowers of Tropaeolum majus L., which also contains quercetin and coffee acid in its composition, showed antioxidant effects (45). Sevastre-Berghian (46) identified that quercetin has an antioxidant effect decurrent the increase of enzyme level as SOD and CAT, and anti-inflammatory with a decrease of cytokines level as interleukin-1α, interleukin-1β, monocyte chemotactic protein-1, interferon, as well reduction of nuclear factor-kappaB (NFκB) and pNFκB.

Given the results presented in terms of a gastroprotective activity, the EHFZJ may promote a physical barrier, due to the present compounds, such as tannins that adhere to the mucous layer of the stomach, forming a protein precipitate, creating an impermeable film that prevents the absorption of toxic substances (47, 48) and for the discreet participation of the EHFZJ through the intraperitoneal route.

In terms of a cicatrizing activity, the EHFZJ presented no significant differences in terms of the percentage of contraction and the speed of wound closure at the time of observation. The healing process occurs naturally, resulting in the restoration of homeostasis and the recovery of damaged tissue (8). However, some endogenous and exogenous factors can delay or impede the cicatrizing process, such as: systemic diseases, corticosteroid use and smoking (49). Karbarz *et al*. (50) it was found that cereal juice presents an adaptive response by means of hormonal mechanism mediated by antioxidant pathways NF-κB/HO-1 and insulin/IGF-1, significantly regulates the wound healing process, activate the response of hormetic adaptation in normal fibroblasts and induces cytotoxic and genotoxic events in cancer cells.

In conclusion, the present study demonstrated the *Ziziphus joazeiro* Mart. leaf hydroalcoholic extract that in terms of an acute preclinical toxicity, the single oral administration of the EHFZJ did not present signs of toxicity and mortality. As for pharmacological activities, the EHFZJ (400 mg/kg) presented a significant gastroprotective effect in gastric lesion models induced by absolute ethanol, indomethacin and acidified ethanol, with the oral route of administration presenting a potential barrier mechanism, however, the EHFZJ at different concentrations did not interfere with the wound cicatrizing process. Therefore, the data suggests the EHFZJ may be considered as a new therapeutic option for the treatment of gastric lesions.

Author contributions: I.R.A. de Menezes and J.G.M. da Costa proposed the experimental design; S.M.O. Brito, A.O.B. Martins, M.R.C. de Oliveira, C.S Vidal, L.J. Lacerda Neto, L.P. da Cruz and E.A. Nascimento carried the *in vivo* experiments addressing the gastroprotective and cicatrizing activity evaluation of the *Ziziphus joazeiro* Mart. leaf hydroalcoholic extract. J.G.M. da Costa, H.D.M. Coutinho, L.J.Quintans and I.R.A. de Menezes provided the facilities and reagents to carry out the present study.

All authors read and approved the final version of the manuscript.

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