Journal of Applied Pharmaceutical Sciences

japhac.wix.com/japhac
ISSN 2358-3495

Submitted:
02/12/2019
Revised version:
19/02/2020
Accepted:
02/03/2020
ORIGINAL ARTICLE

Evaluation of the antioxidant and gastroprotective activity of leaves and juice of *Anacardium Occidentale*

Bárbara Silva Monteiro[1], Wemerson Neves Matias[1], Karla Brehnda Ca bral Liberato[1], Danielle Rocha Silva[1], Gislayne Tacyana dos Santos Lucena[1], George Souza Feitoza[2], Samara Alves Brito[1]*

1- Santa Maria College, Cajazeiras, 58900-000, Brazil. 2- Department of Physiology and Pharmacology, Federal University of Pernambuco, Recife 50670-901, Brazil.
*Corresponding author: samaralvesbritobrito19@gamil.com

Abstract: *Anacardium occidentale* (Anacardiaceae) is used in folk medicine as an anti-inflammatory, antimicrobial and antidiabetic phytotherapic. This study was carried out to evaluate the antioxidant and gastroprotective activity of the hydroalcoholic extract (HEAo) and juice (JAo) of *Anacardium occidentale*. *In vitro* antioxidant activity of HEAo and JAo was evaluated through the 2,2-diphenyl-1-pricylhydrazyl (DPPH) free radical scavenging method. The *in vivo* gastroprotective capacity was assessed in acute gastric ulcer models induced by absolute ethanol and nonsteroidal anti-inflammatory drugs (NSAIDs). HEAo and JAo presented antioxidant capacity *in vitro.* In absolute ethanol-induced gastric lesions, HEAo (200 mg/kg) reduced the ulcerative lesion index by 76.83%, and JAo (50 and 100%) reduced the ulcerative lesion by 98.55% and 67.64%, respectively, compared to the injured control (IC) group. In the indomethacin-induced gastric lesions, the results showed that HEAo (100 and 200 mg/kg) reduced the area of the ulcerative lesion by 82.24% and 93.03%, respectively, and JAo (25, 50 and 100%) reduced it by 97.39%, 94.67% and 98.24%, respectively, compared to the injured control. In conclusion, the study found that the leaves and juice of the pseudo fruits of *Anacardium occidentale* have antioxidant and gastroprotective activity.

Keywords: *Anacardium occidentale*. Gastroprotective activity. Antioxidant activity, Phytoterapy.

INTRODUCTION

Anacardium occidentale (Anacardiaceae) is originally from Brazil and is present in South America, and countries such as India, Nigeria and Vietnam [1, 2]. This species has components that makes it to be considered a functional food, that is, one that meets the nutritional needs of people, because such components have functions linked to health, and are rich in fiber, vitamins, minerals and other bioactive components, such as phenolic compounds [3, 4, 5]. In the Northeast of Brazil, *Anacardium occidentale* is frequently cultivated in view of its nutritional and functional components, being one of the most produced fruits. Cashew is divided into two elements: the fruit, from which the chestnut is obtained and the pseudofruit (peduncle) which is the cashew itself. Cashew is economically important because it is marketed as fresh product, or as sweets, juices, soft drinks, pulps and almonds [6, 7].

The literature reports that cashew has been used by Indians since the time of the arrival of the Portuguese to Brazil, and has been used by Amazonian tribes for centuries to treat diarrhea, colds, gastritis, cramps, and persistent coughs. Different parts of the plant such as leaves, peduncle, bark, and resin are used in

preparations, in teas, infusion, decoction, topical use, and external use [8 - 12].

Scientific studies confirmed several pharmacological activities of the species, such as antioxidant activity of the juice and anti-inflammatory [13 - 15], antidiabetic and antimicrobial activity of the ethanolic extract of leaves [16, 17], and antibacterial activity of the extract of leaves [18, 19].

Gastric ulcer is a chronic disease that affects millions of people in the world and its appearance is associated with the way of life of populations in modern days, characterized by high level of stress, frequent use of non-steroidal anti-inflammatory drugs (NSAIDs), poor diet, alcohol consumption, and the presence of the bacteria *Helicobacter pylori* [20 - 23]. Although there is a diversity of drugs available on the market to control and treat ulcers, there is a high percentage of recurrence after treatment. The continuous use of these drugs causes a number of undesirable effects on individuals [24 - 25]. In view of this problem, there is a need for new alternatives with fewer side effects and guaranteed safety and efficacy. In this context, medicinal plants and their metabolites are promising in the search for new therapies that can be used to treat diseases that affect the gastrointestinal tract [26].

Considering the use of *A. occidentale* in traditional medicine as anti-inflammatory, the present study sought to verify the antioxidant and gastroprotective activities of this species from the hydroalcoholic extract of leaves and the juice of cashew pseudofruits, in view of the need to explore new therapeutic alternatives in the treatment of gastric ulcers.

MATERIALS AND METHODS

Collection of plant material

Leaves and pseudofruits of *A. occidentale* were collected in the city of Cajazeiras - PB in October 2018, in the geographic coordinates 6º55'33" S 38º34'62" W. A sample of the species containing leaves and flowers was collected for botanical identification and deposited in the Caririense Dárdano de Andrade-Lima Herbarium of the Regional University of Cariri (URCA), in the city of Crato - EC.

Obtaining leaf extract and pseudofruit juice

The hydroalcoholic extract of *A. occidentale* (HEAo) was prepared from 100 g of dry leaves by cold extraction with ethanol, based on the methodology described by Matos [27]. The dried leaves were comminuted and passed through the process of maceration in 1000 mL of 70% ethanol solution for seven days at room temperature. Then, the solvent was evaporated under vacuum in a rotary evaporator at 50°C in water bath until ethanol was completely removed. The hydroalcoholic extract was weighed and stored for phytochemical analysis and evaluation of antioxidant and gastroprotective activity.

The juice of fresh samples of *A. occidentale* (JAo) was manually extracted. Part of this juice was dehydrated with the aid of a Spray Dryer (MSD 0.5, Labmaq do Brasil, Ltda.). The parameters used were: drying air flow rate: 4.5 mm³/min; outlet temperature: 95°C; compressed air flow rate: 40 L/min; and peristaltic pump: 0.5 L/h. The other part of the juice was used pure and diluted for biological activities.

In vitro study

Determination of phenolic compound

The Folin-Ciocalteau method was used to determine the total phenolic compounds of HEAo and JAo. The amount of 0.1 mg of the sample and 1 ml of the Folin-Ciocalteau reagent diluted in water in the proportion of 1:10 were added. After 3 minutes of in absence of light, 0.8 mL of 7.5% sodium carbonate was added, and

the solution was left in the dark for 120 minutes. Absorbance readings were performed in a spectrophotometer at wavelength of 765 nm. The results were calculated on the basis of a standard curve of gallic acid and expressed as mg of gallic acid equivalents (GAE)/100 g of the sample [28]. Phytochemical studies (HPLC) are ongoing and will be made available in a timely manner.

Determination of flavonoid content

Aluminum chloride was used for determination of the flavonoid content of HEAo and JAo. To this end, 1 mL of the sample solution was added to 1 mL of 2% ethanolic solution of aluminum chloride. The absorbance of the resulting solution was read at a wavelength of 765 nm after one-hour incubation at room temperature. The result corresponded to the Y of the line obtained through the calibration curve that was made with quercetin (0 - 100 µg/mL), expressed as mg of quercetin equivalent (QE)/100 g of the sample [29].

DPPH free radical scavenging

2,2-diphenyl-1-picrylhydrazyl (DPPH) free radical scavenging activity was analyzed with adaptations for microplate analysis [30]. An aliquot of 40 ml of the extract or juice separately at the concentrations of 1000, 500, 250, 125, 62.50 and 31.25 mg/mL were added to 250 µL of 0.004% methanol solution of DPPH. The mixture was incubated at room temperature for 25 minutes in the dark. Simultaneously, a control solution (0.004% methanol solution of DPPH) and a blank (methanol) were read. The absorbance was measured at wavelength of 517 nm. Each concentration was calculated as follows:

$$\text{SRL (\%)} = \frac{\textbf{(Abs sample − Abs control)}}{\textbf{Abs control}} \text{ x } 100$$

In vivo study

Obtaining animal

Male and female Wistar rats of the species *Rattus norvegicus*, aged two to three months and presenting body mass of approximately 200-230 g, were used to evaluate the gastroprotective activity of the HEAo and JAo. The animals were kept under controlled conditions of illumination (12-12 hr light-dark cycle) and temperature (22 ± 2ºC), and received water and feed (Presence ®) *ad libitum*. The protocols were submitted for appreciation by the Ethics Committee on Use of Animals in Research (CEUA) of the Santa Maria Faculty - FSM, and approved under protocol nº 01/2019.

Evaluation of acute toxicity of the hydroalcoholic extract of leaves of A. occidentale

The median lethal dose (LD_{50}) of the HEAo by oral route was determined according to the OECD-guidelines 423/2001, defined by the number of occurrence of deaths. After 12-hour fast, the animals were divided into two groups (n = 3/group); one group received a negative control (NaCl 0.9%) and the other, the hydroalcoholic extracts of the leaves in a single oral dose (gavage). The objective was to evaluate the behavioral changes on the central nervous system and the autonomic nervous system, as well as the occurrence of death. Behavioral parameters were observed during the first four hours, and once a day, until completing 72 hours, as mentioned by Almeida [31].

During 14 days of observation, water and feed consumption and the body mass of the animals were recorded on a daily basis. On the 14th day, animals were euthanized by cervical dislocation and their organs (stomach, liver, spleen and kidneys) removed, weighed and examined macroscopically to evaluate changes between the HEAo-treated group and the control (0.9% NaCl) group.

Evaluation of gastroprotective activity in absolute ethanol-induced acute gastric ulcer models (as in [32], with modifications)

After a 12-hour fast, eight groups of animals (n = 5-6/group) were pretreated orally (gavage) with HEAo (50, 100 and 200 mg/kg), JAo (25, 50 and 100 %), negative control (0.9% NaCl), and lansoprazole (30 mg/kg). After one hour of pretreatment, the animals were given ethanol p.a. (4 mL/100 g body weight, oral). One hour after administration of alcohol, the animals were euthanized (by cervical dislocation), the stomachs were removed, opened along the greater curvature, the gastric contents were discarded, and the mucosa was carefully washed. The stomachs were pressed into glass plates and photographed to analyze the lesions. The lesions were counted with the help of the *ImageJ* software, and the results were expressed as total area of the ulcerative lesion (AUL) (mm^2) in relation to the total area of the gastric body.

Evaluation of gastroprotective activity in non-steroidal anti-inflammatory drug (indomethacin)-induced acute gastric ulcer models (as in [33], with modifications)

After a 12-hour fast, seven groups of animals (n = 5-6/group) were pretreated orally (gavage) with HEAo (100 and 200 mg/kg), JAo (25, 50 and 100%), negative control (0.9% NaCl), and ranitidine (60 mg/kg, oral). After one hour of pretreatment, the animals were given indomethacin (30 mg/kg, subcutaneous). Six hours after administration of indomethacin, the animals were euthanized (cervical dislocation), the stomachs were removed, opened along the greater curvature, the gastric contents were discarded, and the mucosa was carefully washed.

The stomachs were pressed into glass plates and photographed to analyze the lesions. The lesions were counted with the help of the *ImageJ* software, and the results were expressed as total area of the ulcerative lesion (AUL) (mm^2) in relation to the total area of the gastric body.

Statistics

Results were expressed as mean ± standard error of the mean (SEM). Differences between groups were compared through analysis of variance (ANOVA) followed by the Tukey test. Statistical analyses were performed using the *GraphPad Prism®* 6.0 Software.

RESULTS AND DISCUSSION

In vitro study

Total phenol content

The total phenol content of HEAo and JAo was 314.70 ± 1.83 mg GAE/g and 20.11 ± 1.60 mg GAE/g of juice, respectively. In another study, total polyphenols were also identified in the cashew pulp by HPL. The authors attributed this class of phytochemicals to the antibacterial activity found [34].

Flavonoid content

The content of flavonoids present in HEAo was 41.60 mg of quercetin/100 g of extract, and in JAo was 0.6 mg of quercetin/100 g of juice. The equation of the quercetin calibration curve was y = 0.0196x + 0.0372, where y is the concentration of quercetin (Coefficient of correlation, $R^2 = 0.9923$).

Antioxidant activity

The DPPH free radical scavenging antioxidant assay showed that all concentrations of both HEAo and JAo had the capacity to scavenge free radicals. The concentration that stood out was 1000 mg/mL with 90.42 and 63.68% inhibition, respectively, for HEAo and JAo (Table 1).

In vivo study

Evaluation of toxicity of the hydroalcoholic extract of leaves of A. occidentale

No changes were observed in the central nervous system (hyperactivity, aggressiveness, and hypnosis) and the autonomic nervous system (diarrhea, constipation, and micturition) during the 14 days after treatment of the animals with a single dose of 2000 mg/kg of the hydroalcoholic extract. There was no death of animals, preventing the calculation of the LD_{50} (LD_{50} > 2000 mg/kg). The 14-day observation period showed that administration of the extract studied caused no significant changes in water and feed consumption. Relative body mass gain in relation to the control group no significant changes (Table 2). The LD_{50} aims to avoid the inappropriate use of overdoses that may compromise the health of the animal or cause its death.

The macroscopic analysis of the liver, spleen, kidneys and stomach of animals treated with HEAo at 2000 mg/kg did not show significant differences in relative body mass, color, or texture in relation to the control group. These parameters are important pathophysiological indicators that may be affected by metabolic reactions caused by toxic substances [35]. These results indicate that the acute oral toxicity of the hydroalcoholic extract of *A. occidentale* is greater than 2000 mg/kg.

Evaluation of gastroprotective activity of HEAo in absolute ethanol-induced acute gastric ulcer models

Oral administration of HEAo (200 mg/kg) and lansoprazole (30 mg/kg) were able to significantly protect the gastric mucosa, respectively, by 76.83% and 86.84%, respectively, against absolute ethanol-induced lesions when compared to the control group (200.50 mm^2) (Figures 1 and 2).

Absolute ethanol-induced gastric ulcers are commonly used because ethanol has a rapid effect, promoting the creation of lesions in the gastric mucosa, mainly in the glandular portion of the stomach [36, 37]. Oral administration of ethanol influences muscle activity and reduces gastric blood flow, causing flow arrest, capillary congestion, and increased vascular permeability, leading to hemorrhage, hyperemia, erosion, and necrosis of the tissue with formation of striae [38 - 41]. Ethanol can also cause direct damage to the gastric mucosa cells, causing cell death [42, 43]. A study by Konan and Bacchi [44] analyzed the hydroethanolic extract of the leaves of *A. occidentale* in HCl/ethanol-induced gastric lesions and found that doses above 100 mg/kg significantly inhibited gastric lesions.

Evaluation of gastroprotective activity of JAo in absolute ethanol-induced acute gastric ulcer models

Oral administration of JAo (100 and 50%) and lansoprazole (30 mg/kg) was able to significantly protect the gastric mucosa, respectively, by 67.64%, 98.55% and 86.94% against absolute ethanol-induced lesions when compared to the control group (200.50 mm^2) (Figures 3 and 4).

This is the first work that evaluates the gastroprotective capacity of the juice of the pseudofruit of *A. occidentale*. In a study conducted by Brito [45] with the fruit juice of *Spondias mombin*, belonging to the same family of cashew (Anacardiaceae) and collected in the same region, the juice also showed to have gastroprotective effect, tested through the same model of gastric lesion induction used herein.

Evaluation of gastroprotective activity of HEAo in indomethacin-induced acute gastric ulcer models

Oral administration of HEAo (200 and 100 mg/kg) and ranitidine (60 mg/kg) were able to significantly protect the gastric mucosa by 93.03%, 82.24% and 85.63%, respectively, against indomethacin-induced lesions when compared to the injured control group (10.23 mm^2) (Figures 5 and 6).

Evaluation of gastroprotective activity of JAo in indomethacin-induced acute gastric ulcer models

Oral administration of JAo (100, 50 and 25%) and ranitidine (60 mg/kg) were able to significantly protect the gastric mucosa, respectively, by 98.24%, 94.67%, 97.39% and 90.23%, against indomethacin-induced lesions when compared to the injured control group (13.72 mm2) (Figures 7 and 8).

The high consumption of non-steroidal anti-inflammatory drugs (NSAIDs) increase the expression of intercellular adhesion molecules in the vascular endothelium of the gastric mucosa, thus increasing the neutrophilic adhesion to the vascular endothelium consequently leading to the liberation of free radicals and causing damage to the gastric mucosa. These drugs are capable of producing local and systemic lesions, exerting a local effect by crossing the plasma membrane and penetrating the epithelial cells of the gastric mucosa, causing intracellular damage and systemic damage through reduction of the synthesis of mucosal prostaglandins [46, 47].

Table 1. Percent inhibition of *Anacardium occidentale* extract and juice

[ ] mg/mL	% inhibition of HEAo (mean ± S.D)	% inhibition of JAo (mean ± S.D)
1000	90.42 ± 0.25	63.68 ± 0.57
500	89.50 ± 0.19	54.85 ± 0.07
250	90.80 ± 0.36	52.86 ± 0.43
125	90.30 ± 0.29	47.89 ± 1.39
62.50	77.49 ± 0.92	45.40 ± 1.01
31.25	66.04 ± 1.74	41.04 ± 2.59

Results are expressed as mean ± S.E.M. (n = 3/group). Results were analyzed by one-way ANOVA followed by Tukey's test.

Table 2. Effect of hydroalcoholic extract of A. occidentale on the relative organ mass of female rats after 14 days of oral administration of a single dose of 2000 mg/kg.

Organs (g)	Control (0.9% NaCl)	HEAo (2.000 mg/kg)
Liver	7.94 ± 0.12	7.79 ± 0.41
Spleen	0.44 ± 0.42	0.52 ± 0.52
Kidneys	1.62 ± 0.01	1.53 ± 0.06
Stomach	1.34 ± 0.09	1.33 ± 0.12

Results are expressed as mean ± S.E.M. (n = 3/group). Results were analyzed by one-way ANOVA followed by Tukey's test.

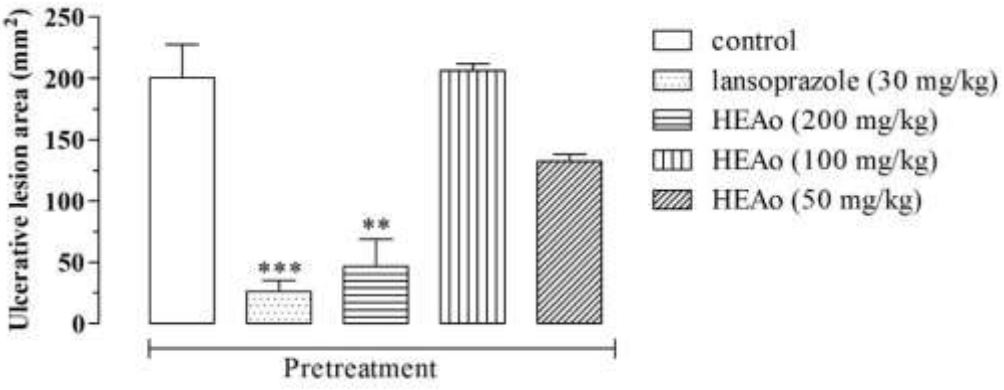

Figure1.Effect of oral pretreatment of HEAo on absolute ethanol-induced gastric lesions in rats. Results are expressed as mean ± S.E.M. (n = 5/group). Results were analyzed by one-way ANOVA followed by Tukey's test, **p < 0.01.

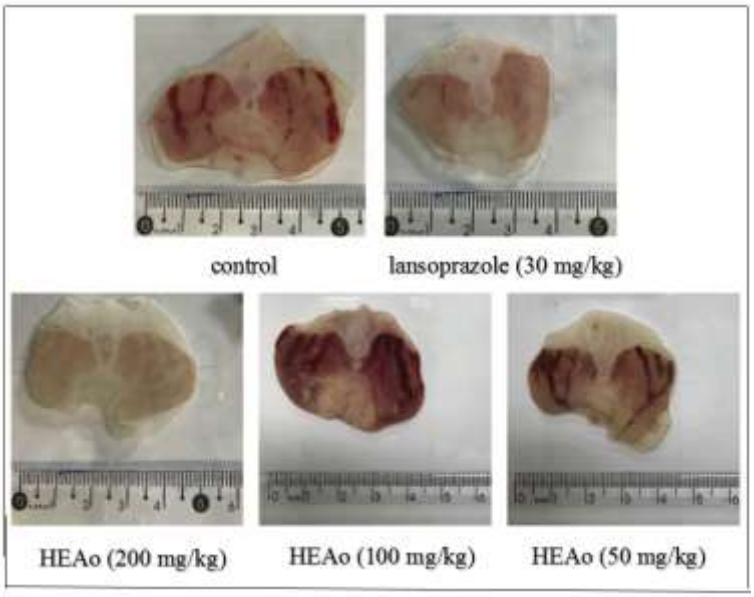

Figure 2. Stomachs of rats orally treated with HEAo for absolute ethanol-induced gastric lesions.

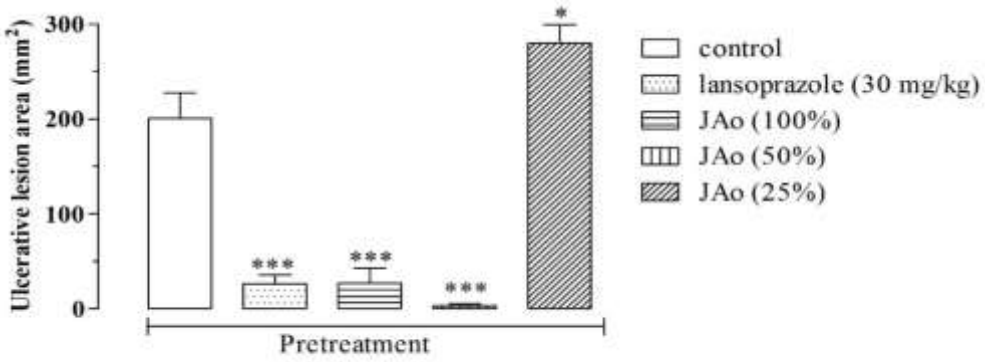

Figure 3. Effect of oral pretreatment of JAo on absolute ethanol-induced gastric lesions in rats. Results are expressed as mean ± S.E.M. (n = 5/group). Results were analyzed by one-way ANOVA followed by Tukey's test, *p < 0.05 ***p < 0.001.

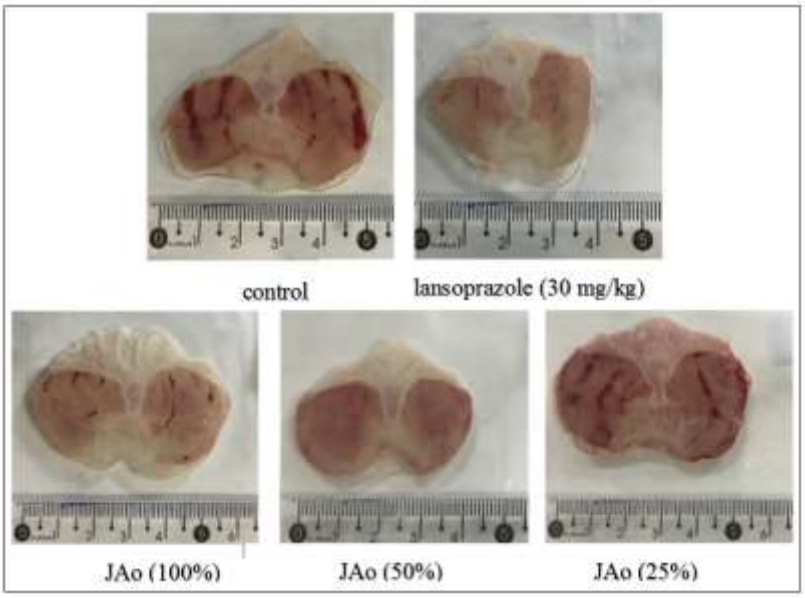

Figure 4. Stomachs of rats orally treated with JAo for absolute ethanol-induced gastric lesions.

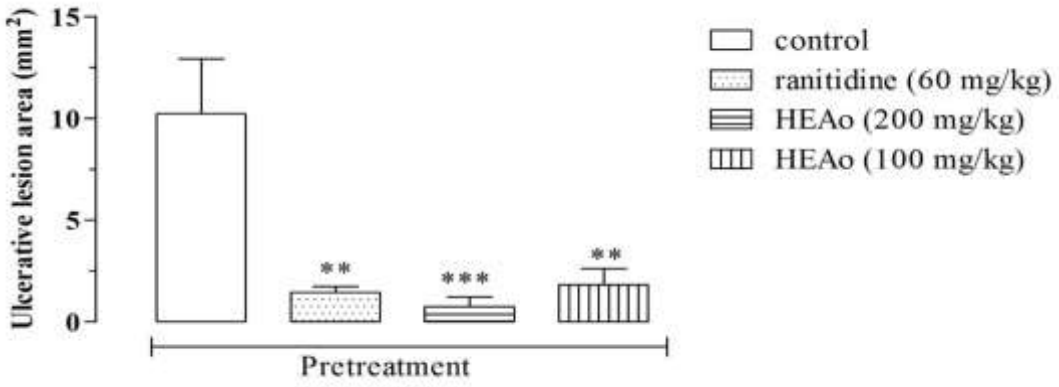

Figure 5. Effect of oral pretreatment of HEAo for indomethacin-induced gastric lesions in rats. Results are expressed as mean ± S.E.M. (n = 5/group). Results were analyzed by one-way ANOVA followed by Tukey's test, **p < 0.01 ***p < 0.001.

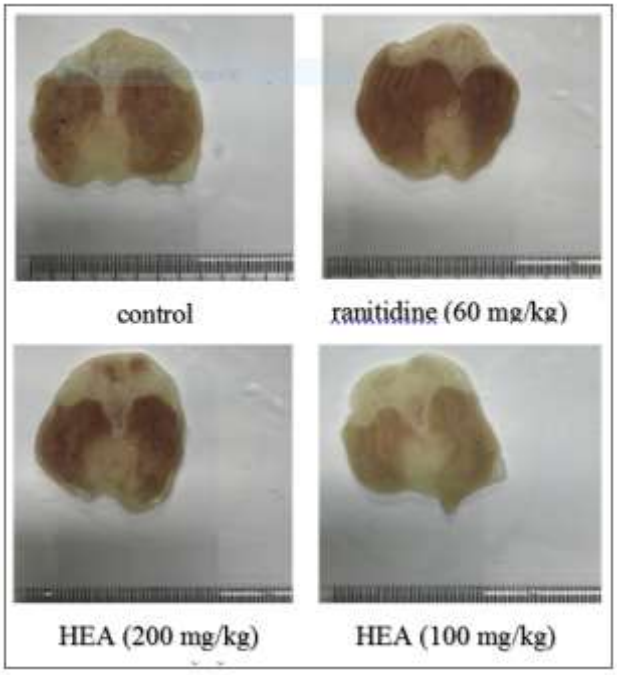

Figure 6. Stomachs of rats orally treated with HEAo for indomethacin-induced gastric lesions.

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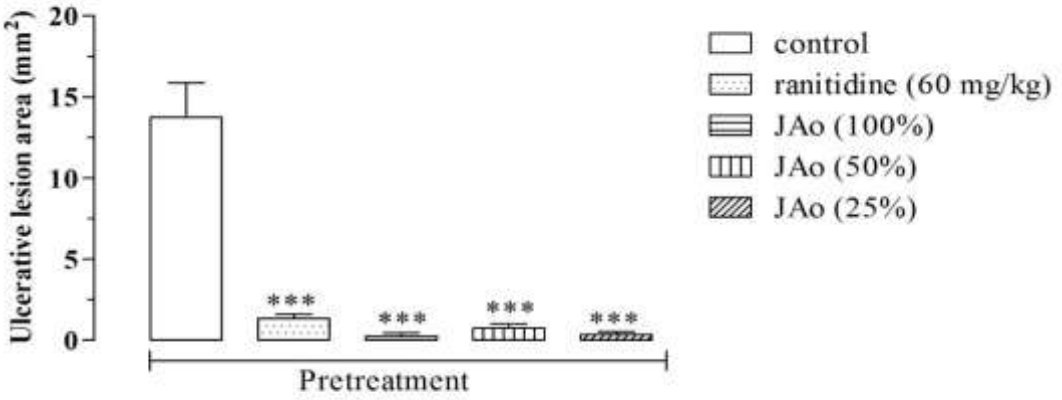

Figure 7. Effect of oral pretreatment of JAo for indomethacin-induced gastric lesions in rats. Results are expressed as mean ± S.E.M. (n = 5/group). Results were analyzed by one-way ANOVA followed by Tukey's test,***p < 0.001.

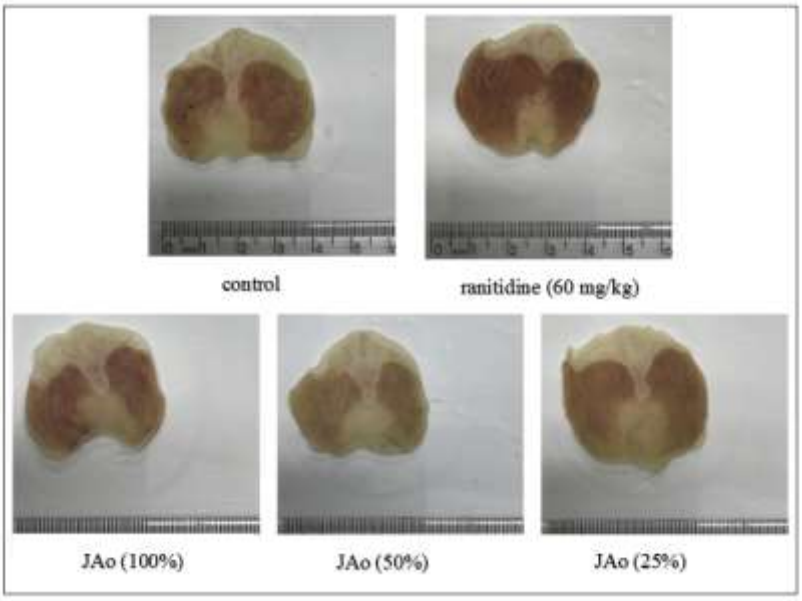

Figure 8. Stomachs of rats orally treated with JAo for indomethacin-induced gastric lesions.

CONCLUSION

The results of the present study show that the hydroalcoholic extract of the leaves and the juice of the *Anacardium occidentale* pseudofruits have a gastroprotective effect in the model of gastric lesions induced by ethanol and indomethacin in rats the hydroalcoholic leaf extract (200 mg/kg) and pseudofruit juice (100 and 50%) were able to significantly reduce gastric lesions induced by absolute ethanol. Hydroalcoholic leaf extract (200 and 100 mg/kg) and pseudofruit juice (100, 50 and 25%) were able to significantly reduce indomethacin-induced gastric lesions.

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