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Effect of Crude Extracts from the Bark and Leaf of *Cinnamomum Zeylanicum* (*Cinnamon*) on the Levels of Glucose, Triglycerides and Fatty Live

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ABSTRACT

Introduction: considering the benefits of cinnamon consumption, the objective was to evaluate the action of crude extracts of the leaf and bark of the cinnamon stem (*Cinnamomum zeylanicum*) on biochemical parameters and histological, morphometric, and stereological aspects of the liver of obese rats.

Methods: thirty male Wistar rats were divided into two groups: normonourished and obese. The normonourished received standard diet and the obese group received hypercaloric diet, from the 21st to the 120th day of life. After 99 days of diet intake, the rats were divided into six subgroups and treated with extracts by gavage for 21 days at a dose of 200 mg/kg. At the end of the treatment, the animals were euthanized, the blood collected for biochemical measurements and the liver tissue for histological, morphometric, and stereological analysis.

Results: cinnamon extracts improved the plasma triglyceride profile, reduced blood glucose levels, reduced AST levels, and decreased the volume of the middle lobe of the liver of obese rats. In the histological analysis, it was observed that the extracts acted by reducing the hepatic steatosis.

Conclusion: the results found demonstrate that the dose and time of use of cinnamon extract can reverse some adverse effects of an obesogenic diet and, consequently, the development of non-alcoholic fatty liver disease (NAFLD). However, these findings should be continued with complementary studies, such as the isolation of specific substances from the extracts, diversified doses, and longer usage times for better use of raw cinnamon extracts in the prevention and development of NAFLD.

Keywords: Obesity; Non-alcoholic fatty liver disease; *Cinnamomum zeylanicum*.

Introduction

Obesity, a serious public health problem, is a complex disease, characterized by abnormal or excessive accumulation of fat that can become an aggravating factor for the health of the individual and cause death. Its etiology is multifactorial, it is believed that biological, genetic, psychological, economic, social, behavioral, and environmental factors are interrelated, causing the emergence of high levels of body fat[1,2].

Obesity is considered a risk factor for the development of several chronic diseases, such as metabolic syndrome, type 2 diabetes mellitus, diseases of the cardiovascular system, cerebrovascular diseases, cancer, and non-alcoholic fatty liver disease (NAFLD)[3,4].

Non-alcoholic fatty liver disease (NAFLD) has its prevalence particularly associated with obesity. Its pathogenesis based on the accumulation of lipids in the hepatocytes of individuals who do not consume alcohol, triggered by the imbalance between absorption, hepatic synthesis, and excretion, constitutes the first step towards the development of the disease[5]. The second step is based on lipid deposition that contributes to an increased production of reactive oxygen species and gives rise to oxidative stress that, associated with the inflammatory process, becomes responsible for the appearance and evolution of liver lesions[6]. Based on these statements, blocking both the accumulation of lipids and the onset of oxidative stress is an efficient alternative to prevent the development of NAFLD.

Cinnamon has phenolic compounds in its barks, and it is known that these compounds act by reducing serum concentrations of insulin[7], triacylglycerol[8] and cholesterol[9]. In this context, cinnamon (*Cinnamomum zeylanicum*) has attracted attention in recent years due to the beneficial effects associated with its ingestion[10,11,12,13].

Cinnamon intake has been linked to a reduction in oxidative stress[14], an improvement in the plasma lipid

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profile[15], blood pressure levels[11] and a decrease in blood glucose levels[16]. However, until that date, few studies on the effects of consumption of the phenolic compounds present in the cinnamon bark and leave (*Cinnamomum zeylanicum*) on the progression of NAFLD have been published.

Considering the benefits of cinnamon consumption, the objective of this study was to evaluate the action of the crude leaf extract and the crude stem bark extract of the cinnamon (*Cinnamomum zeylanicum*) on biochemical parameters and histological, morphometric, and stereological aspects of the liver of obese rats.

Material and Methods

Obtaining and treating plant material

The plant material consisting of the leaves and barks of the stem of *Cinnamomum zeylanicum* was collected on the Campus of the Federal University of Pernambuco and the specimens were deposited in the herbarium Dárdaro de Andrade-Lima at the agronomic institute of Pernambuco with the number of Tombo 91609. The material was submitted to drying in a circulating air oven and later, the dry materials were sprayed separately.

Production of crude extracts from cinnamon

The extracts of cinnamon leaves and barks were obtained separately by turbolysis with the aid of an industrial blender (Metvisa®) for 20 minutes, with intervals of 30 seconds for each extractive cycle of 5 minutes. For each 40 g of vegetable drug, dried and ground, 400 mL of the acetone mixture were used: water (7:3, v/v) extracting liquid, with a vegetable drug/solvent ratio of 10% (w/v). The extracts were filtered on cotton with the aid of a vacuum and later concentrated on a rotary evaporator under reduced pressure (40ºC) to eliminate the organic solvent. Finally, the two types of crude extracts were frozen in a freezer (T = -80ºC) for 3 days and lyophilized (L101, Liotop®), thus obtaining the crude extracts (CE) of the leaves and stem barks of the cinnamon.

Biological Test

Animals and experimental design

The study was approved by the Ethics Committee on the Use of Animals of the Biological Sciences Center of the Federal University of Pernambuco (CEUA/CCB/UFPE) under the registration: 23076.041253/2016-21. Thirty male albino *Wistar* rats, 21 days old (post-weaning) and weight varying between (60 - 75g) were used.

Throughout the experiment, the animals were kept in standard vivarium conditions (temperature of 22 ± 2ºC) receiving their diets without restriction and water *ad libitum*, according to ethical recommendation by the National Council for Animal Experimentation Control (CONCEA).

The animals were separated into cages forming two large groups, thus established: Normonourished Groups (15 animals) and Obese Groups (15 animals), with five animals being distributed per cage. The animals in the normonourished groups after weaning received the standard Presence® rodent diet, from the 21st to the 120th day of life. The animals in the obese groups after weaning were fed a high-calorie diet (westernized diet), from the 21st to the 120th day of life.

After 99 days of ingesting the high-calorie diet, obesity was established (animals between 15-20% more weight than normonourished water - NW animals). Then, the rats were randomly divided according to treatment with leaf extract or cinnamon bark or water filtered by gavage for 21 days, forming 6 subgroups: Normonourished water - NW group (n=05) – daily administration intra-gastric (gavage) filtered water; Normonourished Crude Leaf Extract of Cinnamon - NL (n=05) – daily administration by intra-gastric (gavage) dose of crude extract of cinnamon leaf (*C. zeylanicum*); Normonourished Crude Extract Bark of the Cinnamon - NB (n=05) - daily administration by intra-gastric route (gavage) of dose of the crude extract of the cinnamon bark (*C. zeylanicum*); Obese water - OW (n = 05) - daily administration by intra-gastric route (gavage) of filtered water; Obese Extract Crude Leaf of Cinnamon - OL (n = 05) - daily administration by gastric route (gavage) of dose of crude extract of cinnamon leaf (*C. Zeylanicum*); Obese Crude Extract Bark of the Cinnamon - OB (n=05) - daily administration by intra-gastric route (gavage) of dose of the crude extract of the cinnamon bark (*C. zeylanicum*). The dose used in the study was 200 mg/kg/day established after the acute oral toxicity test in rats with the crude extracts of leaves and barks cinnamon according to the OECD 423 guide.

Diets used

The standard diet used in the experiment, consisted of commercial feed developed for Presence® laboratory rats, composed of corn bran, wheat bran, soy bran, calcium carbonate, phosphate, dicalcium, sodium chloride, amino acid, and vitamin mineral premix. It has an average of 24.3 g% protein, 56.0 g% carbohydrate, 3.2 g% lipids and 5.0 g% fibers with a caloric supply of 3.3 kcal/g.

The hypercaloric diet (westernized diet) used has purified/semi-purified industrialized ingredients (laboratory Rhoster Indústria e Comércio) and natural foods. It was prepared at the UFPE Nutrition Department, produced for the growth and maintenance phases of the rats with use according to the age of the animal, with variations in its palatable components for each of them, but with preservation of the nutritional contents. In the growth phase, the diet had protein values around 18 g%, lipids 17 g%, carbohydrates 44 g% and fibers 5.2 g% with an average supply of 4.0 Kcal/g;

The maintenance diet had a composition of around 14% proteins, 18% lipids, 47% carbohydrates and 5.2% fibers with an average supply of 4.0 Kcal/g.

Collection of blood and tissues

At 120 days of life, the animals were fasted for 12 hours and anesthetized with ketamine (45 mg/kg) and Xylazine (5 mg/kg) intramuscularly. Then, blood was collected by cardiac puncture to obtain the serum and biochemical measurements. In sequence, a trans-cardiac perfusion was performed to obtain the liver, which was dissected, weighed, and conditioned in pots identified in a 10% buffered formaldehyde fixing solution for histological analysis.

Analysis of biochemical parameters

The concentrations of aspartate aminotransferase (AST), alanine aminotransferase (ALT), total cholesterol, triglycerides, and glucose, were determined in plasma, using commercial kits (LABTEST®), in a biochemical analyzer AU680 Chemistry Analyzer. The tests were performed at the Laboratory of Needy and Metabolic Diseases - UFRPE.

Histological, morphometric, and stereological evaluation of livers

After fixing in 10% formaldehyde solution for 24 hours, some morphometric parameters were analyzed, the livers were weighed and the measurement of the hepatosomatic index (HSI) was obtained by the relationship between liver weight and body weight on the day of euthanasia (HSI = liver weight/body weight X 100), in addition to the relationship between the weight of the average liver lobe and body weight on the day of euthanasia. The middle lobe was separated from the others and selected to measure weight and length; a caliper was used to measure the length (Figure 1B). After obtaining the measurements, the lobe was cross sectioned into several slices of 2mm each (Figure 1C). Since the first slice of each lobe is always neglected, after this stage a fraction of 1/2 was applied to select the slices to be studied (Figure 1D).

Then the selected slices were photographed, and the photos were added to the imageJ program (version 1.3.4.67) for the stereological quantification of the volume of the middle lobe of the liver, using the Cavalieri principle, which consists of a quadratic test system to calculate areas and volumes. This Principle was used in macroscopic sections, arranged in transverse slices using the following formula: $V = \sum P \; x \; (a/p) \; x \; t$. Where, $\sum P$ corresponds to the sum of points in the test system that touch the desired structure (sectional area of any organ), (a/p) is the area associated with each point in the test system, and t is the thickness of each slice[17].

Then, each slice was divided into 6 fragments (Figure 1E) and all fragments were distributed on a flat surface from the smallest to the largest fragment one and, from there to the smallest again, forming a "U" shape (Figure 1F and 1G), where it was applied a fraction of 1/3 was and then the fragments were selected to be analyzed (Figure 1H and 1I).

An average of 10 to 12 fragments were sampled to be inserted in the histological cassettes and arranged for processing in routine histological techniques

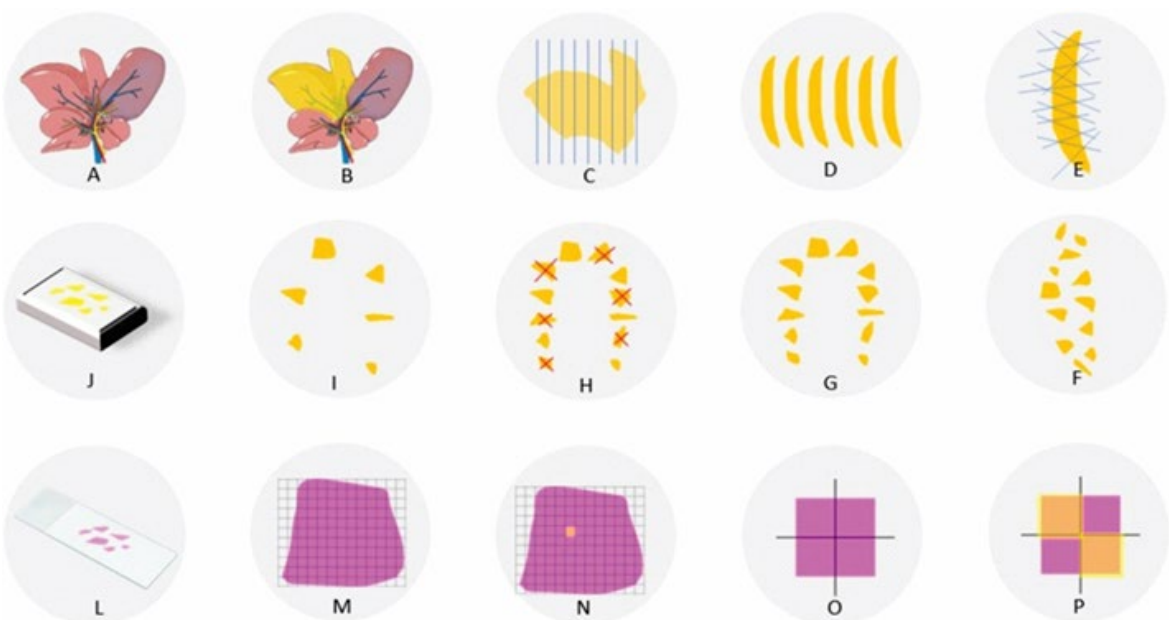

Figure 1. Scheme showing obtaining and sampling fragments of hepatic tissue from rats for histological analysis.
A. illustration of whole rat liver. B. Medium lobe of rat liver. C. Medium lobe of the rat liver sectioned transversely in several slices of 2mm each. D. Selected slices of the middle lobe of the rat liver. E. Selection of 6 fragments from each slice of the middle lobe of the rat liver. F. All fragments of the slices. G. Distribution of hepatic tissue fragments on a flat surface from the smallest to the largest fragment and from there to the smallest again in a "U" shape. H. Application of a fraction of 1/3 for sampling the fragments. I. Selected fragments. J. Fragments on histological cassettes. L. Slide stained using the hematoxylin-eosin technique. M. Field superimposed by a transparent grid with squares of 1mm2 observed in the 100x objective. N. Selection of a square for each field. O. Square divided into four quadrants that were photographed with the 400x objective. P. Selection of two photos per square for analysis.
Source: Elaborated by the authors.

(Figure 1J). The Leitz microtome model 1512 was used to perform the 5μm thickness cuts. All histological sections were stained using the hematoxylin-eosin technique to observe the histomorphological aspects of the tissue (Figure 1L).

After this procedure, the samples were sent for histological reading by a researcher experienced in animal pathology for qualitative analysis. From the slides of each animal, five fields were selected through randomization to be photographed by the camera (Moticam 1000 1.3 MP) coupled to the Labomed brand microscope using Motic Image Plus 2.0 software. Each field was superimposed on a transparent grid with squares of $1mm^2$ (Figure 1M). Through a new randomization, only one square was selected for each field (Figure 1N). This square was divided into four quadrants that were photographed in the 400x objective using the Motic Image Plus 2.0 software (Figure 1O). Subsequently, a new fraction of 1/2 was applied for the selection of two photos per square (Figure 1P). In total, 300 photos were analyzed, 10 photos of each animal, from the 150 selected fields.

Using morphometric parameters, the area of the hepatocyte nucleus and the number of binucleated hepatocytes were quantified. From the slides of each animal, photomicrographs of 5 fields were performed as previously described. In total, 25 fields from each group were photomicrographed.

To measure the area of the nuclei of the hepatocytes, the ImageJ program (version 1.3.4.67) was used, which allows by means of the outline of the studied structure through the ellipse tool, that is, the nucleus of the hepatocytes, the verification of the area, in this case in μm^2.

The same program was used for the numerical quantification of the binucleated hepatocytes, the binucleated hepatocytes were quantified by group in a semi-automatic way, using the program counting plugin.

Volume Density (Vv) of the following liver components was checked using stereological parameters: nucleus of hepatocytes, sinusoid capillaries, hepatocyte with microsteatosis. Thus, the relative occupation of the test area was determined by the area of images of the studied structure, since the basic law of stereology is that the relative number of points that touch the structure is comparable to the volume of this structure in the test area. Volume Density (Vv) was calculated according to the formula: $Vv[structure] = (P[structure])/P_T$, where *P[structure]* is the number of points that cross the tested structure and P_T the total number test points of the test system. The *Vv[structure]* can be multiplied by 100 to express the result as a percentage[18].

From the slides of each animal, photomicrographs of 5 fields were also performed with a 400x magnification as previously described, 25 fields of each group were photomicrographed.

For the analysis, a 352-point test system available in the ImageJ program (version 1.3.4.67) was used. The points were superimposed on the images and by counting the points it was possible to determine the volume density (Vv) of the structures.

Statistical Analysis

The data were recorded in Excel spreadsheets for analysis of the parametric data: mean and standard deviation. To verify differences in mean values and between groups, the One-Way Statistical Test (ANOVA) was used, complemented with the Bonferroni Multiple Comparison Test. For data processing, the statistical program Graphpad Prism 6.0 (GraphPad Software Inc., La Jolla, CA, USA) was used. Most results were considered significant with $p<0.05$.

Results

Effect of using crude cinnamon leaf and cinnamon bark extracts on the biochemical parameters

After analyzing the biochemical profile of the animals, the parameters were expressed in Table 1. Regarding liver function, there was an increase in the levels of aspartate aminotransferase (AST) in the animals of the obese water group, moreover an improvement was also observed in AST levels in obese animals treated with crude extracts of cinnamon leaf and bark. In normonourished water animals and normonourished animals treated with extracts, the AST values remained within the normal range (61-210 U/L), there was no statistical difference between the groups. In addition, a decrease in alanine aminotransferase (ALT) was identified in the animals in the obese bark group, while animals in the other groups showed ALT levels within the desirable parameters (38-82 U/L), with statistical difference between the groups obese water *versus* obese bark (55.17±8,994; 37.41±4.48, p: 0.0039) (Table 1).

As for the lipid profile, there was no statistical difference between the groups with respect to cholesterol, they remained within the normal parameters (45 – 76 mg/dL) both in the animals of the normonourished groups and in the animals of the obese groups. As for triglyceride levels, high values were observed in animals in the obese water group, as well as a decrease in triglyceride levels to desirable values in animals in obese groups treated with crude cinnamon bark and leaf extracts. The animals of the normonourished water groups and those treated with cinnamon extracts remained with normal triglyceride values. The statistical difference was present between the groups normonourished water *versus* obese water (82.09±13.68, 157.23±42.09, $p <0.0001$), obese water *versus* obese leaf (157.23±42.09, 57.10±10.25, $p <0.0001$) and obese water *versus* obese bark (157.23±42.09, 93.08±41.08, $p <0.0001$) (Table 1).

Regarding the glycemic profile, only the animals in the obese water group had high glycemic levels,

Table 1. Biochemical values after treatment with crude extracts from cinnamon leaves and bark.

Parameters evaluated	Groups						RV
	Normo			Obese			
	Water	Leaf	Bark	Water	Leaf	Bark	
AST (U/L)	162.69±32.5	170.10±30.99	147.42±16.3	225.22± 97.38↑	158.22±21.42	203.15±53.7	61-210
ALT (U/L)	58.61±11.4	55.15± 6.31	50.30±5.71	55.17± 8.99*	43.18± 10.93	37.41± 4.48*	38-82
TC (mg/dL)	57.81±9.93	68.21± 8.47	62.72±5.29	75.76± 14.94	60.19± 5.37	70.32± 9.81	45-76
TG (mg/dL)	82.09±13.68*	81.10±10.56	65.23±16.14	157.23±42.09*↑	57.10±10.25*	93.08±41.08*	22-100
Glucose (mg/dL)	149.37±23.58	161.68±38.51	168.12±38.08	197.78±57.17↑	170.92±47.07	162.14±45.28	72-193

Values represent mean ± SD (n= groups of 5 animals). * The same line indicates the statistical differences between groups ($p<0.05$) (One-Way ANOVA followed by Bonferroni Post-Test, $p<0.05$). VR- Reference value; AST-aspartate aminotransferase; ALT-alanine aminotransferase; CT-Total cholesterol; TG-Triglyceride.
Source: Elaborated by the authors.

whereas the obese animals treated with crude cinnamon extracts showed a decrease in glycemic levels to normal values. In the groups of normonourished animals, glycemia remained within the desirable parameters (72-193 mg/dL). There was no statistical difference between the groups.

3.2 Effect of using crude cinnamon leaf and cinnamon bark extracts on histopathological and morphometric and stereological parameters.

Regarding the histopathological study of the liver tissue of normonourished animals treated or not with cinnamon extracts, it can be noticed the presence of hypertrophic and hyperplastic hepatocytes with single nucleus and binucleate, atrophy of the hepatocyte cords, activated Kupffer cells, in addition to the presence of mild to moderate microsteatosis. As for the obese animals, it was possible to identify in the histopathological analysis the same changes found in the normonourished animals, in addition to the presence of moderate/intense macrosteatosis and microsteatosis, diffuse and moderate coagulation necrosis and dilation of sinusoid capillaries in obese animals that did not received treatment with the extracts. In the obese animals that received cinnamon bark and leaf extracts, there was an improvement in the pattern of macrosteatosis and microsteatosis, which became mild to moderate, as well as a decrease in coagulation necrosis becoming focal and no longer diffuse and moderate (Figure 2).

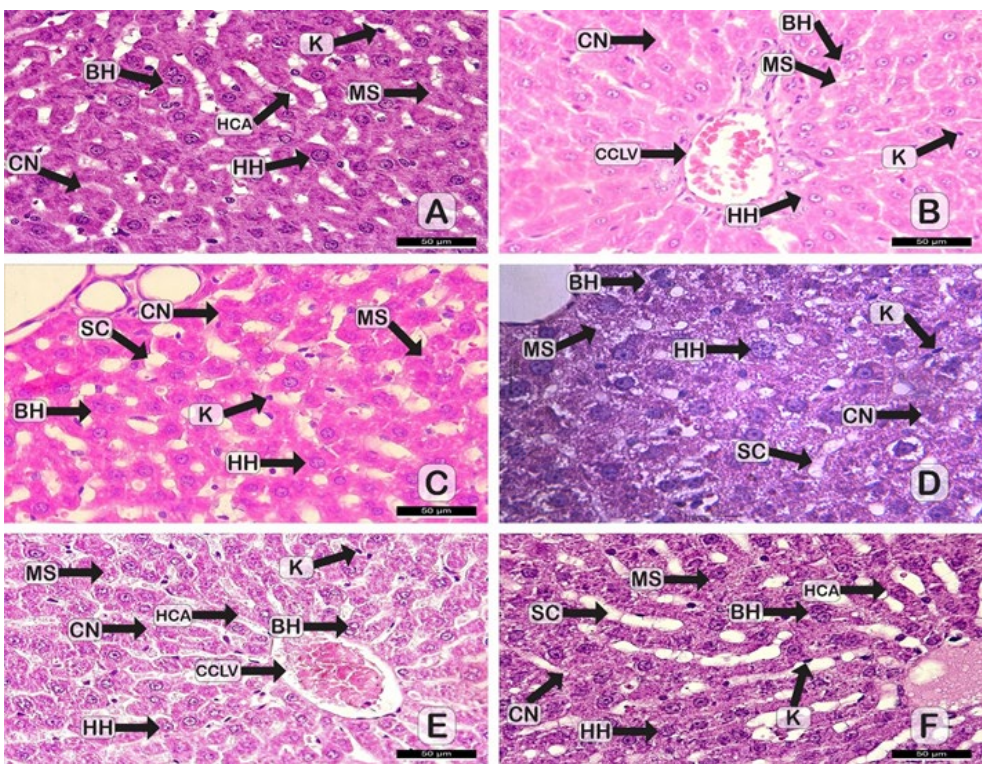

Figure 2. Liver of rats after treatment with crude extracts of the leaves and barks of cinnamon. Hematoxylin-eosin stain.
2A - Liver of normonourished water rats. Observe: hyperplastic and hypertrophic hepatocytes with single or binucleated nucleus (HH, BH), hepatocyte cords atrophy (HCA), presence of discrete microsteatosis (MS), coagulation necrosis (CN) and discrete and presence of activated Kupffer cells (K).
2B - Liver of normonourished rats treated with crude extract of leaves of Cinnamomum zeylanicum. Observe: hypertrophic hepatocytes with single or binucleated nucleus (HH, BH), coagulation necrosis (CN), discrete microsteatosis (MS), congestion in the centrilobular vein (CCLV) and presence of activated Kupffer cells (K).

2C - Liver of normonourished rats treated with crude extract from the barks of Cinnamomum zeylanicum. Observe: hyperplastic and hypertrophic hepatocytes with single or binucleated nucleus (HH, BH), coagulation necrosis (CN), dilation of sinusoid capillaries (SC), activated Kupffer cells (K) and presence of mild to moderate microsteatosis (MS).
2D - Liver of obese water rats. Observe: diffuse (moderate), macro and microvesicular (MS) steatosis, presence of activated Kupffer cells (K), diffuse and moderate coagulation necrosis (CN), dilation of sinusoid capillaries (SC) and hypertrophic hepatocytes with single or binucleated nucleus (HH, BH).
2E - Liver of obese rats treated with crude extract of leaves of Cinnamomum zeylanicum. Observe: diffuse steatosis (mild to moderate), microvesicular (MS), presence of activated Kupffer cells (K), congestion in the centrilobular vein (CCLV), focal coagulation necrosis (CN), hepatocyte cord atrophy (HCA) and hypertrophic hepatocytes with single or binucleated nucleus (HH, BH).
2F - Liver of obese rats treated with crude extract from the barks of Cinnamomum zeylanicum. Note: Mild diffuse steatosis, microvesicular (MS), dilation of sinusoid capillaries (SC), focal coagulation necrosis (CN), atrophy of hepatocyte cords (HCA), presence of activated Kupffer cells (K) and hypertrophic hepatocytes with nucleus single or binucleated (HH, BH).
Source: Elaborated by the authors.

Regarding the hepatosomatic index of liver weight and middle liver lobe weight, there was no statistical difference between the groups, as well as there was no difference between the groups regarding the length of the hepatic middle lobe.

The density of hepatocytes with steatosis (Vv steatosis) was higher in the obese group compared to the normonourished one, it is also observed that after the treatment with the extracts of the leaf and bark of the cinnamon there was a reduction in the density of hepatocytes with steatosis in both normonourished animals and in obese animals, with a significant reduction in Vv steatosis in normonourished animals treated with cinnamon leaf extract, with a statistically significant difference between the normonourished leaf group and the obese leaf group (9.10±2.8, 21.70±1.5, p = 0.0011) (Figure 3).

The volume of the medium lobe of the liver or reference volume (RefV) estimated by the Cavalieri method showed no statistical difference between the experimental groups, but the obese water group (0.714±0.14, p = 0.0734) showed a greater volume when compared to the normonourished water animals (0.499±0.07, p = 0.0734). In addition, there was a decrease in the volume the obese animals' livers treated with cinnamon leaf extract (0.544±0.06, p = 0.0734) and bark (0.554±0.09, p = 0.0734) (Figure 4).

Regarding the quantity of binucleated hepatocytes, it was observed that the obese water (8.12±1.19, p <0.0001) and obese groups treated with cinnamon leaf extract (8.06±0.82, p <0.0001) showed an increase in binucleation of the hepatocytes, when compared to normonourished groups. It was also observed that both the treatment with the bark and with the

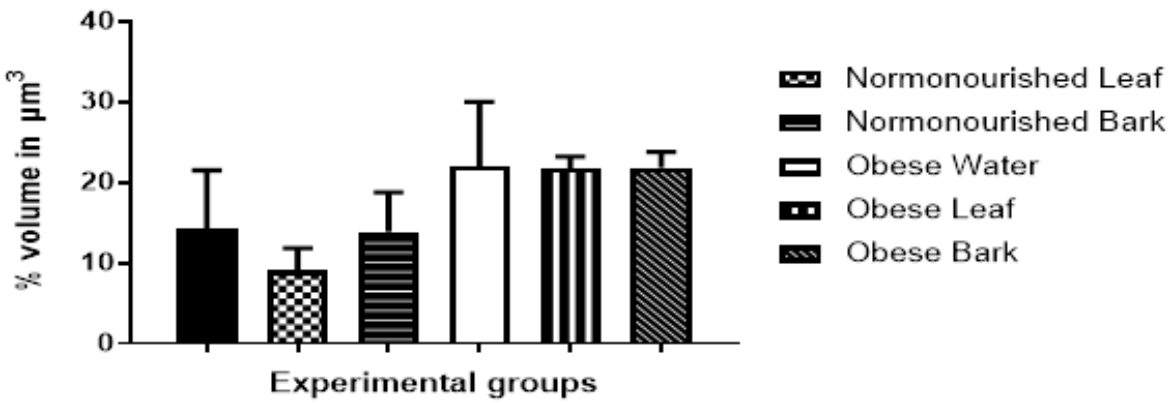

Figure 3. Hepatocyte volume density with steatosis Vv [steatosis] from the liver of Wistar rats. Values are represented by mean ± standard deviation (SD). Significance obtained from the One-Way ANOVA Test followed by the Bonferroni Post-Test (p <0.05). ** (p = 0. 0011) normonourished leaf versus obese leaf.
Source: Elaborated by the authors.

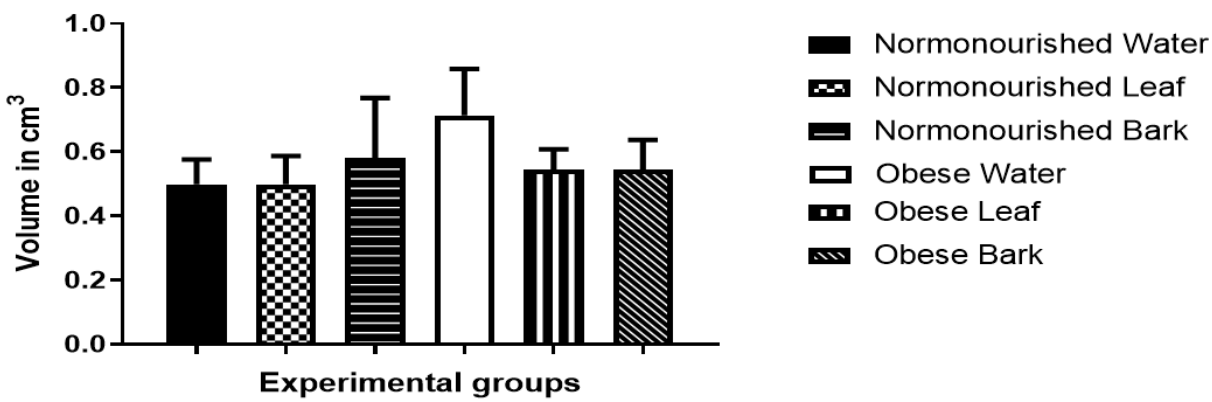

Figure 4. Volume of the middle lobe of the liver of rats (Vref) using the Cavalieri method. Values are represented by mean ± standard deviation (SD). There was no significant difference between groups. Significance obtained from the One-Way ANOVA Test followed by the Bonferroni Post-Test (p: 0.0734).
Source: Elaborated by the authors.

cinnamon leaf decreased the binucleation in both normonourished and obese animals. There was a statistically significant difference between the groups normonourished water *versus* normonourished bark (6.92±1.26, 3.64±1.30, p <0.0001), normonourished leaf *versus* obese leaf (4.98±0.90, 8.06±0.82, p <0.0001), normonourished bark *versus* obese bark (3.64±1.30 5.96±0.53, p <0.0001), and obese water *versus* obese bark (8.12±1.19, 5.96±0.53, p <0.0001) (Figure 5).

When using stereological analyzes to verify changes promoted either by obesity or by treatments, it was found that the obese animals had values of volume density (Vv) of the nuclei of the hepatocytes smaller than the normonourished animals and that the treatment with cinnamon extracts cause an increase in the Vv of the nucleus of hepatocytes in obese and normonourished animals, with a statistically significant difference in this increase between animals obese water *versus* obese bark (8.02±0.69, 10.47±1.45, p = 0.0012) (Figure 6).

Regarding the measurement of the nuclear area of hepatocytes, it was identified that the group of obese water animals (42.42±5.08, p <0.0010) showed a decrease in the nuclear area of hepatocytes in relation to the group of normonourished water animals (53.35±4.48, p <0.0010), but without statistical difference. In addition, it was possible to identify that cinnamon leaf and bark extracts increased the nuclear area in both normonourished and obese animals. There was a statistical difference between the obese water *versus* the obese bark group (42.42±5.08.60.70±7.43, p <0.0010) (Figure 7).

Regarding the results of the sinusoidal Vv, it was noted that the animals in the normonourished and obese groups treated with cinnamon bark and leaf extract obtained an increase in the sinusoidal Vv and that the sinusoidal Vv was decreased in the animals in the obese water group to animals in the normonourished water group, with no statistically significant difference (Figure 8).

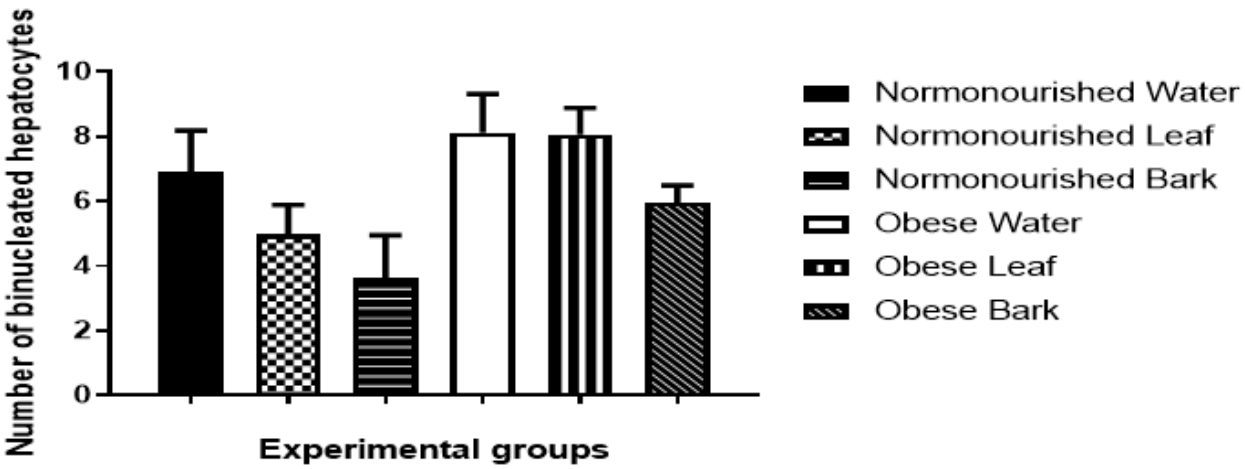

Figure 5. Binucleated hepatocytes from the liver of *Wistar* rats. Values are represented by mean ± standard deviation (SD). Significance obtained from the One-Way ANOVA Test followed by the Bonferroni Post-Test (p <0.05). *** (p <0.0001) normonourished water versus normonourished bark; **(p <0.0001) normonourished leaf versus obese leaf; *(p <0.0001) normonourished bark versus obese bark and *(p <0.0001) obese water *versus* obese bark.
Source: Elaborated by the authors.

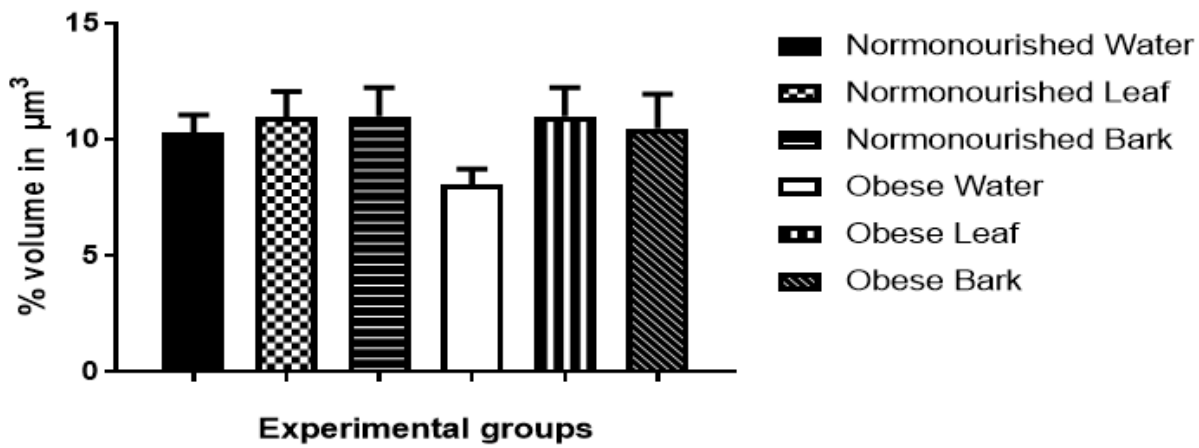

Figure 6. Volume density of Vv nucleus [hepatocyte nucleus] in the liver of Wistar rats. Values are represented by mean ± standard deviation (SD). Significance obtained from the One-Way ANOVA Test followed by the Bonferroni Post-Test (p <0.05). *(p: 0.0012) obese leaf versus obese bark.
Source: Elaborated by the authors.

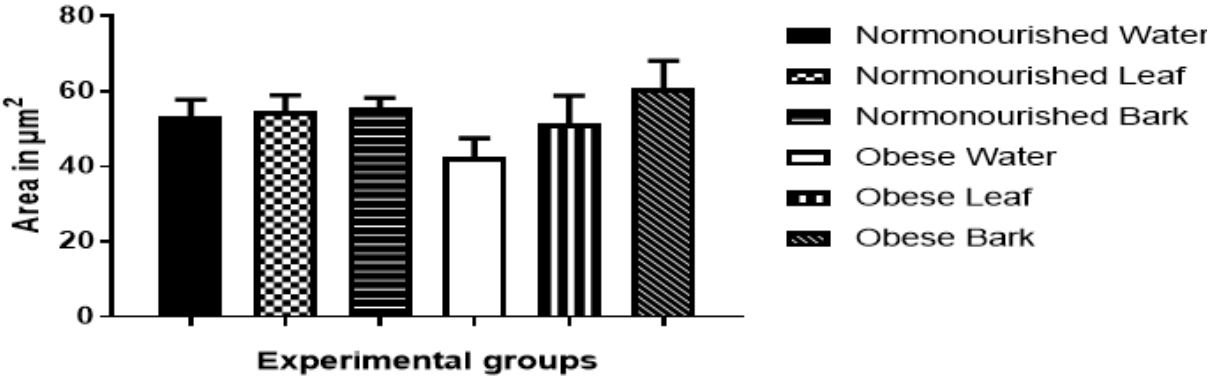

Figure 7. Core area of liver hepatocytes from Wistar rats. Values are represented by mean ± standard deviation (SD). Significance obtained from the One-Way ANOVA Test followed by the Bonferroni Post-Test (p <0.05). ***(p <0.0010) obese water versus obese bark.
Source: Elaborated by the authors.

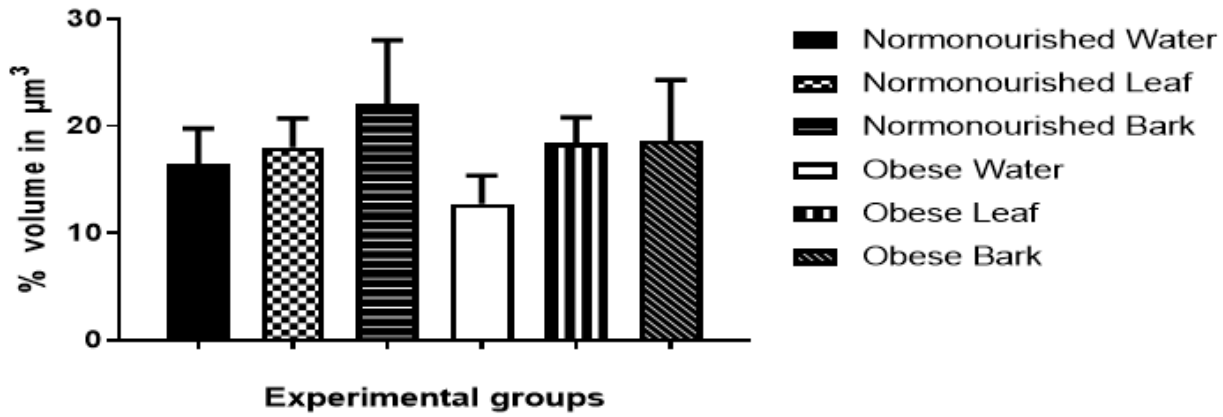

Figure 8. Density of sinusoid volume Vv [sinusoid] of the liver of Wistar rats. Values are represented by mean ± standard deviation (SD). There was no significant difference between groups. Significance obtained from the One-Way ANOVA Test followed by the Bonferroni Post-Test (p <0.05).
Source: Elaborated by the authors.

Discussion

Several pathophysiological disorders (systemic arterial hypertension, diabetes, atherosclerosis, hyperlipidemia, hepatic steatosis, and cancer) are directly caused or aggravated by obesity, with consequent increased morbidity and reduced life expectancy and quality of life[19,20]. In recent years, the interest in studying phytotherapics has been focused on the potential biological effects of polyphenols, present in these compounds, on the prevention of obesity and associated clinical manifestations[21,22]. Recently, studies have suggested that cinnamon (*Cinnamomum zeylanicum*) may be effective in reducing blood pressure, blood glucose levels, and serum cholesterol[11,12,13,] in addition to having hepatic protective effects[10, 11].

In the present study, the action of cinnamon leaf or bark extract on biochemical parameters of obese rats shows beneficial effects on serum triglyceride and enzyme levels related to tissue damage liver and other organs. In the liver in particular, the greatest finding is in the regression of hepatic steatosis in obese rats treated with cinnamon extract.

Changes in serum enzyme rates related to tissue damage have also been reported by Song *et al.* (2016). These authors supplemented hypercholesterolemic mice daily with blackberry extracts and suggested a hepatoprotective effect due to the decrease in serum levels of ALT and AST[23]. Similarly, daily administration of açaí decreased plasma ALT and hepatic TG concentrations in animals submitted to a high-fat diet[24]. The authors related these protective effects to the content of bioactive compounds in these fruits, known as natural sources of phenolic compounds and flavonoids, which are also compounds found in cinnamon extract. A study that induced hepatic damage in rats using acetaminophen showed a significant reduction and restoration of ALT and AST levels by supplementing these animals with cinnamon extract[14].

These enzymes are considered markers of nonspecific hepatocellular lesion and, according to Pacífico *et al.* (2007) the increase in the levels of transaminases is directly related to the increase in the fraction of hepatic fat[25]. Csonka *et al.* (2017), however, observed that in rats fed a high-fat diet for 12 weeks, there was an increase in the levels of alkaline phosphatase and the AST and ALT concentrations remained within normal parameters[26]. Yet, because alkaline phosphatase is not a specific hepatic damage

enzyme, its isolated increase requires further investigation.

In obese animals, treatment with cinnamon leaf and bark extract was effective in reducing blood triglyceride levels. This result is in accordance with the literature, Kassaee *et al*. (2017) demonstrated that cinnamon (*Cinnamomum zeylanicum*) significantly reduces triglyceride, total cholesterol, and LDL cholesterol (low density lipoprotein), while significantly increasing the HDL cholesterol (high density lipoprotein) in hyperlipidemic hamsters[27]. Another study carried out in Sri Lanka by Ranasinghe and Gunatilake *et al*. (2012) demonstrated that cinnamon (*Cinnamomum zeylanicum*) significantly reduces total cholesterol and LDL cholesterol, whereas no significant changes in triglyceride and HDL cholesterol levels were observed in healthy rats, differing from the present findings[15]. The possible reason for the different findings in these studies is that cinnamon (*Cinnamomum zeylanicum*) has the potential to reduce triglyceride levels and improve HDL cholesterol only in dyslipidemic participants. The mechanism for the effects of lipid reduction has not yet been clarified in the literature. Probable mechanisms for this effect include the high content of dietary fiber in cinnamon, which reduces intestinal absorption of lipids and the high content of vitamins and antioxidants, which increases lipid metabolism[28]. In addition, insulin plays a key role in lipid metabolism, and it can be postulated that the consumption of cinnamon improves lipid levels through its stimulatory effect on insulin, as demonstrated by the increase in serum insulin levels after the administration of *Cinnamomum zeylanicum*.

When assessing glucose levels according to the table of biochemical parameters for male *wistar* rats, high rates were found in the obese water group. Being observed in the present study anti-hyperglycemic action of the cinnamon leaf and bark extract of the cinnamon in obese animals. These findings corroborate with another study by Beji *et al*. (2018) who reports that cinnamon (*Cinnamomum zeylanicum*) has the potential to reduce fasting glycemia in diabetic animals[29]. In another study by the same group of researchers in Sri Lanka, a decrease in fasting blood glucose levels was also identified in diabetic animals that received cinnamon supplementation. However, in the same study, there was no decrease in fasting glycemia among healthy rats (Ranasinghe and Gunatilake *et al*., 2012)[15]. Thus, it is possible that cinnamon (*Cinnamomum zeylanicum*) does not cause a reduction in fasting glycemia among healthy normoglycemic individuals, as in the present study.

Some *in vitro* studies have shown how cinnamon (*Cinnamomum zeylanicum*) acts to reduce glucose levels, according to Adisakwattana *et al*. (2011), cinnamon has the potential to reduce postprandial intestinal glucose absorption by inhibiting the activity of enzymes involved in the metabolism of carbohydrates (pancreatic α-amylase and α-glycosidase)[30], already Anand *et al*. (2010) states that cinnamon stimulates cellular glucose uptake by translocation of the glucose-4 (GLUT4) transporter membrane[31]. Another study by Soonham *et al*. (2010) argues that cinnamon stimulates glucose metabolism and glycogen synthesis, inhibiting gluconeogenesis due to the effects on the main regulatory enzymes[32].

Regarding the histological findings in the livers of normonourished and obese rats, small hepatic changes were observed in all groups analyzed, but mainly in obese animals.

In the analysis of the livers of obese animals, one of the most important findings of this research was found. A mild to moderate pattern of steatosis was observed in the obese groups that were treated with cinnamon extracts, contrasting with the liver findings of the obese water animals, where steatosis (moderate - intense), macro and microvesicular steatosis and diffuse coagulation necrosis were observed (moderate). Cinnamon extracts may have reduced hepatic damage due to the accumulation of fat, due to its antioxidative power, or even prevented the accumulation of fat. These findings corroborate those of Eidi *et al*. (2012) who demonstrate the hepatoprotective, curative and antioxidant effects of cinnamon extract against liver damage induced by carbon tetrachloride (CCl4) in rats[33].

This accumulation of fat was also evidenced by stereological parameters, where the volume density of steatosis (Vv steatosis) was increased in the animals of the obese groups when compared to the animals of the normonourished groups. It was evidenced in the present study that the treatment with the extracts acted in reducing the Vv steatosis in the obese animals, even though there was no statistically significant difference. Also, in the normonourished animals the treatment with leaf extract showed statistically significant difference in reducing the levels of the Vv steatosis compared to the obese animals treated with leaf extract. It is believed that the action of cinnamon leaf extract is due to the presence of phytoconstituent, such as: flavonoids, terpenes, tannins, steroids and saponins. These same phytoconstituent are found in cinnamon bark extracts[34]. A review by Nabavi *et al*. (2015) further specifies the constituents of the extracts of cinnamon leaves and barks, claiming that the authors claim that the oil in the leaf has a predominant component, the eugenol, while the oil in the bark presents cinnamaldehyde as its main constituent[35].

In addition, an increase in the volume of the liver was also observed in the animals of the obese water group, fed with a Westernized diet, this increase seems to reflect the accumulation of fat in this tissue, probably resulting from the high content of lipids present in the hypercaloric diet, since the consumption of a high-fat diet results in an increase in lipid infiltration in hepatic tissue[36]. In addition, it was observed that the

obese groups treated with extracts of the cinnamon bark and leaf had hepatic volume close to that of the normonourished water group, suggesting that the treatment with the extracts may have reversed possible changes caused by the ingestion of the hypercaloric diet.

Still dealing with the analysis of hepatic tissue, it was possible to identify in the studied animals that there was a decrease in the binucleation of hepatocytes in the normonourished and obese animals treated with the different extracts, with a greater reduction in the binucleation in the animals treated with bark extract. These findings may suggest that cinnamon bark extract has greater potential for protection of hepatic tissue, regarding hepatocyte ploidy, when compared to cinnamon leaf extract. In this sense, a study by Gentric *et al*. (2015) states that the change in the ploidial profile of hepatocytes, that is, the binucleation of hepatocytes is associated with the regenerative capacity of the liver against damage (stress, fat, metabolic overload) and the amount of binucleated hepatocytes is proportional to the exposed damage[37.] Therefore, we can infer that the cinnamon stem bark extract had a more significant role considering hepatic protection against the damage of obesity and against the damage caused by the toxicity of the crude extract itself.

These findings differed from a study by Lannes *et al*. (2018) who observed little hepatocyte binucleation in rats simultaneously treated with streptozotocin and with a high-fat diet[38].

The results obtained in the stereological analysis referring to the volume density (Vv) of the hepatocyte nucleus allowed to verify that the nucleus Vv was lower in obese animals and that the treatment with the extracts promoted an increase in the nucleus Vv in both normonourished and obese groups. These findings can be justified by the presence of a greater amount of fat in obese animals that can cause damage to hepatocytes and consequently a decrease in the amount of nuclei[39]. In this study, the damage was visible in the histological analysis, where the presence of diffuse coagulation necrosis in the tissue of these animals was identified, thus making nuclear visualization difficult.

As for the increase in hepatocyte nucleus Vv in the animals that received the extracts, it may be related to the increase in cellular metabolism due to the action of the extracts[40]. In addition, in obese animals this increase can also be justified due to the large quantities of phenolic compounds present in these extracts that act to attenuate inflammation and reduce the oxidative stress resulting from obesity, thus restoring nuclear function[28,10]. Similar results were found found in another study by Rafiei, Omidian, and Bandy (2017) that demonstrated that phenolic compounds act in decreasing the generation of reactive oxygen species, in increasing the number of mitochondria, in decreasing inflammation and, in the accumulation of fat in the liver, thus preventing damage to hepatocytes caused by excess lipids[41].

The analysis of the area of the nuclei of the hepatocytes allows to expand the considerations, since the area of the nucleus decreased in the obese water group in relation to the normonourished water group, thus identifying a possible action of fat on the reduction of hepatocyte nuclei. In addition, it was observed that the treatment with the extracts promoted an increase in the nuclear area of hepatocytes in normonourished and obese animals. According to Silva 2006, this increase in the nuclear area is an indicator of the functional activity of the cel[42]. Hence, it can be inferred that the higher nuclear area value is due to the higher metabolism of hepatocytes exposed to cinnamon extracts[40].

Consequently, the increase in the nuclear area and the Vv of the nucleus of the hepatocytes in both normonourished and obese animals treated with cinnamon extracts, may be related to the increase in cellular metabolism resulting from the action of the extracts in this organ.

In addition, the animals in the obese water group also had lower volume density of the sinusoids compared to the animals in the normonourished water group. This also suggests that the high-fat diet leads to lesions in the sinuses, a finding that corroborates with another study carried out by Neves *et al*. (2006), which demonstrates a decrease in the sinusoid Vv in animals fed with diets with a high lipid content[39]. Moreover, another previous study also showed that long-term administration of a high-fat diet caused the accumulation of hepatic fat with a reduction in the Vv values of the sinusoid[43]. It is important to note that sinusoids play a fundamental role in providing blood flow to hepatocytes, especially during the hepatic regeneration process. Thus, the decrease in the sinusoidal Vv can lead to impaired adequate cellular functioning[43].

The sinusoid Vv showed higher levels in the animals that were submitted to the use of the extracts, this result highlights the potential of the phenolic compounds present in the cinnamon leaves and barks against possible cellular alterations present in the hepatic tissue[33].

Conclusion

The crude extracts of the leaves and crude extracts of the stem barks of the cinnamon (*Cinnamomum zeylanicum*) were able to promote the improvement of biochemical parameters such as triglycerides, AST, and glucose in obese rats. Regarding histopathological aspects, a reduction in the hepatic steatosis triggered by obesity was observed, however, there was also the presence of different liver lesions in the studied groups, possibly related to the diet and the dosage of the extracts used.

In view of the results obtained, it is concluded that *Cinnamomum zeylanicum* improves hepatic damage triggered by obesity. However, complementary studies with isolation of specific substances from the extracts, diversified doses and for a longer time, are necessary for a better evaluation of the raw extracts of cinnamon in the prevention and development of NAFLD.

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