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Coconut oil effect in the complications of hepactic disease

Efeito do óleo de coco nas complicações da doença hepática

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ABSTRACT

Background: In the prevention of the progression of cirrhosis with dietary supplementation has been in focus the coconut oil.

Objective: To evaluate the effect of coconut oil supplementation in cirrhotic rats.

Method: Forty Wistar rats were divided into control (CO), control + coconut oil (CO + OC), thioacetamide (TAA) and thioacetamide + coconut oil (TAA + OC). TAA was added to water for 13 weeks and coconut oil was administered by gavage. Blood, liver, muscle and nervous tissue samples were obtained for laboratory and histopathological analyses.

Result: AST, ALT, total cholesterol, HDL, LDL, triglycerides were not statistically significant. In histology, even without differences, there was a tendency towards significance regarding the proliferation of bile ducts, fibrotic septa and muscle fiber atrophy between the TAA and TAA + OC groups.

Conclusion: No significant protective effect of coconut oil on liver disease was found.

KEYWORDS: Liver cirrhosis. Experimental. Coconut oil.

RESUMO

Introdução: Na prevenção da evolução da cirrose com suplementação dietética tem estado em foco óleo de coco.

Objetivo: Avaliar o efeito dessa suplementação em ratos cirróticos.

Método: Quarenta ratos Wistar foram divididos em controle (CO); controle + óleo de coco (CO+OC); tioacetamida (TAA) e tioacetamida + óleo de coco (TAA + OC). O TAA foi adicionado à água por 13 semanas e o óleo de coco administrado por gavagem. Amostras de sangue, fígado, tecido muscular e nervoso foram obtidas para análises laboratorial e histopatológica.

Resultado: AST, ALT, colesterol total, HDL, LDL, triglicerídeos não foram estatisticamente significantes. Na histologia, mesmo sem diferenças, houve tendência na significância quanto a proliferação de ductos biliares, septos fibróticos e atrofia da fibra muscular entre os grupos TAA e TAA + OC.

Conclusão: Não foi encontrado efeito protetor significativo do óleo de coco na doença hepática.

PALAVRAS-CHAVE: Cirrose hepática. Experimental. Óleo de coco.

Central message

The literature has currently shown benefits of virgin coconut oil in relation to the reduction of body fat and other aspects in the area of health. The therapeutic benefits of extra virgin oil are due to its composition of 92% saturated fatty acids, with a predominance of medium chains, phospholipids, tocopherol (which are natural antioxidants) and other volatile constituents. This study sought to gather evidence of this protection referred to by coconut oil.

Perspective

This study evaluated the use of coconut oil, based on its widespread use, easy access, and as a potential therapeutic adjuvant, since there are benefits in the use of other fatty acids in cirrhotic liver disease. The knowledge about the indication of coconut oil for therapeutic purposes aims to enlighten the population about inappropriate or inefficient dietary indications.

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INTRODUCTION

Cirrhosis is responsible for nearly 2 million deaths worldwide each year,1 and is currently the 11th most common cause of death in the world. It results from the process of hepatic fibrosis, which is characterized by the progressive accumulation of extracellular matrix, which destroys the physiological architecture of the liver.[2,3] This disease can be defined as the final stage of fibrosis of the hepatic parenchyma, which results in the formation of nodules with altered liver function and leads to hepatocellular failure and portal hypertension.[4,5]

As a way of investigating complementary treatments, it is important to establish animal models of cirrhosis induction that mimic the liver disease found in humans. Some of the main induction methods include thioacetamide (TAA). It requires metabolic activation by CYP450 enzymes to become toxic. A proteomic analysis of thioacetamide and cirrhosis induced by hepatotoxic rats that determine the administration of TAA, can be regulated as enzymes of primary metabolic pathways such as b-fatty acid oxidation, branched-chain amino acids, and methionine breakdown, as well as upregulated stress-related oxidative proteins and even lipid peroxidation.[6,7] Considered a complication of liver disease, malnutrition is often associated with cirrhosis, representing an important factor in the prognosis and progression of the disease. In this context, nutritional intervention plays an important role.[8]

The literature has currently demonstrated benefits of virgin coconut oil (OC) in relation to the reduction of body fat and other aspects in the area of health.[9] The therapeutic benefits of extra virgin oil are due to its composition of 92% saturated fatty acids, with a predominance of medium chains, phospholipids, tocopherol (which are natural antioxidants) and other volatile constituents. Between 40-50% of coconut oil's composition is lauric acid, which unlike other saturated fats, is quickly absorbed, transported to the liver, and oxidized, rather than stored.[10] A study showed that the use of coconut oil, as it is quickly broken down and absorbed into the portal circulation, would be a good indication for those with cirrhosis. It is capable of reducing steatorrhea, in addition to promoting balanced energy balance.[11]

Thus, the objective of the present study was to evaluate the supplementation of coconut oil in a cirrhotic induction model by means of thioacetamide.

METHOD

The research was based on ethical precepts and approved by the Ethics Committee on the Use of Animals of the Mackenzie Evangelical College of Paraná - CEUA/FEMPAR number 1582/2017, as well as with the Declarations of Helsinki and the International Standards for the Protection of Animals.

Forty 5-week-old male Wistar rats, with an average weight of 250 g, were used in the research. They were kept in the vivarium in propylene cages measuring 47x34x18 cm, lined with wood shavings, in a controlled photoperiod of 12 h light/dark (light from 7-19 h) and at room temperature of 22 ± 2 ºC. After 3 weeks of acclimatization with water and adequate feed ad libitum, the rats were identified and separated into the cages according to the experimental group.

Experimental groups

The rats were divided into 4 groups of 10 animals each, namely: 1) CO, a control group fed with feed and water for 13 weeks; 2) CO+OC, group fed with feed and water, in addition to intragastric administration of coconut oil for 13 weeks; 3) TAA, group fed with feed and water with thioacetamide for 13 weeks; 4) TAA+OC, group fed with feed and water with thioacetamide, in addition to intragastric administration of coconut oil for 13 weeks.

Experimental model of liver cirrhosis

Thioacetamide (Sigma-Aldrich) was administered in the drinking water of animals in the TAA and TAA+OC groups for a period of 13 weeks, based on the protocol of Al-Bader et al.[12] The initial concentration was 0.$^{06\%}$ for 17 days. In the 18th, the concentration was reduced to 0.$^{03\%}$ and maintained for 5 weeks. After that, the highest and lowest doses were swapped for 1 week each. On the 67th day, the 0.$^{06\%}$ dose was restarted and maintained until the end of the experiment. Care was taken in handling the TAA, use of mask, gloves, apron and goggles to avoid skin contact and inhalation. For control, the animals were weighed weekly and their physical and behavioral characteristics were observed.

The animals in the CO+OC and TAA+OC groups received 0.^{5} ml of liquid extra virgin coconut oil per 200 g of body weight by intragastric gavage and the animals in the CO and TAA groups received the vehicle (water) in the same amount, during all days of the experiment.

After 13 weeks, the animals were submitted to 12 h of fasting and anesthetized with intraperitoneal application of ketamine hydrochloride at a dose of 90 mg/kg body weight and xylasine hydrochloride at a dose of 10 mg/kg body weight. Blood was then collected in heparinized glass tubes, kept on ice for later analysis of liver integrity and lipid profile.

Subsequently, after trichotomy and disinfection of the abdominal region, the liver was removed and the animals were euthanized by exsanguination while anesthetized. The brain was dissected to collect the hippocampal formation after decapitation, and the gastrocnemius was removed after the right leg was dissected. The liver and gastrocnemius were then weighed on a precision scale and measured with a caliper for later evaluation. The hepatosomatic index (HSI) was calculated by dividing the weight of the liver by the weight of the animal and multiplying the result by 100, and the same relationship was used to analyze the proportion of gastrocnemius.

Liver integrity tests, lipid profile and histological evaluation

From the blood samples, aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels were determined, in addition to the evaluation of total cholesterol, HDL, LDL, VLDL and triglycerides.

For histological processing, the removed organs were fixed in 10% formaldehyde dissolved in 0.1M PBS and pH 7.4. Then, they were cleaved for processing according to conventional histological technique, using fragments of the left lobe of the liver, longitudinal sections of muscle tissue and hippocampal formation. The specimens were stained with H&E for analysis of histopathological alterations under light microscopy, without the identification of each slide being known to ensure impartial results.

Statistical analysis

The information obtained was tabulated according to the data protocol and then expressed through graphs and tables. For the description of the qualitative variables, frequencies and percentages were considered, and for the description of the quantitative variables, the statistics of mean ± standard deviation were considered, according to normality. The test used to verify the normality of the quantitative data was the Shapiro-Wilk test. For the variables that had a normal distribution, the One-Way ANOVA test followed by the Tukey test, if a significant result was obtained. For the variables that did not present normal distribution, it was performed the Kruskal-Wallis test followed by the Dunn test if significant result. $p<0.05$ was considered statistically significant and statistical analyses were performed using GraphPad Prism version 7.04.

RESULT

Preliminary observations

During the induction of cirrhosis by TAA, the animals were observed. From the second week on, it was possible to notice weight loss and yellow coloration in the coats of the rats in the TAA and TAA+OC groups. They also showed aggressive behavior and irritability, in contrast to the mice in the CO and CO+OC groups, which allowed for easier manipulation.

From the third week of cirrhotic induction, several animals in the TAA and TAA+ OC groups showed reddish discharge around the eyes and nose associated with apathy and poor responsiveness to manipulation.

One animal in the TAA+OC group and one in the CO+OC group presented tumors in the cervical region, evolving to pus drainage. The total mortality during the experiment was 2.5% (1 animal in the TAA+OC group on the 22nd day of the experiment, representing 10% within this group and 5% among the 20 animals submitted to TAA).

Body weight

The animals were weighed weekly during the experiment to verify the conditions of cirrhotic induction. From the 3rd week until the end of the experiment, it was noted that the average weight of the TAA+OC group was higher than those of the TAA group, which did not receive supplementation with coconut oil. Also, in these groups, the peaks of weight gain in the 3rd and 10th weeks corresponded to the reduction of the dose of TAA. When comparing the average weight among the 4 groups throughout the experiment period, it was possible to observe a higher value of the CO group (average of 339.5 g) in relation to the others. The average weight of the animals in the TAA+OC group (250.7 g) was also higher than in the TAA group (241.1 g, $p<0.0001$). When comparing groups 2 to 2, statistical significance was observed between the weightings of groups CO and TAA, CO and TAA+OC, CO+OC and TAA, and CO+OC and TAA+OC ($p<0.0001$).

Using only the last weighing before euthanasia as a parameter of comparison, similar results were obtained. Similarly, the TAA group had a lower mean weight (238.8 g) than the TAA+OC group (243.1 g). Compared to the control groups, the animals that received coconut oil supplementation had lower mean weight (373 g) when compared to those in the CO group (384.5 g, $p = 0.001$).

Macroscopy

The livers of the CO and CO+OC groups showed normal color and texture, with no macroscopic changes. In relation to the TAA and TAA+OC groups, it was noted that the livers were atrophied and had a yellowish color. Micronodules, macronodules and hemorrhagic lesions were also found. Regarding the gastrocnemius muscles, it was not possible to notice macroscopic changes other than the length and width measurements.

Liver weight and hepatosomatic index

When analyzing the absolute value of liver weight obtained in euthanasia, a higher mean was observed in the TAA group (12.04 g) in relation to the TA+ OC (11.57 g), CO (9.69 g) and CO+OC (9.36 g, $p = 0.003$) groups.

Comparing the results group by group, statistical significance was obtained between the groups CO and TAA, CO and TAA+OC, CO+OC and TAA, and CO+OC and TAA+OC. The chelation of absolute liver and body weight at the end of the experiment by the hepatosomatic index (HSI) showed similar results between both. The TAA group obtained the highest mean (5.1%) when compared to all the other groups, including the TAA+OC (4.85%, $p<0.0001$).

Analyzing the correlation between each of the groups, statistical significance was found between all comparisons except for the CO and CO+OC groups between themselves ($p>0.9999$) and the TAA and TAA+OC groups ($p>0.9999$).

Gastrocnemius measurements and proportion

Regarding gastrocnemius, the highest means were obtained in the CO+OC (2.12 g) and CO (2.11 g, $p<0.0001$) groups. Relating the groups separately, a statistically significant result was observed in all correlations, except between the control groups ($p = 0.9999$) and between the cirrhotic groups ($p = 0.9912$). When analyzing the proportion between gastrocnemius muscle weight and the animal's body weight, no statistical significance was found ($p = 0.3091$).

Similarly, using the length of the gastrocnemius as a parameter, there was also no statistically significant result ($p = 0.2257$). On the other hand, when comparing the measurement of the width of the gastrocnemius muscle, a higher mean was obtained among the animals in the

CO group (14 mm) when compared to all the others (p = 0.0119). Comparing groups 2 to 2, there was statistical significance only between the CO and and TAA groups (p = 0.0256).

Histopathological findings

For histopathological analysis, the tissue samples were visualized at 40, 100 and 400x magnifications according to the data from each slide. The histopathological findings were statistically analyzed according to the presence and intensity of the alterations observed in each organ of each group of animals.

In the histological analysis of the liver, 5 parameters were analyzed: presence of fibrotic septa, presence of regenerative nodules, inflammatory infiltrate, proliferation of bile ducts and hepatocellular necrosis. Each criterion was classified as absent, mild, moderate, and marked.

Regarding the presence of fibrotic septa, 50% of the samples in the TAA group showed marked alterations, in addition to moderate 20%. In the TAA+OC group, 44.44% also had a marked presence of septa, followed by 33.33% mild and 22.22% moderate.

When analyzing the presence of regenerative nodules, 30% of the samples in the TAA group were classified as marked alterations, as well as 30% as moderate alterations and 10% as discrete nodules. In relation to the TAA+OC group, 44.44% of the livers had a marked presence of regenerative nodules, 11.11% moderate, and 44.44% were mild or absent.

Inflammatory infiltrate was found in 100% of the samples in the TAA and TAA+OC groups. Evaluating the intensity of this alteration, 7 animals in the TAA group (70%) showed mild infiltration, followed by 20% moderate and 10% severe. As for the TAA+OC group, 55.56% of the samples were classified as mild infiltrate, 33.33% as moderate, and 11.11% as severe.

Using bile duct proliferation as a parameter, the alteration was absent in 30% of the samples in the TAA group, while in the TAA+OC group, it was not found in more than half of the samples (55.56%). Also comparing the 2 groups, ductal proliferation was accentuated in 30% of the livers analyzed in the TAA group, while the same intensity occurred in 22.22% of the samples in the TAA+OC group.

Regarding the evaluation of hepatocellular necrosis, the results were similar between the cirrhotic groups. Mild necrosis was found in 60% of the animals in the TAA group and in 66.67% of the TAA+OC group.

Regarding the gastrocnemius muscle, the following criteria were used: atrophy of muscle fibers, proliferation of connective tissue, and infiltration by adipose tissue. Again, the alterations were classified as absent, mild, moderate and severe. The results regarding muscle fiber atrophy, observed under microscopy, occurred in the AAT group slightly 60%, moderately 20% and markedly 10%. In relation to the TAA+OC group, the change was absent in 33.33% of the samples, mild in 55.56% and accentuated in 11.11%.

Regarding the proliferation of connective tissue between muscle fibers, only 1 sample (10% of the TAA group) showed slight alteration. The infiltration of adipose tissue into the muscle was not found in any sample.

In the hippocampal analysis, the parameter used as a suggestive of hepatic encephalopathy was the swelling of the astrocytes. No swelling was observed in 60% of the animals in the CO, CO+OC and TAA+OC group. In the CO+OC group, the change was slight in 60% of the samples.

Clinical analysis

Laboratory analysis included results of ALT, AST, total cholesterol, HDL, LDL, VLDL and triglycerides. The TAA group had a mean of 65.38 IU/L, while the TAA+OC group had a mean of 51.6 IU/L. The results, however, were not statistically significant (p = 0.7507). Regarding AST levels, the TAA group had a mean of 182.8 IU/L while the TAA+OC group had a mean of 127.4 IU/L, although there was no statistically significant p-value (p = 0.0940).

Regarding the cholesterol results, it was observed that the cirrhotic groups had lower total cholesterol values compared to the controls. The mean value of the AAT group was 100.2 mg/dL and the mean value of the AAT+OC group was 94.7 mg/dL (p = 0.0959).

Regarding HDL, the mean value of the TAA group was 56.21 mg/dL, while in the TAA + OC group, the mean HDL value was 46.98 mg/dL (p = 0.1069).

Regarding the results of LDL measurement, the TAA+OC group had the highest mean score of 38.28 mg/dL, while the TAA group had a mean score of 33.02 mg/dL. The p-value, however, was not significant (p = 0.0531).

Regarding the VLDL, there was statistical significance (p = 0.0066) when comparing the results between the 4 experimental groups. The mean was higher among the control groups (mean CO of 20.4 mg/dL and mean CO+OC of 18.6 mg/dL) than in the cirrhotic groups (mean TAA of 10.8 mg/dL and mean TAA+OC of 9.4 mg/dL).

When comparing the groups separately, it was observed that a significant result was found only in the correlation of the data from the CO and TAA+OC groups (p = 0.0293).

Regarding serum triglyceride values, the mean in the TAA group was 54.64 mg/dL, while in the TAA+OC group it was 47.04 mg/dL. The values found in the control groups were higher, with a mean of 101.5 mg/dL in the CO group and 92.12 mg/dL in the CO+OC group (p = 0.0045).

When performing the multiple comparisons test, it was observed that the significant result found on triglyceride values was the correlation between the CO and TAA, CO and TAA+OC, as well as CO+OC and TAA+OC groups.

DISCUSSION

Malnutrition in cirrhotic patients ranges from 50-90%, which is concerning due to its association with increased mortality. Malnutrition is associated with a higher number of complications, such as hepatorenal syndrome, encephalopathy, and poorer liver function. Among

malnourished patients, 20% had a 1-year mortality rate.[13] Mortality rates were not statistically evaluated. There are several factors that contribute to nutrient malabsorption, especially fats and proteins. Bile acids are produced by the liver with the function of absorbing fatty acids into the lymphatic system. Bile acid deficiency is common in cirrhotic patients, leading to lower bile production capacity.[13]

In this context, nutritional therapy has great potential in cirrhosis.[5] Recommendations for nutritional intervention aim to provide substrates such as proteins, lipids, carbohydrates, vitamins and minerals. Early identification and correction of malnutrition in patients with liver disease leads to better disease progression and prevents complications of cirrhosis.[14]

Coconut oil is a source of lauric acid and medium-chain triglycerides (MCTs), containing 90% saturated fats.[15] They have been cited in the literature as a suggestion for the complementary treatment of cirrhosis, as they are quickly absorbed into the portal circulation and are capable of promoting energy balance and reducing steatorrhea.[11]

Thus, the aim of this study was to develop a cirrhosis induction model to evaluate coconut oil supplementation on macroscopic, microscopic and laboratory parameters of liver cirrhosis and its complications. No studies were found in the literature directly analyzing the effect of coconut oil on the histological and laboratory parameters of cirrhosis. Given the high prevalence and importance of cirrhosis in the current context, many experimental models of induction and forms of prevention and treatment have been developed and described in the literature.[5] The model used in this study was through the administration of thioacetamide to promote liver cirrhosis similar to involvement in humans, with low mortality in relation to bile duct ligation and with more evident histological parameters than induction with carbon tetrachloride. It has safety of use and greater reproducibility. Oral administration in drinking water is a non-invasive and practical method for experimentation in large number of animals5, although there is difficulty in controlling the dosage of the drug administered by the animal, due to the fragility of the model itself.[9]

The mechanism of action of TAA in liver injury is based on metabolic activation after metabolism by the CYP2E1 isoenzyme, resulting in a highly reactive product (S-thioacetamide dioxide). This product, derived from biotransformation, causes TAA to have cytotoxic activity, resulting in reduced antioxidant activity in the liver and increased lipoprotein peroxidation. Then, oxidative stress is established, culminating in necrosis and cellular fibrosis.[16.17]

The initial dose of TAA used was 0.06% in drinking water, which was then reduced to 0.03% for 5 weeks, with the 2 doses interspersed, and the highest maintained in the last 3 weeks of the experiment. It was decided to reduce the dose initially proposed due to the great weight loss and fragility of the animals submitted to TAA. However, continuous weight gain was observed with the reduction of the dose, and 0.06% was reintroduced by the end of the experiment.

The protocol used is similar to described by Al-Bader et al.[12,] through the oral administration of 0.05% TAA for 12 weeks, showing an increase in liver function markers and a decrease in the weight of the animals. Another study used a dose of 0.03% in water for 12 weeks, being effective in inducing cirrhosis at macro and microscopy in 90% of the animals, without mortality.[18] The literature suggests, however, that greater efficiency is obtained by adjusting the dose according to the individual weight of each animal, minimizing mortality and providing homogeneous cirrhosis.[5]

In the observation of clinical signs in the animals during cirrhosis induction, reddish discharge was observed around the eyes and muzzle in the TAA and TAA+OC groups. This alteration is compatible with porphyrinemic secretion from Harder's gland.[17] The manifestation of porphyrin is called chromodacryorea, occurring in situations of stress due to chronic diseases.[19] In addition, there was yellowing of the coat of animals submitted to the administration of TAA - including those that received coconut oil - described in the literature as a consequence of the involvement of the biliary system.[19] The mortality rate found was consistent with other experiments described in the literature.[17,19,20]

The weight changes recorded during the experiment were compatible with those of the TAA dose. In the first 2 weeks of exposure to the dose of TAA 0.06%, intense weight loss was observed in the rats of the TAA and TAA+OC groups. After the reduction in the following 5 weeks, the animals began to gain weight in a similar way to the constituents of the control group. From the 7th to the 8th week, with an increase in the concentration of TAA, there was a slight weight loss, followed by recovery from the 8th to the 9th week with a decrease in the dose. In the 11th week, intense weight loss was observed with the reintroduction of 0.06% TAA, with no major weight variations until the end of the experiment with dose maintenance. The chosen dose of coconut oil intake, saturated fats, was limited to less than 10% of daily calories approximately.[21]

Regarding body weight at the end of the experiment, the difference between the CO group (384.5 ± 10.14) and the TAA (238.8 ± 10.78) was significant in 37%. The result found is compatible with the literature, a difference of approximately 31% described between a control group and a cirrhotic group due to exposure to TAA.[14] Regarding the comparison between the TAA and TAA + OC groups, the mean final weight was higher in the group supplemented with coconut oil (243.1 ± 15.61) than in the animals in the TAA group (238.8 ± 10.78), but this difference was not statistically significant. The literature argues that the appetite of animals is decreased when liver damage is excessively installed, limiting water intake with AAT. This may be associated with lower mortality and slower weight loss after a certain period of exposure to TAA.[18]

The mean final weight between the CO and CO+OC groups was similar (339.5 and 336.2, respectively) and there was no significant difference. This result coincides with the study by Santana et al.[22] and Schumacher et al.[23,] who concluded that coconut oil is not related to weight

gain.[22,23] This last study, however, analyzed peritoneal fat and described a reduction of 25.6% with the use of coconut oil. The macroscopy found in cirrhotic livers is in agreement with the literature, demonstrating modularity, in contrast to the smooth surface of the livers of control animals.[24] In a study with intraperitoneal administration of TAA, congestion, micro and macronodules were also observed.[25] Hemorrhagic lesions in this sample were also observed in the Lima TC19 study, which correlated this finding with increased pressure in the hepatic capillaries due to hypertension. The macroscopic characteristics of cirrhosis were also found, in similar intensity, in the cirrhotic group that received coconut oil supplementation.

Regarding liver measurements and hepatosomatic relationship, in the present study there was a significant difference between the control and cirrhotic groups, but not between AAT and AAT+OC. The increased hepatosomatic ratio in cirrhosis is likely related to collagen accumulation, increasing the weight of the liver.[26]

The histological findings of the liver samples showed the intense presence of fibrotic septa, found in 100% of the samples in the TAA+OC group (44.44% marked) and in 70% of the TAA group (50% marked). Regenerative nodules were also more found in the TAA+OC group (77.77%, 44.44% of which were accentuated) than in the TAA group (70%, 30% of which were accentuated). Inflammatory infiltrate was recorded in all samples from both groups and hepatocellular necrosis had similar proportions (a discrete finding in 60% of the TAA group and 66.67% of the TAA+OC group). Bile duct proliferation was the only criterion with a lower prevalence in the cirrhotic group with coconut oil (present in 44.44%, half mild and half severe). In the TAA group, 70% of the samples showed ductular proliferation, which was accentuated in 30%, moderate in 10% and mild in 30%. Thus, the histology between the TAA and TAA+OC groups was very similar, with a higher frequency of changes in the group supplemented with coconut oil - except for the proliferation of bile ducts.

The histopathological alterations are similar to those found in the study by Guerra et al.[27,] through intraperitoneal administration of TAA: loss of hepatic parenchymal architecture, with formation of fibrotic septa delimiting regenerative nodules, associated with necrosis and proliferation of bile ducts. The study by Silva[17] carried out with induction of cirrhosis by TAA and administration of hepatotrophic factors, demonstrated that the treatment did not alter histological activity, fibrosis staging and bile duct proliferation. Hepatotrophic factors, however, reduced collagen deposition and activation of stellate cells.

According to the literature, AST and ALT aminotransferases are reliable indicators of liver cell alterations, the presence of fibrosis, and tissue injury.[16] A study with 0.03% TAA administration for 12 weeks obtained significantly higher values ($p < 0.001$) of liver enzymes in the cirrhotic group (AST of 271.63 and ALT of 105.38) compared to the control group (AST of 158.88 and ALT of 62.88).[28]

In the present study, however, no increase in liver enzymes was found in the animals in the TAA groups compared to the controls, even with cirrhosis established macro- and microscopically. Coconut oil was also not associated with a significant decrease in markers of liver injury. This condition coincides with other studies found in the literature, where cirrhosis was successfully installed, presenting the typical morphological characteristics of the disease, but without significant changes in liver function tests.[18,29] It may be possible that increased transaminases are a relevant marker during the acute phase of liver injury, but not during the advanced stages of cirrhosis.[29]

In general, plasma lipids and lipoproteins tend to decrease with parenchymal liver disease, with progressive reduction of cholesterol and its fractions according to the severity of the disease. Triglyceride levels are more associated with cirrhosis of alcoholic origin.[30] The study by Chen et al.[31] with induction of cirrhosis by intraperitoneal TAA found a reduction in total cholesterol and triglyceride levels in the cirrhotic group (84.2 mg/dL and 57.4 mg/dL, respectively) compared to the control group (167.1 mg/dL and 83.2 mg/dL).

This difference in lipid profile, however, was not observed in the present study in relation to total cholesterol and its fractions (HDL, LDL, and VLDL) between the CO and TAA groups. Supplementation with coconut oil also had no significant effect. Regarding triglyceride levels, there was a significant difference between the CO group (101.5 ± 15.5) with the TAA (54.64 ± 5.24) and TAA+OC (47.04 ± 3.89) groups. There was also a significant value when comparing triglyceride values in the CO+OC group (92.12 ± 12.98) with TAA+OC. But again, when relating the effect of coconut oil between the 2 cirrhotic groups, no statistical difference was observed.

The isolated effect of coconut oil among the non-cirrhotic control groups was also not statistically significant in the present study. However, most of the randomized controlled trials analyzed suggest a cardiovascular protective effect. They show that coconut oil intake, when compared to other vegetable oils, increases HDL and decreases triglycerides, although the latter finding is questionable.[21,32]

Considered a complication of liver cirrhosis, cachexia is a metabolic disorder associated with the loss of cell mass, rapid protein degradation, and decreased food intake and physical activity. This syndrome is present in several chronic diseases, being an important factor in poor prognosis.[33] Decreased muscle mass is observed in approximately 50% of patients with cirrhosis and is a predictor of mortality in these patients.[34]

The pathogenesis of cachexia in liver disease is related to the imbalance between energy expenditure and intake. Hypermetabolism is identified by increased resting energy expenditure in cirrhotic patients and is associated with proinflammatory cytokines. Nutritional intake is impaired in those with liver disease due to dysregulated appetite. The literature describes that nutritional intervention improves body composition, liver and muscle function, and mortality.[8]

In the present study, the measurement of gastrocnemius weight had a significant result in the comparison of the control groups with the cirrhotic ones, but not in the evaluation of coconut oil supplementation in the TAA

group. The measurements of width, length, and the ratio of muscle weight to total weight did not result in statistically significant differences in most groups. Regarding the histology of the gastrocnemius samples, fiber atrophy was found in 90% of the animals in the TAA group and less frequently (66.67%) in the rats in the TAA+OC group.

Hepatic encephalopathy is a sequelae of chronic liver disease with significant morbidity and mortality and is related to poor prognosis and impaired quality of life. The etiopathogenesis of encephalopathy is multifactorial, but the increase in circulating ammonia and the urea cycle play a crucial role. Nitrogenous compounds are excreted by the gut microbiota and transported to the liver according to the urea cycle for elimination in the urine. In advanced liver disease, however, ammonia bypasses the liver due to portal changes and accumulates in the systemic circulation. When crossing the brain barrier, metabolism by astrocytes and glutamine production occurs.[35]

The nutritional management of hepatic encephalopathy is related to the adequate consumption of proteins, with branched-chain amino acids, fibers, and vegetables, as well as non-absorbable disaccharides and probiotics.[35]

In the present study, the objective was to analyze hepatic encephalopathy through hippocampal histopathology. The literature describes that the accumulation of glutamine in astrocytes leads to progressive and cytotoxic edema, resulting in cellular edema.[36] Astrocytes become larger, with bulky, vesicular nuclei called Alzheimer's type II astrocytes found in gray matter.[37]

This alteration of astrocytes, however, was difficult to characterize in the hippocampal samples and was not found in any group. It is argued that histopathological analysis of changes in astrocytes would be better characterized in samples of cortex and basal nuclei. Another hypothesis is that the induction of cirrhosis by oral TAA would have less toxic effects on extrahepatic organs such as the kidneys and brain, as it is transported directly to the liver through the portal vein after entering the systemic circulation. Subcutaneous or inhaled administration of hepatotoxins may more likely cause more evident extrahepatic findings.[18]

CONCLUSION

This study demonstrated that no protective effect of coconut oil as a supplement in the treatment of liver cirrhosis was found when analyzing markers of liver integrity, lipid profile and histopathology, both hepatic and muscular and also nervous, although there was a trend towards improvement parameters.

Authors' contributions

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