Euterpe edulis pulp products and your effect on liver and kidney of *in vivo* model of colorectal cancer: Histopathological analysis

Produtos da polpa de *Euterpe edulis* e seus efeitos no fígado e nos rins em modelo *in vivo* de câncer colorretal: Análise histopatológica

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ABSTRACT
The objective was to report the injuries on liver and kidney promoted by experimental colorectal carcinogenesis induction in rats and evaluate the effect of supplementation with Euterpe edulis M. pulp products on resolution of this injuries. Colorectal carcinogenesis with 1,2-dimethylhydrazine was induced in young male rats, allocated into: C - induced to carcinogenesis; CJ - induced to carcinogenesis and supplemented with juçara fruit pulp; and CE - induced to carcinogenesis and supplemented with juçara fruit lyophilized extract. Nine animals were a negative control. Supplementation occurred three times a week, totaling 54 days of administration with 1 mg of cyanidin-3-glycoside per kilogram live weight. The hepatic and renal histopathological injuries were assessed at 10 and 23 weeks. In liver, at 10-week biliary hyperplasia was more evident in colorectal cancer induced groups compared to N group ($p = 0.0230$), as well as megalocytosis ($p = 0.0269$), and juçara fruit-based product do not promote cytoprotection. At 23-week biliary hyperplasia continued present, and liver necrosis was evident in C group and CJ group. Hepatic degeneration was greater in C group, and megalocytosis was evident in the cancer-induced groups, without cytoprotection by juçara fruit-based product. In kidney, at 23-week, renal congestion was more evident in CJ group, and tubular degeneration in C and CE groups. Important hepatic and renal injuries were observed in rats induced to colorectal cancer and the supplementation with juçara fruit-based product, in the dose used, did not interfere in the prevention and resolution of these injuries, mainly with the chronic use.

Keywords: carcinogenesis, experimental model, liver injuries, renal injuries, biocompounds

RESUMO
O objetivo era relatar as lesões no fígado e nos rins promovidas pela indução experimental de carcinogênese colorretal em ratos e avaliar o efeito da suplementação com produtos de polpa Euterpe edulis M. na resolução dessas lesões. A carcinogênese colorretal com 1,2-dimetil-hidrazina foi induzida em ratos jovens do sexo masculino, alocada em ratos: C - induzido à carcinogênese; CJ - induzido à carcinogênese e suplementado com polpa de fruta juçara; e CE - induzido à carcinogênese e suplementado com extrato liofilizado de fruta juçara. Nove animais foram um controle negativo. A suplementação ocorreu três vezes por semana, totalizando 54 dias de administração com 1 mg de cianidina-3-glicosídeo por quilograma de peso vivo. As lesões hepáticas e renais histopatológicas foram avaliadas às 10 e 23 semanas. No fígado, com 10 semanas, a hiperplasia biliar era mais evidente nos grupos induzidos por câncer colorretal em comparação com o grupo N ($p = 0,0230$), assim como a megalocitose ($p = 0,0269$), e o produto à base de fruta juçara não promove a citoproteção. Com 23 semanas a hiperplasia biliar continuou presente, e a necrose hepática foi evidente no grupo C e no grupo CJ. A degeneração hepática era maior no grupo C, e a megalocitose era evidente nos grupos induzidos pelo câncer, sem citoproteção pelo produto à base de fruta da juçara. No rim, com 23 semanas, a congestão renal era mais evidente no grupo CJ, e a degeneração tubular nos grupos C e CE. Importantes lesões hepáticas e renais foram observadas em ratos induzidos ao câncer colorretal e a suplementação com produto à base de fruta da juçara, na dose utilizada, não interferiu na prevenção e resolução destas lesões, principalmente com o uso crônico.

Palavras-chave: carcinogênese, modelo experimental, lesões hepáticas, lesões renais, biocompostos

1 INTRODUCTION

Colorectal cancer is among the main causes of morbidity and mortality worldwide, representing a major public health problem (Favoriti *et al*., 2016). According to the *Cancer Today* of the International Agency for Research on Cancer (IARC), colorectal cancer affected 1.849.518 people worldwide in 2018 and the *Cancer Tomorrow* tool estimates for 2040, an increase of 75% in colon cancer cases and 66.3% in rectum cancer cases (IARC, 2020).

It was found that a diet rich in bioactive compounds can assist in cytoprotection (De Barrios Freitas *et al*., 2017), and the vegetables and fruit consumption which are rich in bioactive compounds is directly linked to the oxidation process, because they have antioxidant activities and indicated for the treatment and prevention of cancer (Soares *et al*., 2015).

Juçara palm fruit (*Euterpe edulis* M.) has bioactive compounds and phenolic substances, such as flavonoids and chlorogenic, benzoic, caffeic, ferulic, ρ-coumaric, protocatechuic, syringic and vanillic acids, which favor the antioxidant activity (Rogez, 2000; Borges *et al*., 2013). Studies with *Euterpe oleracea* palm fruit, which belongs to the same family as the juçara palm, have shown the function of cytoprotection in MCF-7 cells (breast cancer) grown under stress with hydrogen peroxide (H_2O_2) (Chin *et al*., 2008), as well as acting in the prevention and potential improvement in acute kidney injury in an experimental model (El Morsy *et al*., 2015; Unis, 2015; da Costa *et al*., 2017).

Colon cancer induced by carcinogenic substance contributes significantly to the understanding of pathogenesis, in addition to sharing many similarities with human neoplasms (Taketo and Edelmann, 2009). Because the tumor induction is fast, reproducible and allows the study of the adenoma-carcinoma sequence, rodents are widely used as experimental models of colorectal carcinogenesis (Balmain and Harris, 2000).

The 1,2-dimethylhydrazine (DMH) and its metabolite azoxymethane (AMO) are procarcinogens and applied to induce and promote colorectal cancer in mice and rats (Corpet and Pierre, 2005; Perše and Cerar, 2011). In addition, DMH is metabolized to methylazoxymethanol (MAM) and the most used agent for carcinogenesis in rats, whose absorption by colon cells is three times greater and its action can be obtained through a single injection or serial injections weekly (Oliveira *et al*., 2001; Gois *et al*., 2012).

In the liver, DMH is metabolized in azomethane (AM), azoxymethane (AOM) and methylazoxymethanol (MAM), and the azomethane metabolite had 0.6% of the

administered dose eliminated throught bile and 12.3 % stored in liver tissue (Wolter and Frank, 1982). Prior to this, was shown that DMH metabolites were also excreted in the urine (Fiala, 1977). As a consequence these substances in these tissues, important histomorphological changes were found in the liver, such as, mild centrilobular necrosis, considerable pleomorphism with anisocariosis and binucleation of the hepatocytes, portal inflammatory infiltrate, vascular dilation, steatosis, necrosis, cystic hyperplasia and neoplasms; and in kidneys, injuries such as inflammation and glomerular congestion, reduction of the Bowman's chamber and neoplasms (Hawks *et al*., 1974; Kobaek-Larsen *et al*., 2004; Kuri-García *et al*., 2019).

As the neoplasic induction with 1,2-dimethylhydrazine causes important histomorphological changes with homeostatic imbalance, which can be minimized with bioactive substances from juçara fruit, a promising therapeutic potential, it is extremely important to investigate through histopathological findings, the effects that supplementation with pulp and lyophilized extract of this fruit has on the liver and kidney tissue of rats induced to colorectal cancer with DMH, given the importance these organs in the metabolism and excretion of xenobiotics, since *in vivo* experimental models of diseases are used for studies of therapeutic application. Thus, the objective was to report the injuries on liver and kidney promoted by experimental colorectal carcinogenesis induction in rats and evaluate the effect of supplementation with *Euterpe edulis* Martius pulp products on resolution of this injuries.

2 MATERIAL AND METHODS

ETHICAL ASPECTS

The experimental procedures were reviewed and approved by the internal Ethics Committee under protocol #043/2013. Animals used in this study were kept in accordance with the National Institute of Health Guide for Care and Use of Laboratory Animals (NIH Publications Nº 8023), 2011 review, and with the Brazilian Law on Procedures for Scientific Use of Animals (#11794/2008).

JUÇARA FRUIT PULP AND PHENOLIC COMPOUNDS EXTRACTION

For this experiment, a single batch of juçara fruit pulp was obtained from commercial supplier in the municipality of Rio Novo do Sul in the southern region of the State of Espírito Santo, Brazil (20º48'30"S and 41º00'00"W). The pulp was free of dyes

and preservatives, hermetically storage at -20ºC protected from light and atmospheric oxygen.

The phenolic compounds extraction and anthocyanin pigment from juçara fruit pulp was carried on Laboratory of Chemical Analyzes of Food of the Agricultural Sciences and Engineer Center - Federal University of Espírito Santo, Alegre/ES, according to adapted methodology of Francis (1982). Briefly, 20 g of juçara fruit pulp was diluted in 200 mL of 70% cereal alcohol and adjusted pH to 2.5 with citric acid. The extract was storage at 4ºC protected from light and atmospheric oxygen for two hours. After that, the extract was filtered, concentrated in a rotoevaporator and lyophilized. Lyophilized samples were hermetically storage at 4ºC protected from light until use.

Total anthocyanin pigment was quantified by spectrophotometry using pH-differential (Giusti and Wrolstad, 2001), using 0.025 M Potassium Chloride Buffer (pH 1.0) and Sodium Acetate Buffer 0.4 M (pH 4.5). The anthocyanins extract were diluted in each buffer in the proportions 1:10 and 1:25 and the absorbances were recorded at 510 nm and 700 nm wavelengths. Total anthocyanin results were expressed in mg cyanidin-3-glucoside per gram of pulp. (Cy-3-glu: molar mass: 449.2 g.mol-1; molar absortivity: 26900 L.mol-1.cm-1.). Thus, was found 107.08 mg of cyanidin-3-glycoside per gram of pulp.

EXPERIMENTAL DESIGN

The study used 36 young male Wistar albino rats (45-60 days old, weighting mean 181 ± 35 g at the beginning of the experimente). Animals were housed in groups of five to six per cage under controlled temperature (21-24°C), humidity (45-55 %) and lighting (12 hours light, 12 hours dark; lights on at 6:45 AM). Food and water were available *ad libitum* throughout the experiments.

Colorectal carcinogenesis was induced with 1,2-dimethylhydrazine (DMH) (Sigma Aldrich, Germany) in 27 animals and nine animals were maintained as a negative control. DMH was dissolved in 0.9% sodium chloride solution containing 1.5% ethylenediaminetetraacetic acid disodium salt solution as a vehicle, adjusted to a final pH of 6.5 with 1 N sodium hydroxide solution and administered subcutaneously once a week for five weeks, totaling 240 mg per kilogram live weight for animal. This protocol was chosen based on previous data from Laranjeira *et al*., (1998).

The animals were randomly divided into four experimental groups, namely: *N* (n = 9): negative control; *C* (n = 11): induced to colorectal carcinogenesis; *CJ* (n = 10):

induced to colorectal carcinogenesis and supplemented with Juçara fruit pulp at 0.1 % (mass / volume), equivalent to 0.10708 mg/mL of cyanidin-3-glycoside, and *CE* (n = 6): induced to colorectal carcinogenesis and supplemented with lyophilized extract diluted in water (1 mg / mL), containing 0.10708 mg/mL of cyanidin-3-glycoside. The number variation of animals between the groups occured by death of some during the experiment.

The supplementation with pulp and lyophilized extract of the juçara fruit started one week after the neoplasic induction stage (sixth week) and the availability of this supplement occurred three times a week, by gavage, until completing 23 weeks, totaling 54 days of administration (adapted methodology from dos Reis *et al*., 2020). For supplementation, 1 mg of cyanidin-3-glycoside per kilogram live weight for animal was considered for each administration.

Euthanasia of the animals occurred in two moments for analysis of liver and kidney injuries. In the 14th week (corresponding to pre-neoplasic lesions), four animals from *N* group, four animals from *C* group, four animals from *CJ* group and two animals from *CE* group were euthanized. In the 23rd week (corresponding to developed colorectal neoplasms period) the rest of the animals were euthanized.

The animals were necropsied and kidneys and a fragment of the left medial hepatic lobe were collected. The samples were fixed in 10 % formalin and processed by the paraffin inclusion method for histopathological analysis by light microscopy. Slides from each sample were stained with Hematoxylin-Eosin (HE) and blindly analyzed by veterinary pathologist.

In the liver, portal fibrosis, biliary fibrosis, biliary hyperplasia, inflammatory infiltrate, vascular stenosis, necrosis, congestion, hepatic degeneration and megalocytosis were investigated. In the kidneys, tubular degeneration, congestion, tubular necrosis, dilatation of Bowman's capsular space, inflammatory infiltrate, renal brush border loss, interstitial edema, tubular dilation, dendrites and hyaline cylinder were investigated.

Morphological changes were assessed for distribution (absent, focal = +, multifocal = ++ and diffuse = +++) and intensity (absent, discrete = +, moderate = ++ and severe / intense = +++). Sample scoring was performed by multiplying the intensity score by the distribution score. The score for each sample was determined to be 0 for absence; 1 for discrete/focal; 2 for moderate/multifocal and 3 for severe/diffuse (Trivilin *et al*., 2017).

STATISTICAL ANALYSIS

Scores from histopathological analysis were performed by Kruskal-Wallis test followed by the Student-Newman-Keuls *post-hoc* test. Data are presented as median and upper and lower limit. For test, the level of significance was set a 5 %, the calculations were performed in GraphPad Prism® 7.00 demo software.

3 RESULTADOS

HEPATIC INJURIES

At 10 weeks (five of induction and five of supplementation), biliary hyperplasia was observed (Fig. 1a), which was more evident in colorectal cancer induced groups (*C*, *CJ* and *CE* groups) compared to negative control group (*N* group) ($p = 0.0230$), without interference from supplementation with juçara fruit-based product, as the supplemented groups with pulp and lyophilized extract (*CJ* and *CE* groups) did not differ from induced and non-supplemented group (*C* group) ($p = 0.7353$ and $p = 0.4687$, respectively), as shown in Table 1. Mononuclear inflammatory infiltrate, hepatic degeneration, necrosis and congestion were also found, however, none difference between groups was observed (Table 1). Megalocytosis (Fig. 1d) was observed in all colorectal carcinogenesis induced groups (*C*, *CJ* and *CE* groups) and showed a significant difference between the studied groups ($p = 0.0269$). However, it was evident that supplementation with juçara fruit-based product did not reduce the occurrence of this injury (Table 1).

After 23 weeks of experiment, despite to present biliary hyperplasia, mononuclear inflammatory infiltrate and hepatic congestion did not differ between groups ($p = 0.8624$; $p = 0.2934$ and $p = 0.2457$, respectively) (Table 1). Liver necrosis (Fig. 1c) was evident in rats induced to colorectal carcinogenesis and non-supplemented (*C* group) and in the induced colorectal cancer and supplemented with pulp of juçara fruit group (*CJ* group). Regarding hepatic degeneration (Fig. 1b), there was a difference between the groups ($p = 0.0074$), which was greater in the cancer-induced group with no supplementation (*C* group) compared to the other groups. Megalocytosis was evident in the cancer-induced groups, without interference from supplementation with juçara fruit-based product, as the supplemented groups (pulp and lyophilized extract) did not differ significantly from the induced and non-supplemented group (Table 1).

Fig. 1. Photomicrography of liver histological sections from rat induces to colorectal carcinogenesis supplemented with pulp or lyophilized extract of juçara fruit. (A) Biliary hyperplasia (asterisk); (B) Hepatic degeneration area with accumulation of substance in hepatocyte cytoplasm (arrow). (C) Small necrosis area with pyknosis (arrow), karyorrhexis (arrowhead) and karyolysis (asterisk), as well liver congestion. (D) Cells with abnormal nucleus/cytoplasm ratio due to hepatic megalocytosis (arrow), in addition to a small mononuclear inflammatory infiltrade area (arrowhead). H&E staining. Bar: 22µm.

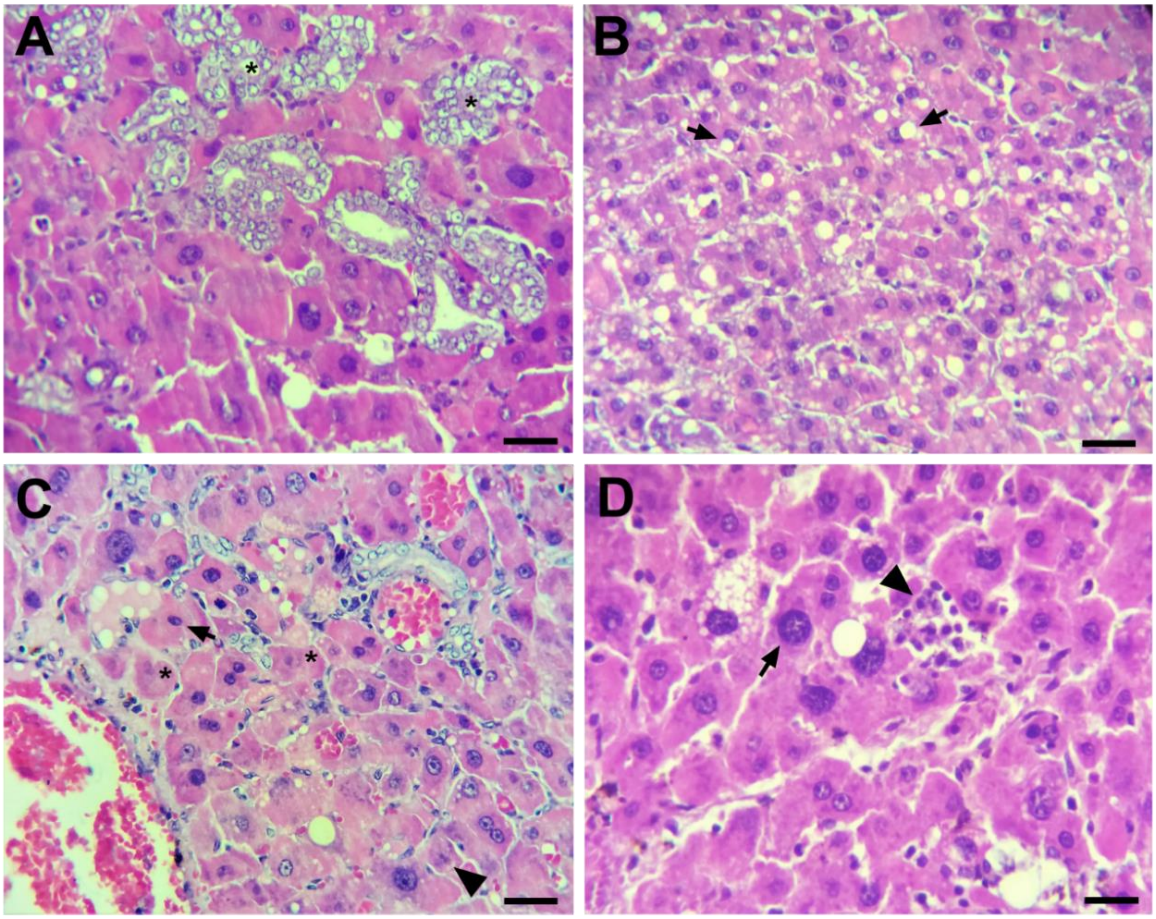

Table 1. Hepatic injuries score assessed at 10 and 23 weeks in rats induced to colorectal carcinogenesis, supplemented or not with juçara fruit-based product (pulp and lyophilized extract). Scores described in median (Lower-Upper Limit).

Hepatic Injuries	10 WEEKS					23 WEEKS				
	N (*n = 4*)	C (*n = 4*)	CJ (*n = 4*)	CE (*n = 2*)	*p*-value*	N (*n = 5*)	C (*n = 7*)	CJ (*n = 6*)	CE (*n = 4*)	*p*-value*
Portal Fibrosis	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	-	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	-
Biliary Fibrosis	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	-	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	-
Biliary Hyperplasia	0 (0-0)	3 (2-6)	4 (2-6)	2 (2)	**0.0230**	1 (1-2)	2 (2-6)	2 (2-6)	2 (2-6)	0.8624
Inflammatory Infiltrate	0.5 (0-1)	3 (0-6)	2 (0-2)	2 (2)	0.1863	0 (0-2)	2 (0-2)	2 (0-4)	2 (0-4)	0.2934
Vascular Stenosis	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	-	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	-
Necrosis	0 (0-0)	2 (0-4)	3 (0-4)	3 (2-4)	0.1235	0 (0-0)	2 (0-4)	2 (0-4)	0 (0-0)	**0.0067**
Congestion	4 (2-6)	4 (4-6)	4 (4-6)	3 (2-4)	0.5051	2 (2-4)	4 (2-4)	2 (2-4)	4 (0-4)	0.2457
Hepatic Degeneration	1 (0-1)	1 (0-6)	4 (2-4)	6 (6)	0.0625	1 (0-1)	4 (4-6)	2 (2-4)	4 (2-6)	**0.0074**
Megalocytosis	0 (0-0)	4 (4-6)	6 (2-6)	4 (4)	**0.0269**	0 (0-0)	6 (2-6)	5 (0-6)	6 (4-6)	**0.0092**

N: negative control; C: induced to colorectal carcinogenesis; CJ: induced to colorectal carcinogenesis and supplemented with Juçara fruit pulp at 0.1% (mass / volume), equivalent to 0.10708 mg/mL of cyanidin-3-glycoside; CE: induced to colorectal carcinogenesis and supplemented with lyophilized extract diluted in water (1 mg / mL), containing 0.10708 mg/mL of cyanidin-3-glycoside. * Kruskal-Wallis test (α = 5%)

RENAL INJURIES

After 10 weeks, the only histopathological changes found in the experimental groups were congestion and hyaline cylinder, with no difference for them between the experimental groups (p = 0.1027 and p = 0.2319, respectively) (Table 2).

After 23 weeks of experiment, tubular degeneration was observed in colorectal carcinogenesis induced and supplemented (pulp and lyophilized extract) groups (p = 0.0031) (Fig. 2c), being more evident in animals supplemented with lyophilized extract of juçara fruit (Table 2). All experimental groups had renal congestion, however this was more evident in supplemented with juçara fruit pulp group (p = 0.0345). Mononuclear inflammatory infiltrate was also observed (Fig. 2b), however, it did not differ between groups (p = 0.2563), as well as hyaline cylinders (p = 0.8396) (Fig. 2a) and dendrites (p = 0.3709) (Fig. 2d) (Table 2).

Fig. 2. Photomicrography of kidney histological sections from rat induces to colorectal carcinogenesis supplemented with pulp or lyophilized extract of juçara fruit. (A) Hyaline cylinders (arrow) and renal congestion (asterisk) are observed. (B) Mononuclear inflammatory infiltrate near the glomerulus (arrow), in addition to renal congestion. (C) Tubular degenerations are observed (asterisk). (D) Presence of dendrites inside the renal tubule (arrow) and congestion. H&E staining. Bar: 22µm.

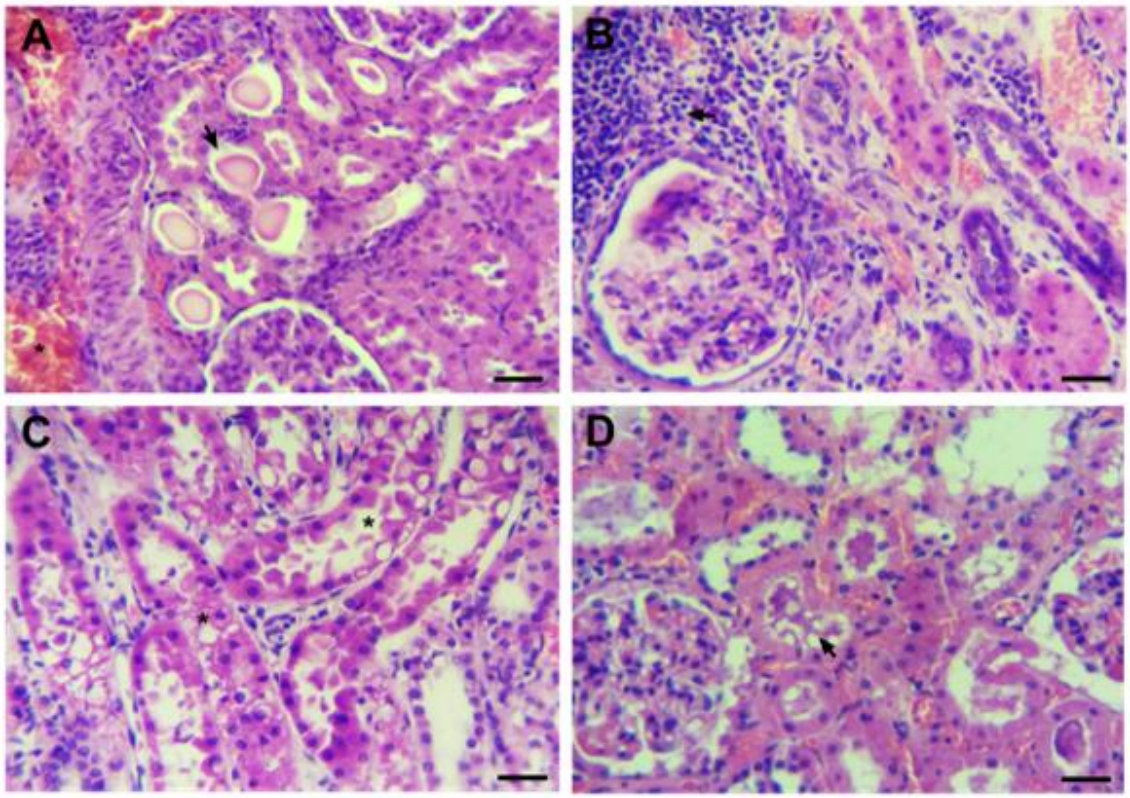

Table 2. Renal injuries score assessed at 10 and 23 weeks in rats induced to colorectal carcinogenesis, supplemented or not with juçara fruit-based product (pulp and lyophilized extract). Scores described in median (Lower-Upper Limit).

Renal Injuries	10 WEEKS					23 WEEKS				
	N *(n=4)*	**C** *(n=4)*	**CJ** *(n=4)*	**CE** *(n=2)*	***p*-value***	**N** *(n=5)*	**C** *(n=7)*	**CJ** *(n=6)*	**CE** *(n=4)*	***p*-value***
Tubular Degeneration	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	-	0 (0-0)	0 (0-0)	2 (0-4)	4 (2-4)	**0.0031**
Congestion	6 (4-6)	5 (4-6)	5 (4-6)	2 (2)	0.1027	2 (2-4)	4 (2-6)	4 (4-6)	4 (4)	**0.0345**
Tubular Necrosis	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	-	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	-
Dilatation of Bowman's Capsular Space	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	-	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	-
Inflammation Infiltrate	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	-	0 (0-2)	2 (0-6)	2 (0-6)	4 (0-6)	0.2563
Renal Brush Border Loss	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	-	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	-
Intersticial Edema	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	-	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	-
Tubular Dilatation	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	-	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	-
Dendrites	0 (0-0)	0 (0-0)	0 (0-0)	0 (0-0)	-	0 (0-0)	0 (0-0)	1 (0-2)	0 (0-0)	0.3709
Hyaline Cylinder	0 (0-0)	0 (0-2)	1 (0-2)	2 (2)	0.2319	2 (0-2)	2 (0-2)	1 (0-2)	1 (0-6)	0.8396

N: negative control; C: induced to colorectal carcinogenesis; CJ: induced to colorectal carcinogenesis and supplemented with Juçara fruit pulp at 0.1% (mass / volume), equivalent to 0.10708 mg/mL of cyanidin-3-glycoside; CE: induced to colorectal carcinogenesis and supplemented with lyophilized extract diluted in water (1 mg / mL), containing 0.10708 mg/mL of cyanidin-3-glycoside. * Kruskal-Wallis test (α = 5%)

4 DISCUSSION

This study revealed that important hepatic injuries were observed in rats induced to colorectal carcinogenesis with 1,2-dimethylhydrazine (DMH), as well as in rats induced to ccolorectal carcinogenesis and supplemented with pulp or lyophilized extract of juçara fruit, both at 10 weeks and 23 weeks of experiment. The injuries found were biliary hyperplasia, mononuclear inflammatory infiltrate, necrosis, congestion and hepatic degeneration, as well as megalocytosis.

Other studies have evaluated both the effect of DMH (Sharma and Sharma, 2011), as well as effect of eating açaí (*Euterpe oleracea* Mart.) on the liver (Ribeiro *et al*., 2010; Marques *et al*., 2016; Alessandra-Perini *et al*., 2018), as well as on experimental models of kidney injury (El Morsy *et al*., 2015; Unis, 2015; da Costa *et al*., 2017). After oral administration of DMH in mice there was a significant increase in enzymes indicating liver damage and in histopathological examination, cell necrosis, fatty infiltration, pleomorphic nuclei and aberrant mitotic figures were observed, indicating damage and the appearance of hepatic pre-neoplasic lesions (Sharma and Sharma, 2011). Thus, it is evident that neoplasic induction process using DMH affects liver tissue considerably and it is important to consider these changes in physiological homeostasis in studies involving the experimental model of colorectal carcinogenesis.

The investigation of açaí (*Euterpe oleracea* Mart.) effect, a species belonging to the same family as the juçara palm, in breast cancer induced by 7,12-dimethylbenzanthracene (DMBA) showed that histopathological samples of liver and kidneys revel a cytoprotective effect of açaí on the observed organs, since in the control group, which did not ingest the fruit, there was a marked fibrosis, atypical cells and hemorrhagic microenvironment compared to the açaí treated group, indicating that these injuries are related to DMBA (Alessandra-Perini *et al*., 2018). In our study, there was no portal fibrosis and biliary fibrosis in the experimental groups at 10 and 23 weeks, and it is believed to be related to carcinogen agent, as well as carcinogenesis induction methodology.

An important injury found in hepatic tissue of the animals in our study was megalocytosis. This injury was observed both at 10 weeks and 23 weeks of study, showing that its persistence in an experimental model of colorectal carcinogenesis is a considerable finding. Megalocytosis is defined as increase in the size and nucleus/cytoplasm ratio of hepatocytes, with much larger cells than the expected normal size, and it is one of the first lesions observed in the liver of intoxicated animals (Torres and Coelho, 2008).

Enlarged hepatocytes (megalocytosis) are unable to perform the functions, influencing the development of liver failure. This change was associated with several causes, such as poisoning by *Senecio* sp. in cattle (Grecco *et al*., 2010) and ivermectin in dogs, for example (Turra Pimpão *et al*., 2005). Thus, it is believed that once induced to colorectal carcinogenesis with DMH, the rats presented and persisted with megalocytosis indicating a process of chronic intoxication. In addition, the fact that supplementation with products based on juçara fruit, rich in bioactive compounds, was not able to modify the megalocytosis, as the supplemented groups (*CJ* and *CE*) did not differ from the induced and not supplemented group.

An investigation of the cytotoxicity of açaí in the liver and kidney, as well as other organs of mice, has shown that the administration of açaí by gavage was not cytotoxic to animals and the components of this product can be exploited as promoters of good health (Ribeiro *et al*., 2010). Corroborating this result, a study also showed that açaí did not have significant toxic effects on the liver and other organs (Marques *et al*., 2016). These results suggest that açaí is a safe food ingredient and that the megalocytosis in the cancer-induced groups (*C*, *CJ* and *CE*) of this study is related to carcinogenesis and not with juçara-based

products. However, contrary to expectations, supplementation with juçara-based products did not promote cytoprotection in the concentration used.

Hepatic degeneration observed at 23 weeks of experiment revealed that colorectal carcinogenesis with DMH directly affects the liver of the animals. This result was expected, since that DMH causes severe liver damage, like necrosis and hepatocellular degeneration (Comstock *et al*., 1954). However, we also found that during this observation period, supplementation with pulp or extract of juçara fruit in the concentration used tended to reduce hepatic degeneration. A study has proved that rats submitted to a diet with açaí had decreased hepatic damage and fatty liver degeneration, suggesting the use of açaí as a potential therapy for liver injuries (Pereira *et al*., 2016). Dyslipidemic mice experienced a reduction in fat accumulation after a diet enriched with lyophilized *E. edulis* pulp (Marques Cardoso *et al*., 2015). Thus, we believe that other concentrations of juçara-based products could elucidate the role of reducing hepatic degeneration in rats induced to colorectal cancer.

Hepatic necrosis, as well as degeneration, had a higher score in groups C and CJ in this study. It should also be noted that supplementation with the lyophilized extract of the juçara fruit protected against hepatic necrosis in animals induced to colorectal cancer. Hepatic necrosis has been described as a consequence of direct toxicity to hepatocytes generated by the conversion of a xenobiotic to active toxin, which can even lead to liver disease (Blatt *et al*., 2016). In this sense and according to the findings of this work, it is evident that the carcinogenesis process using DMH is directly linked to hepatic necrosis. In addition, there is evidence that uncontrolled oxidative stress, which is defined as an imbalance between the oxidative challenge and the antioxidant defense capacity and which may be caused by a xenobiotic, may be a key factor in the occurrence of hepatocellular apoptosis and necrosis (de Oliveira *et al*., 2015).

Another important organ evaluated in this study, and responsible for maintaining homeostasis is the kidney, because it is the main route of excretion of xenobiotics. However, exogenous compounds can cause kidney damage due to the synthesis of reactive oxygen species and oxidative stress, which can induce cell death or necrosis (Gupta *et al*., 2016).

At 10 weeks of experiment, the only lesions found in the experimental groups were congestion and hyaline cylinders, without significant difference between groups. However, at 23 weeks of experiment, mononuclear inflammatory infiltrate, dendrites and hyaline cylinders was observed in renal tissue, also without significant difference. But,

tubular degeneration and congestion score at 23 weeks showed diferences between groups, especially in those supplemented with juçara-based product.

For the congestion found in this study, we believe it to be a non-specific finding. A study has show that the use of proanthocyanin extract from grape seed in an experimental model of contrast-induced nephropathy alleviated renal congestion in these animals (Ozkan *et al*., 2012). In another study using *Punica granatum* L. in rats induced to oxidative stress with lead acetate, showed that low and high dose *P. granatum* juice reduced renal congestion (Aksu *et al*., 2017). Thus, it is evident that this injury in rats induced to colorectal cancer supplemented with *E. edulis* needs further studies, mainly regarding the type of product and dose used.

As noted, tubular degeneration was a lesion found in animals supplemented with juçara fruit. However, in animals with glycerol-induced acute renal failure (ARF) there was a significant improvement in tubular degeneration in the groups treated with açaí extract when compared to the non-supplemented group (Unis, 2015). The açaí seed extract, through its antihypertensive, antioxidant and anti-inflammatory properties, was able to substantially reduce kidney damage and prevent kidney dysfunction, reducing inflammation, oxidative stress and improving the renal filtration barrier (da Silva Cristino Cordeiro *et al*., 2018). Thus, it is understood that depending on the product of fruits of the genus *Euterpe*, on the time of administration and dose, beneficial and harmful effects can be observed.

It is important to note that there are a limited number of studies analyzing the possible effects of fruit-based products of *E. edulis* Mart. under liver and kidney injuries, neither associated with colorectal cancer. Thus, this study presents important results on lesions in liver and kidney tissue that were observed in experimental modelo of colorectal carcinogenesis with DMH, as well as in those animals induced and supplemented with pulp or lyophilized extract of juçara fruit.

5 CONCLUSIONES

This study showed that important hepatic and renal injuries were observed in rats induced to colorectal cancer with 1,2-dimethylhydrazine and the supplementation with pulp or lyophilized extract of Juçara fruit, in the dose used, did not interfere in the prevention and resolution of these injuries, mainly with the chronic use of fruit-based products of *E. edulis* Mart.

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