The potential of pomegranate peel (*Punica granatum*) in the treatment of obese and glucose-intolerant mice

O potencial da casca de romã (*Punica granatum*) no tratamento de ratos obesos e intolerantes ao glucos

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ABSTRACT
Obesity is a pandemic condition, absolutely out of control. Food assistance is among the main proposals for the management of the disease. Thus, the aim of this study was to evaluate the potential of aqueous pomegranate extract in a model of obesity, glucose intolerance and hepatic steatosis induced by high-fat diet. The pomegranate extract was obtained from the association of green extraction techniques. Through the crude extract containing punicalagin and ellagic acid (main compounds), extracts with isolated fractions of these compounds were obtained. For the experimental study, Swiss mice were subjected to the obesity induction period for eight weeks. After, were treated for another 30 days with crude extract of 250 mg/kg (group HF+[1]EXT); punicalagin extract isolated 8.35 mg/kg (HF+Punica group); isolated extract of ellagic acid 0.208 mg/kg (group HF+EA), via gavage. Both the crude extract and its subfractions reduced the body weight gain for the HF+Punica group (1.1 g); HF+EA (0.92 g) compared to HF, while the HF+EXT showed significant weight loss ($P<0.001$). In the glycemic parameters, all extracts were able to reduce blood glucose when compared to the group with HF. Histological data of liver tissue showed improvement in hepatic steatosis, mainly in the crude extract group. Therefore, it was possible to demonstrate that the aqueous extract of the pomegranate peel, obtained by innovative extraction techniques, can be a potential strategy for the treatment and control of obesity.

Keywords: Obesity, Hepatic Steatosis, pomegranate, punicalagin, ellagic acid, emerging extraction technologies.

RESUMO
A obesidade é uma condição pandémica, absolutamente fora de controlo. A assistência alimentar está entre as principais propostas para a gestão da doença. Assim, o objectivo deste estudo era avaliar o potencial do extracto aquoso de romã num modelo de obesidade, intolerância à glicose e esteatose hepática induzida por uma dieta rica em gorduras. O extracto de romã foi obtido a partir da associação de técnicas de extracção verde. Através do extracto bruto contendo punicalagina e ácido elágico (principais compostos), foram obtidos extractos com fracções isoladas destes compostos. Para o estudo experimental, os ratos suíços foram submetidos ao período de indução da obesidade durante oito semanas. Depois, foram tratados durante mais 30 dias com extracto bruto de 250 mg/kg (grupo HF+ EXT); extracto de punicalagina isolado 8,35 mg/kg (grupo HF+Punica); extracto

[1] abbreviations:EXT – extract, EA- acid ellagic, PUNICA – punicalagin.

isolado de ácido elágico 0,208 mg/kg (grupo HF+EA), via gavage. Tanto o extracto bruto como as suas subfracções reduziram o ganho de peso corporal para o grupo HF+Punica (1,1 g); HF+EA (0,92 g) em comparação com HF, enquanto que HF+EXT mostrou uma perda de peso significativa (P<0,001). Nos parâmetros glicémicos, todos os extractos foram capazes de reduzir a glucose no sangue quando comparados com o grupo com HF. Os dados histológicos do tecido hepático mostraram uma melhoria na esteatose hepática, principalmente no grupo do extracto bruto. Assim, foi possível demonstrar que o extracto aquoso da casca da romã, obtido por técnicas de extracção inovadoras, pode ser uma estratégia potencial para o tratamento e controlo da obesidade.

Palavras-chave: Obesidade, Esteatose Hepática, romã, punicalagina, ácido elágico, tecnologias de extracção emergentes

1 INTRODUCTION

Obesity is characterized by a chronic and low-grade inflammatory process mediated by an imbalance in gene expression and protein content of inflammatory cytokines (Hotamisligil, 2017). This inflammation influences cell signaling and the pathophysiology of maintenance and progression of fat accumulation, development of insulin resistance, type 2 diabetes mellitus, dyslipidemia, increased oxidative stress and uncontrolled hunger and energy expenditure, coordinated by the hypothalamus (Hotamisligil, 2017; Van de Sande-Lee, Velloso, 2012).

The damage associated with the obesogenic process extends to other organs and systems. Liver steatosis and its progression to nonalcoholic steatohepatitis, fibrosis, and cirrhosis are increasing outcomes. As treatment for steatohepatitis associated with obesity and cardiometabolic risk are the control of dyslipidemia and weight loss (Parise, 2002; Cotrim et al., 2016).

However, nutritional strategies based on experimental studies with pomegranate are being applied to explore the functional capacity of the fruit's bioactive compounds. The peel is full of polyphenolic compounds such as flavonoids, phenolic tannins, proanthocyanidins, and complex polysaccharides (Fischer, Carle, Kammerer, 2011). Potent specific antioxidants are also found in the pomegranate peel, such as ellagic acid (EA) and punicalagin, the latter having an antioxidant potential 5-10 times greater than ellagic acid, in addition to being in higher concentration (Fischer, Carle, Kammerer, 2011; Lan et al., 2009; Bialonska et al., 2010; Gil et al., 2000).

When considering the representativeness of the portion constituted by the pomegranate peel, its weight represents 50% of the total volume of the fruit, which implies the formation of a large amount of residue. There are several pieces of evidence

that point to the potential of punicalagin and related compounds to the prevention of some diseases, such as cardiovascular outcomes and certain types of cancer (Larrosa, Tomás-Barberán, Espín, 2006; Seeram, Lee, Heber, 2004). Thus, the objective of this study was to evaluate the effect of the aqueous extract of pomegranate, produced from the development of advanced techniques of extraction and purification of compounds, with the purpose of applying the extracts in an experimental model of obesity, in order to observe its effects on hepatic ectopic fat accumulation and glucose intolerance associated to obesity.

2 MATERIAL AND METHODS

2.1 RAW-MATERIAL

The pomegranate samples (*Punica granatum*) were purchased on the market and processed immediately. The sample processing consisted of separating the seeds/arils from the shell and carpel membranes by means of a commercial pulper (Des-60 Braesi, Caxias do Sul, RS, Brazil). The peel (exocarp, albedo, and membranes), excluding the edible part (arils and seeds), was dried at 40 °C in an oven with forced air circulation (JP Selecta, Barcelona Spain) for 48 hours before being ground in a knive mill. The samples of ground pomegranate peel were placed in amber glass bottles, identified and stored at -20 °C until they were used as samples in the extraction process.

2.2 EXTRACTION AND SEPARATION OF BIOACTIVE AND ANTIOXIDANT COMPOUNDS

The extraction process was carried out using the "EXTRACT-US" system (Project FAPESP #2013/04304-4 – Patent pending), which is an integrated extraction and analysis system (Sumere et al., 2018). In this system, it is possible to perform an extraction with liquids at high pressure, combined with the use of ultrasound, and associate the use of expansion gas to the process. To obtain the crude extract containing punicalagin and ellagic acid, 1.5 g of sample were used in the extraction process under the combination of the applied experimental conditions of initial pressure of N_2 (N_2-Pi: 0 – 15 bar) (0 – 15 Kg / cm²) and ultrasound power (US-Pwr: 0 – 600 W). The pressure of system condition was fixed at 200 bar / 200 Kg / cm², with the temperature at 40 °C, totaling 60 min to collect 60 mL of aqueous extract, according to Santos et al., 2019.

The extracts containing the compounds isolated from punicalagin and ellagic acid were obtained using the pressurized liquid extraction (PLE) process coupled to the solid-

phase extraction column (SPE), it was also carried out in the "EXTRACT-US" system, adapted from Souza et al., 2020 . In this step, 1.5 g of sample were used, and the adsorbent C18 (Sepra C18-E, Silica Base, 50 µm, at a flow rate of 2 mL / min., at 40 °C), of water for the collection of the punicalagin compound and later elution was made with 30 mL of ethanol for the extraction of ellagic acid.

2.2.1 Identification of phenolic compounds by high-performance liquid chromatography

For the development of the high-performance liquid chromatography (HPLC) analysis method, a representative extract of the pomegranate peel was used. The analysis of the compounds present in the sample was also performed in the "Extract-US" system. The separation of the compounds was carried out using the developed method, a Fused-core column (Kinetex C18, 2.6 µm, 100 A, 100 × 4.6 mm, Phenomenex, Torrance, CA, USA). The mobile phase is composed of water (1% phosphoric acid) (solvent A) and acetonitrile (1% phosphoric acid) (solvent B). The optimized gradient profile was as follows: 8.15 min, 10% B; 12.6 min, 30% B; 15.6 min, 37% B; 18 min, 90% B; 20 min, 90% B; 21 min; 4% B. Ultraviolet (UV) absorbance was monitored between 260 and 400 nm, and the peaks were integrated at 370 nm. The flow rate is 0.9 mL/min.

The identification of each compound was carried out by comparing the retention times and UV spectra of the separated compounds as well as by co-elution with authentic standards. The stock solutions of the punicalagin (100 mg / L) and ellagic acid (110 mg / L) standards were prepared in methanol. The standard curves for punicalagin (five points: 100, 50, 25, 10, 5 ppm) and ellagic acid (five points: 110, 55, 22.5, 11.15, 5.625 ppm) were prepared using the graphical representation correlating the concentration *versus* the area. All analyses were performed in duplicate, and the results were expressed as mg of phenolic compound / g of dry sample.

3 EXPERIMENTAL MODEL OF OBESITY INDUCTION

3.1 PROTOCOL FOR THE TREATMENT OF ANIMALS

The experiment was conducted according to the rules of the Ethics Committee on Animal Experimentation at the University (CEUA / IB - UNICAMP) – protocol #4596-1/2017. For this, 45 male Swiss mice, 4 wk of age, obtained from the Multidisciplinary Center for Biological Research (CEMIB) at UNICAMP, were placed in individual polyethylene cages, kept in ventilated shelves (Insight®), at (21 °C ± 2), with controlled

photoperiod (12/12 hours light/dark), with water and feed *ad libitum*. Initially, they were separated into two experimental groups, where one group (control – CT – n=9) received commercial feed (Labina®) and the other group (obese – HF – n=34) high-fat diet, modified from the AIN93-G standard (Reeves, Nielsen, Fahey, 1993) to contain 35% fat. To compose 35% of total lipids, 31% came from lard, and 4% from soy oil (Table 1). Both groups were maintained for eight weeks on these diets so that the high-fat diet would induce obesity phenotype and glucose intolerance.

At the end of the obesity-inducing period, the mice consuming the high-fat diet were separated into new four groups, according to a random distribution in Score Z for body mass. The CT group was kept in commercial food (n=9). One group was kept on the high-fat diet (HF – n=9) and received 140 µL of distilled water per gavage. The HF+EXT group received 140 µL of crude extract (containing ellagic acid and punicalagin) from the pomegranate peel (250 mg/kg) by gavage. The HF+Punica group received by gavage 140 µL of the isolated extract of Punicalagina (8.35 mg / Kg). The HF+AE group received 140 µL of ellagic acid isolated extract (0.208 mg/kg) by gavage. The treatments took place once a day, for 30 days.

Table 1. Ingredients and Nutritional Composition of the High-fat (HF) Diet.

Ingredients	HF diet (g/Kg)	Kcal/Kg
Maize starch	115.5	462
Casein	200	800
Sucrose	100	400
Dextrinated starch	132	528
Lard	312	2808
Soy oil	40	360
Cellulose	50	-
Mixture of minerals	35	-
Mix of vitamins	10	-
L-cystine	3	-
Choline Bitartrate	2.5	-
Total	1000 g	5358 Kcal

Legends: Diet based on AIN-93 G – American Institute of Nutrition – 1993. HF – high-fat. .

3.1.1 Preparation of Extracts

Both the crude extract of the pomegranate peel and the isolated extracts (punicalagin and ellagic acid) were diluted in distilled water. The dosages used were 250 mg/kg of the crude extract; 8.35 mg/kg of isolated punicalagin extract; 0.208 mg / Kg of the isolated extract of ellagic acid. The quantity offered of the isolated extract of punicalagin and ellagic acid was based on the amount of the same compounds present in the crude extract of the pomegranate peel, from the analysis made by HPLC. After the experimental period, on the last day, the animals were fasted for 8 hours and euthanized by a deep dose of anesthetics.

3.1.2 Glucose tolerance test (GTT)

Both in the post-obesity induction period and in the post-supplementation period with extracts from the pomegranate peel, the animals were subjected to the glucose tolerance test. For this, they were kept on an 8-hour fast. The first blood collection was performed at time 0 (zero) through a caudal puncture. Then, the 25% glucose solution (2 g / kg) was injected intraperitoneally into the mice, and blood samples were collected at 15, 30, 60, 90, and 120 min to determine blood glucose. Glycemia was determined using glucometer reagent strips (Accu-Chek Active – Roche®). Glucose tolerance was assessed by analysis of blood glucose decay, plotting the area under the curve (AUC).

3.1.3 Liver Histology

Liver fragment specimen was dehydrated with ethanol, cleared with xylene, and embedded in paraffin wax (Histosec® - Merck, Germany), and 4 µm sections were obtained (Olympus microtome) (Cintra et al., 2006). The sections were stained with hematoxylin and eosin. Analysis and documentation of results were performed using a Leica FW 4500 B microscope. The quantification of lipid droplets was carried out using the Image J Software v1.52a, where at least five images were captured from sequential slices from each liver fragment per mouse, separated in the same fields for all images.

3.2 STATISTICAL ANALYSIS

Initially, all the results were submitted to the Kolmogorov-Smirnov test to check for symmetry. The Student's *t*-test was applied for comparison of two independent groups (CT and HF). Analysis of variance (ANOVA) was used to compare three or more

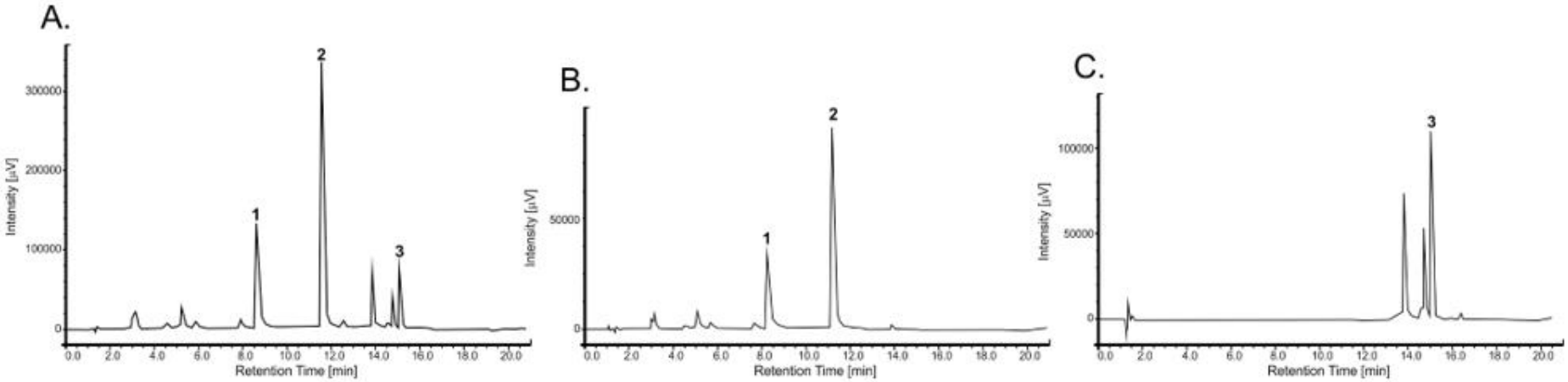
independent groups. Mean values ± SD were compared using Bonferroni's *post-hoc* test, and $P<0.05$ was accepted as statistically significant. Prism v5.0 was used to run the tests.

4 RESULTS

4.1 IDENTIFICATION OF PHENOLIC COMPOUNDS BY HPLC

The PLE-SPE method used to obtain extracts of isolated compounds of punicalagin and ellagic acid was able to separate the compounds perfectly and produce extracts with well-defined characteristics, as illustrated in the sample's chromatogram. Figure 1.

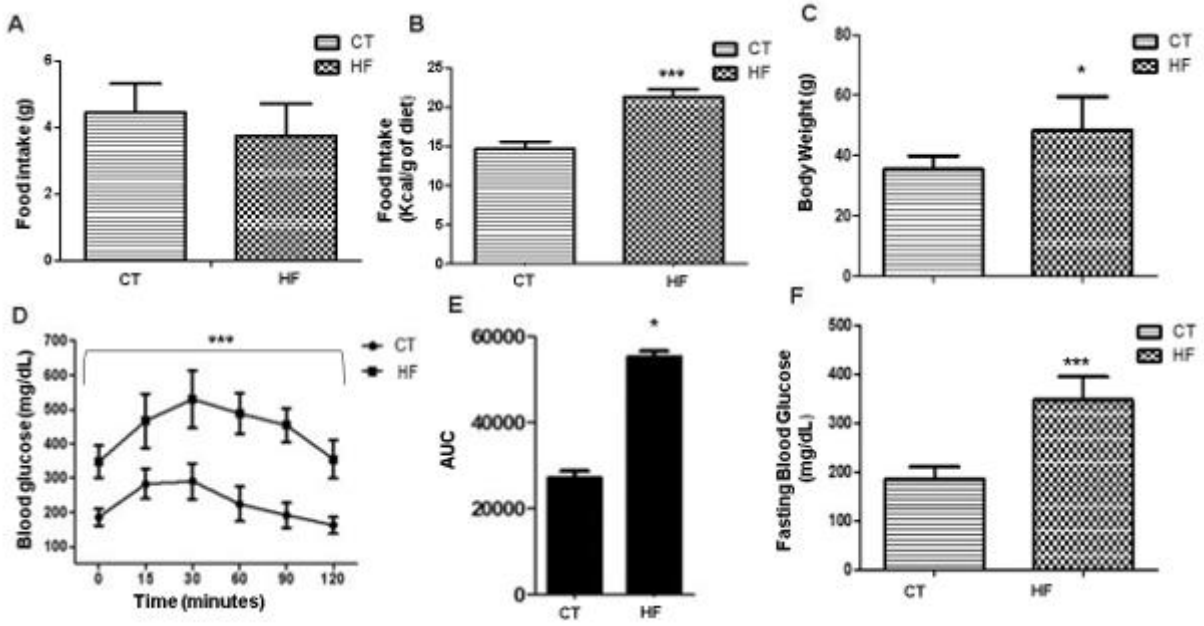
Figure 1 – Chromatograms representative of the compounds identified in the pomegranate peel extract. Chromatogram of the crude extract (A). Chromatograms of the separation of compounds represents the isolated extract of punicalagin and ellagic acid, respectively (B-C). The major compounds were identified: punicalagin alfa (1), punicalagin beta (2), and ellagic acid (3) the others quantified as derivatives of ellagic acid and punicalagin. The samples were analyzed by HPLC (370nm).



4.2 TREATMENT WITH POMEGRANATE PEEL EXTRACTS IN AN OBESE-MICE MODEL

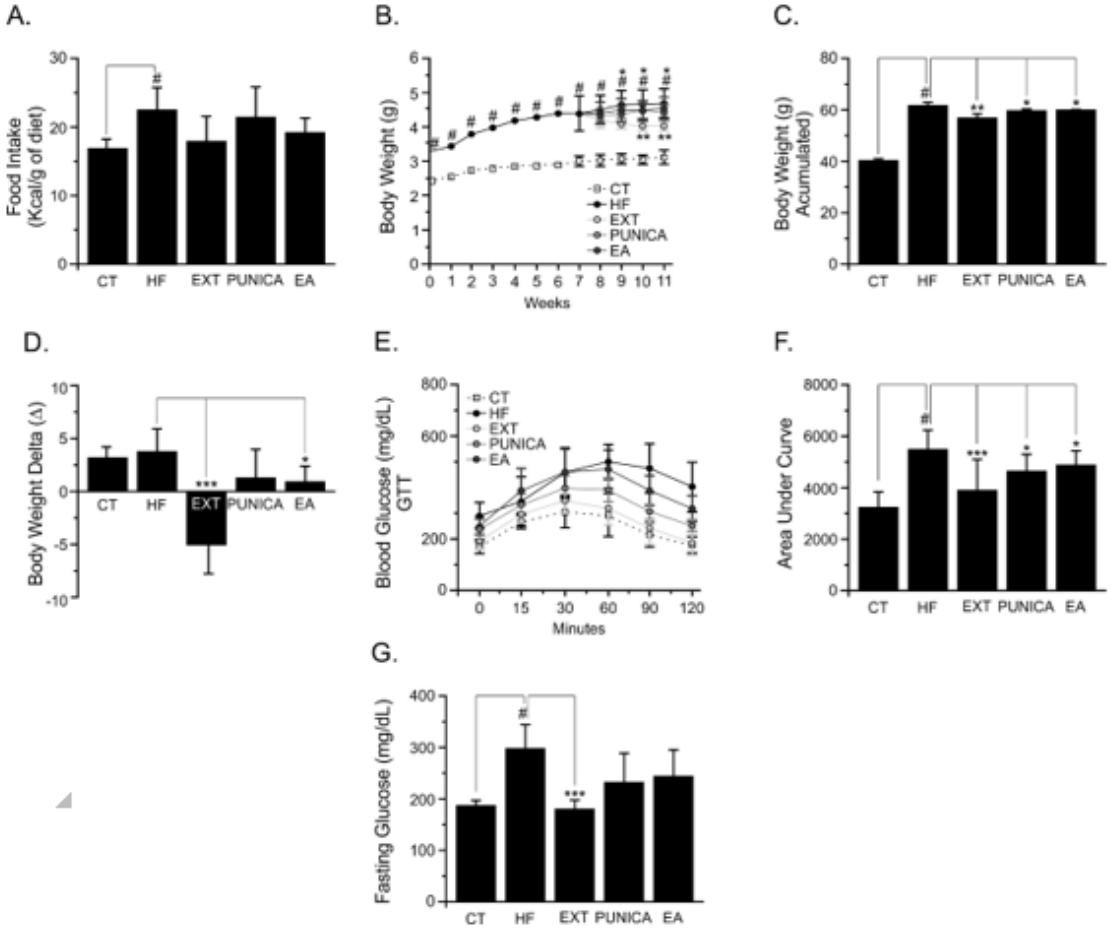
The HF diet was efficient in inducing obesity and the dysmetabolism experimental model. Mice under the HF-diet showed caloric intake and body weight gain significantly higher ($P<0.05$) when compared to animals in the CT group **(Figure 2A-C)**. Following, fasting hyperglycemia and glucose intolerance **(Figure 2D-F)** were also characteristics effectively implemented by the experimental model compared to the CT group ($P<0.05$).

Figure 2 – Metabolic characterization during the obesity induction period. During the obesity period induction, the food intake (A-B) and body weight gain (C) were measured twice a week. The energy density of the standard diet corresponds to 3.31Kcal / g and 5.35Kcal / g of the high-fat diet. At the end of obesity period induction, after 8 hours of fasting, glucose parameters were monitored (D-F). Student's "t" test considered *P<0.05, ***P<0.0001 significant. CT – Control; HF – Obese. N=9 per group.



After the effective induction of the experimental model, the new experimental groups were composed ofobese mice and treated with the products obtained from the pomegranate peel. The food consumption of the group treated with the crude extract was, on average, 16.42 Kcal/day, 20% lower than the obese group (20.54 Kcal/day). The punicalagin and ellagic acid groups consumed 18.99 Kcal and 19.37 Kcal/day, respectively (figure 3), there was no statistical difference for consumption ($P>0.05$).

Figure 3 – Food intake, ponderal evaluation, and glycaemic control of mice treated with pomegranate extracts. (A) Accumulated food intake. (B-D) Ponderal evaluation. (E-F) In the last week before euthanasia, the glucose tolerance test was carried out in mice after 8 hours of fasting. (G) Fasting glucose at the end of the experimental period. Bonferroni's test considered # or *P<0.05, **P<0.01) and ***P<0.0001 significant. CT – Control; HF – High-fat; EXT – Extract; PUNICA Punicalagina; AE – Ellagic acid. N=9 per group.

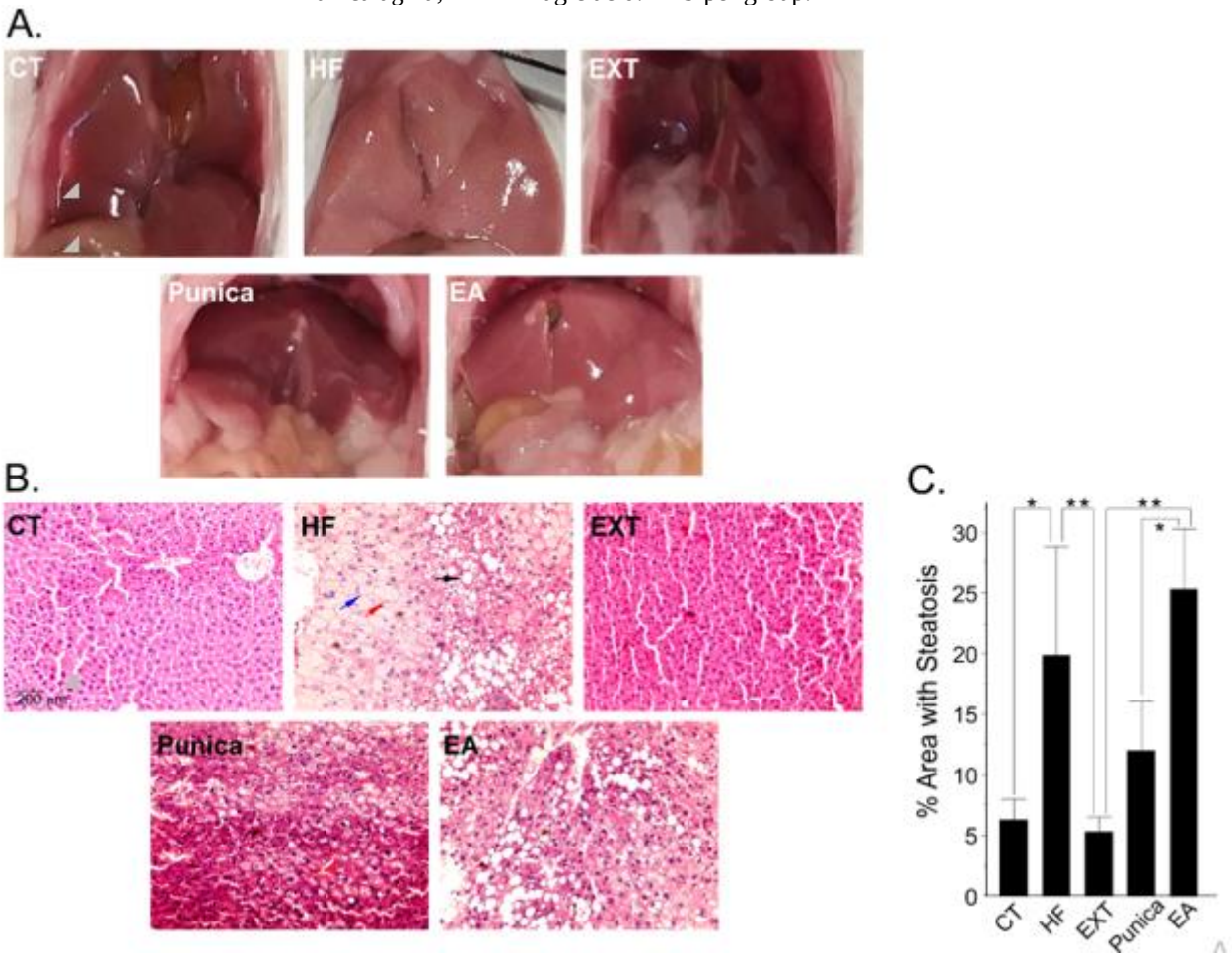


At the end of the experimental period, the different treatments have influenced all groups differently. At the end of the trial period, the total food intake average was increased on the HF group in comparison with the CT group ($P<0.05$); nonetheless, pomegranate extracts did not influence the food behavior **(Figure 3A)**. The high-fat diet has induced even more weight gain in HF-group when compared to CT **(Figure 3B)**. Interestingly, the different treatments with pomegranate extracts influenced the body weight gain in different manners. The animals in the crude extract group had a reduction of 5.16 g at the end of the experimental period, while the punicalagin and ellagic acid groups did not lose weight, however, decreased significantly the intensity of gain ($P<0.05$), with 1.1 g (71%) and 0.92 g (76%) respectively lower than the HF group (3.83 g) **(Figure 3B-D)**.

The glucose tolerance test shows that treatments with crude extract, as well as fractionated extracts, were able to improve glucose tolerance in treated animals **(Figure 3E-F)**. The crude extract group had 179 mg / dL fasting blood glucose, a value 40% lower than that found in the obese group (296 mg / dL) ($P<0.05$), very similar to the finding for the CT group 182 mg / dL **(Figure 3G).**

In the assessment of the macroscopic view of the liver during the surgery **(Figure 4),** the aspect of liver tissue color and size was clearly changed. The natural reddish-brown color and the preserved size in the liver of the CT group were very different from mice treated with the HF diet, in which yellowish and hepatomegalic characteristics suggest hepatic steatosis.

Fig.4. Liver histology analysis. (A) After 8h fasting, mice were euthanized, and the images from the internal abdomen were obtained immediately. (B) The liver fragment was sliced at four µm and stained with hematoxylin-eosin for histological evaluation. Blue and black arrows show a micro and macrovesicular steatosis, respectively. The red arrow shows a strangled nucleus. (C) Lipid infiltration in liver parenchyma. *P<0.05 **P<0.01 (Bonferroni's test). CT – Control; HF – High-fat; EXT – Extract; PUNICA – Punicalagina; AE – Ellagic acid. N=9 per group.



During the quantitative analysis of fat droplets in the liver of mice, the HF group showed a prominent ($P<0.05$) lipid droplets infiltration (20.6 ± 9.6) per field when compared to the CT group 6.27 ± 1.71. The extracts induced different responses in each group. The purified ellagic acid (26.52 ± 5.38) did not protect against liver lipid infiltration induced by the HF diet ($P>0.05$) but worsened it ($P<0.05$) when compared to punicalagin (12.32 ± 4.32). Surprisingly, the crude pomegranate extract (5.36 ± 1.18) has absolutely protected the liver against HF diet damages ($P<0.01$), also similar to the CT group.

5 DISCUSSION

Obesity is a world pandemic condition, without the perspective of satisfactory treatments. The development and improvement of new surgeries or drugs have been failing (Van de Sande-Lee, Velloso, 2012). The search for new strategies has been emerging and needs to be encouraged. Thus, in the present study, we adopted the obese and dysmetabolic mice model induced by a high-fat diet with 35% of fat, predominantly from lard, to test the experimental treatments with natural compounds from pomegranate fruit. The model was successfully implemented with increment in weight gain, adiposity, and glucose intolerance. Buettner et al., 2006 attributed to the saturated fraction of lipids present in lard, more than an unsaturated fraction, to be a more significant impact on the development of obesity, insulin resistance, and glucose intolerance phenotypes. According to Ramirez, Tordoff, Friedman, 1989 and Hariri, Thibault, 2010 , weight gain in animals that receive a high-fat diet is attributed to the energy density and type of fat that make up the diet, and not increase in food consumption, as observed in the present study.

Pomegranate has been increasingly used in metabolic studies, due to its high concentration in functional compounds (Lan J, et al., 2009; Lei et al., 2007; Harzallah et al., 2016; Zhao et al., 2014). The extraction method adopted here showed a high degree of efficiency and, mainly, maintaining the original integrity of the molecules of the fruit compounds in the final extracts. An extraction method used by Sumere et al., 2018 have shown similar efficiency in the obtention of these extracts when compared to the method adopted in the present study. Based on the safety and quality obtained from the extracts, we determine the dose of each extract to be used in the experiment. The concentrations of punicalagin and ellagic acid adopted in this study were based on the proportional offering to that found in the pomegranate's own crude extract.

The significant reduction in the food intake was observed in mice treated with the crude extract (-20%), punicalagin (-7.6%), and ellagic acid (-5.7%) compared to obese mice. Despite these results had been acquired using extracts obtained directly from peel fruit, Lei et al., 2007, observed similar effects using extracts from 800 g / kg of pomegranate leaves. Harzallah et al. (2016) also corroborate both of these observations. Some studies propose the possibility of modulating the pituitary axis acting on satiety control, something that in obesity can be modified by the low-grade pro-inflammatory process. Thus, the result of the present study appears to be promising for improving satiety control, the central pillar in weight loss handling (Van de Sande-Lee, Velloso, 2012; Bialonska et al., 2010; Larrosa, Tomás-Barberán, Espín, 2006; Sumere, et al., 2018).

All pomegranate extracts were efficient to induce the loss of body weight or reduce the intensity of gain. While isolated punicalagin or ellagic acid reduced the weight gain ($P<0.05$), the crude extract induced the weight loss very significantly ($P<0.001$) corresponding to approximately 11% (5 g) compared to the untreated obese group, this significant weight loss highlights the potential for using pomegranate peel as an adjunct to the treatment of obesity. These first results demonstrated so far, point to the imminent need to advance the molecular mechanisms of action of the pomegranate extract. It is important to test its effects on the hypothalamic nuclei, in which exert a fine regulation on food intake and energy expenditure in order to elucidate such pathways in detail (Harzallah A, et al., 2016).

Dysfunctions on the glucose homeostasis is directly associated to the obesogenic process, which could culminate in type 2 diabetes (Van de Sande-Lee, Velloso, 2012). Herein, we demonstrated an important improvement in fasting glucose levels in mice treated with purified punicalagin and ellagic acid ($P<0.05$) and extremely significant with crude extract ($P<0.0001$). In 2016, Harzallah and colls showed reduced fasting glycemia in obese animals when treated with pomegranate seed oil. Thus, it is not a novelty the potential use of parts from pomegranate could reduce the hyperglycemia; however, for the first time, we demonstrated that the crude or purified extracts from pomegranate peel exhibit a potential to recovery or, at least in part, to protect against hyperglycemia induced by the high-fat diet. In this sense, another observation reinforces the role of pomegranate fruit as a potential functional food involved in the control of glycemic homeostasis. During the intraperitoneal glucose tolerant test (GTT) the crude extract group stood out

with a positive response extremely significant when compared to the HF group ($P < 0.0001$).

In obese animals, it is expected glycemic impairment, possibly due to increased production of inflammatory cytokines that can alter the cascade of the insulin pathway in the muscle and liver. For example, insulin resistance in both tissues, is commonly observed a decrement in the glucose uptake by skeletal muscle and the stimulated glyconeogenesis (Hotamisligil, 2017; Van de Sande-Lee, Velloso, 2012). Not distant, the imbalance in the glucose homeostasis can be responsible by liver damage, which can intensify the hyperglycemia (Cintra et al., 2006). In the present study, we assumed these both conditions along with the direct actions of excessive saturated fatty acids in the high-fat diet, carrying out the nonalcoholic fatty liver disease (NAFLD) analysis.

The hyperglycemia associated with obesity state is currently considered a *sine qua non*-condition to lipids accumulation in the liver parenchyma (Van de Sande-Lee, Velloso, 2012), and this aspect needs to be found in the global treatment of obesity, in order to avoid complications (Parise, 2002; Cotrim et al., 2016). Herein, we considered this aspect of investigation where treatments with extracts from the pomegranate peel were efficient in to reduce liver lipid accumulation.

The HF-diet was very effective in to induce fat liver infiltration, changing macro and microscopic parameters such as characteristic liver yellowish, with the presence of micro and macrovesicular steatosis and followed by the loss of hepatocyte architecture. Despite the treatments with isolated pomegranate compounds, punicalagin and ellagic acid did not protect the liver against lipids infiltration induced by HF-diet, the crude pomegranate peel extract had a potent effect. The liver parenchyma of mice treated with the crude extract group was morphologically similar to the CT group, without significant lipid droplets depot with hepatocytes structure highly preserved. Studies *in vivo* and *in vitro* have been showing the potential of pomegranate polyphenols, among them, punicalagin and ellagic acid and others, attenuating liver lipogenesis and reducing the uptake and generation of fat droplets in hepatocytes (Zhao et al., 2014).

From the data presented, it is possible to infer that the obesity-inducing model triggered morphophysiological changes, which were modified through treatments with pomegranate peel extracts. The evidence presented suggests a benefit of using these extracts, especially the crude extract, containing a greater variety of phenolic compounds, showing the importance of the synergistic effect for the modulation of the changes evaluated in this study.

An interesting finding from the nutritional sciences was the evaluation of the functionality of the different extracts produced. A less pronounced effect of the isolated ellagic acid extract was observed on the evaluated changes. It is possible to mention the work carried out by Polce et al., 2018; and Yoshimura et al., 2013, who also administered isolated ellagic acid and observed a decrease in glucose levels and attenuation of hepatic lipid accumulation in an experimental model of animals with hyperglycemia. However, this divergence may be related to the low aqueous solubility of the ellagic acid compound, a factor that can infer about its biodisponibility and bioaccessibility (Kang et al., 2016). According to Kang et al, 2016 the hydrolysis of ellagitannins and the release of ellagic acid occurs in the small intestine, influenced by pH conditions of the medium in which could favor the part of the ellagic acid absorption in that portion of the intestine. On the other hand, most ellagitannins are metabolized by the gut microbiota and biotransformed into urolithins (Long et al., 2019).

Furthermore, the clearance of EA in plasma may be mostly dependent of the EA/ ellagitanninratio. According to Kang et al., 2016 when comparing different amounts of ellagitannins and EA in an extract and free EA only, when increasing the amount exclusively of EA in the extract containing ellagitannins there was no increase in maximum serum concentration (Cmax) of EA bioavailability. Long et al., 2019, proposed another interesting point of view, after comparing the effects of pomegranate juice versus encapsulated free EA in humans. They showed that the EA present in the pomegranate juice is more bioavailable than in the isolated EA form, demonstrating the importance of the presence of ellagitannins to increase the bioavailability of EA.

This synergistic effect between bioactive compounds and nutrients is essential when exploring the functional capacity of food. Studies have been showing that the preservation of the food matrix is mandatory to ensure the correct absorption of nutrients or non-nutrients in effective concentration. The isolation of many compounds and the loss of food matrix could compromise its absorption. The nutritional balance of a diet is linked to the variety of foods consumed daily. However, the consumption of supplements can also alter the balance of a nutritionally adequate diet. Currently, there is indiscriminate consumption of food supplements, due to the appeal of its health benefits. However, it is necessary to evaluate the functional capacity of isolated compounds, to the detriment of the synergistic effect of bioactive compounds. It is understood that isolated extracts as well as extracts rich in compounds of different varieties, have important effects on prevention and health promotion, but have different mechanisms of action.

6 CONCLUSION

The evidence found in the present study demonstrated an important impact of the treatment of pomegranate peel extract under the obesity-inducing model. The findings showed a reduction in food intake, a slowdown in gain and decrease in body weight, an improvement in glucose tolerance, and a reversal of the conditions that characterize hepatic steatosis.

However, in addition to this evidence, the innovative aspect of this work was to demonstrate the potential for the biological activity of three extracts, with different phenolic compositions, obtained by innovative extraction and purification techniques, using emerging technologies and biodegradable solvents. From this study, they showed the potency of the extract produced in view of the critical aspects of obesity treatment.

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CONTRIBUTIONS

MPS, MAR and DEC conceived and designed the experiments.
MPS, MCS, MRS, CBV and CR performed the experiments. MPS, MAR and DEC wrote the paper

CONFLICT OF INTEREST

The authors declare that they have nothing to disclose.

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