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High-fat diet effects on the prostatic adenocarcinoma model and jaboticaba peel extract intake: protective response in metabolic disorders and liver histopathology

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ABSTRACT

Prostate cancer (PCa), overweight and obesity are frequent worldwide health problems. Clinical studies have shown that increased high-fat diet (HFD) consumption is associated with higher incidence of PCa. Brazilian berries, such as *Myrciaria jaboticaba* (Vell.) Berg, present high polyphenol concentration in the peel and exhibit positive effects on metabolic disorders and hepatic lesions. Therefore, the aim of the study herein was to investigate the patented jaboticaba peel extract effects (PJE) on different metabolic parameters and liver histopathology in the transgenic adenocarcinoma of the mouse prostate model, receiving a either normolipid diet or HFD for 8 weeks. The results showed that PJE reduced insulin resistance and glucose intolerance, decreased hepatic lipid accumulation, and inflammatory markers such as PPARγ and TNFα, respectively. In conclusion, the PJE treatment promoted protective effects in the metabolism of insulin and glucose and liver imbalance caused by HFD intake in the PCa model, suggesting that it may be a good protector against metabolic disorders present in overweight and associated with PCa.

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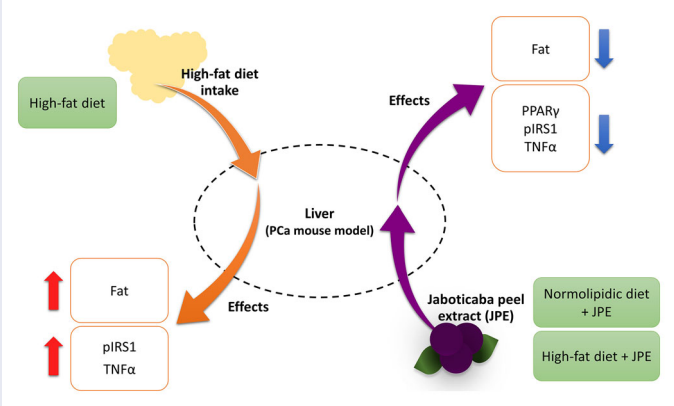
GRAPHICAL ABSTRACT



1. Introduction

Prostate cancer (PCa) and obesity are a significant worldwide health problem and are associated with mortality increase (1,2). In addition, overweight is associated with several diseases such as type 2 diabetes, cardiovascular disease and cancer development (3). Literature has shown that patients under treatment for metastatic PCa are commonly overweight, and the adipose tissue inflammation is linked to aggressive PCa in men (4,5).

The relation between PCa and overweight could be attributed to cancer cell biology effects on the lipid and glucose metabolism (6). Other authors verified metabolic alterations such as aberrant lipogenesis and

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increased glucose synthesis during cancer (7). The insulin systemic imbalance pathway can promote oncogenic signal propagation and cellular proliferation, leading to PCa development (7). Furthermore, it is known that metabolic alterations induced by high-fat diet (HFD) intake can lead to hepatosteatosis formation and, hence, can affect prostate size and the glandular microenvironment (8,9). Insulin is a growth factor and its elevated concentrations can activate the PI3K/Akt/mTOR and MAP/ERK-kinase signaling pathways and lead to cell proliferation and invasion (10).

Different studies, in obese rats, have pointed out that diets, rich in polyphenols, can prevent metabolism disorders and improve insulin resistance (IR) and antioxidant imbalance (11,12). According to Tsuda et al., mice treated with cyanidin3-glucoside-rich purple corn color and/or HFD for 12 weeks showed significant obesity development suppression and hyperglycemia improvement due to hyperlipidic diet ingestion. And also, the same authors verified that the purple corn color led to a suppression of mRNA levels of the enzymes involved in the fatty acid and triacylglycerol synthesis, indicating advantages in obesity and diabetes prevention (13). Also, a Brazilian berry, *Myrciaria jaboticaba* (Vell.) Berg, which presents high polyphenol concentration in its peel and seed has been the focus of these studies (14,15). It is known that one of the most frequent phenolic compounds present in *M. jaboticaba* peel is anthocyanin, such as cyanidin 3-O-glucoside and delphinidin 3-O-glucoside, which are part of the flavonoid group and provides the purple color of the fruit (16,17). Studies showed that anthocyanins were able to reduce IR, TNFα and lipid accumulation in an in vitro adipocyte assay (18).

Research has demonstrated that the 4% freeze-dried jaboticaba peel, added to the diet, increased the GPx and SOD activities in the liver and also increased the antioxidant activity in the plasma, brain and kidney of the rats fed with a HFD (11,19). Nowadays, Lamas and collaborators showed that patented jaboticaba peel extract (PJE) administration, decreased hyperglycemia, IR and dyslipidemia besides preventing hepatic steatosis in HFD senile FVB mice.

Thus, several studies have focused on the HFD and polyphenol effect on PCa progression, taking into consideration that obesity and weight gain are related to tumor progression (20–22). However, the relation of the glucose and insulin metabolisms at the beginning of PCa, as well as the polyphenol effects on this condition still need to be better explained (10, 22). In addition, transgenic adenocarcinoma of the mouse prostate (TRAMP) model has been used in different PCa research, presenting progressive lesions and invasive adenocarcinoma (23). Therefore, the aim of the study herein was to investigate the PJE effects on the metabolic parameters and liver histopathology in the TRAMP model submitted to HFD intake.

2. Materials and methods

2.1. Experimental procedures

Forty male TRAMP mice (C57BL/6-Tg (TRAMP) 8247/Ng/J X FVB/Unib/F1/J) were provided by the Multidisciplinary Center for Biological Investigation on Laboratory Animal Science (CEMIB) in the University of Campinas after approval by the Ethics Committee on Animal Use (CEUA) – UNICAMP/Protocol: 4323-1. The mice received water and solid ration *ad libitum* (Nuvilab, Colombo, PR, Brazil) and were housed at constant temperature (23 °C) and standard dark/light cycle (12–12 h) up to the beginning of the experimental period. At 8 weeks of age, the animals were weighed and randomly distributed into the following experimental groups ($n = 10$): the C group fed on a normolipid diet and treated with vehicle; the JC group fed on a normolipid diet and treated with PJE; the H group fed on a HFD and were treated with vehicle; the JH: group fed on a HFD and treated with PJE. The PJE treated groups received 5.8 g PJE/kg body weight and tap water (vehicle) five days per week. The treatment was performed for 8 weeks by gavage.

The TRAMP mice presented prostatic lesions in a short time (24). From 6 to 12 weeks of age, the prostate exhibits different lesion degrees such as low-grade prostatic intraepithelial neoplasia (PIN) and high-grade PIN (23,25). Around 16 weeks of age, we observed invasive proliferation points called well-differentiated adenocarcinoma (24,26,27). The different prostatic lesions observed in the TRAMP mice are displayed in Fig. 1.

The normolipid diet was prepared according to the American Institute of Nutrition (AIN 93 M) (28) with an adaptation for protein content (12%) (29). For HFD preparation, AIN 93 M formulation was modified with 35% fat (11). Diet compositions are shown in Table 1.

The PJE of the *Myrciaria cauliflo*ra (Vell.) Berg used in the present study was the same which has already been used in another study by our research group. Thus, the detailed characterization of this extract, which was patented, and also the nature of the bioactive compounds can be found in a published

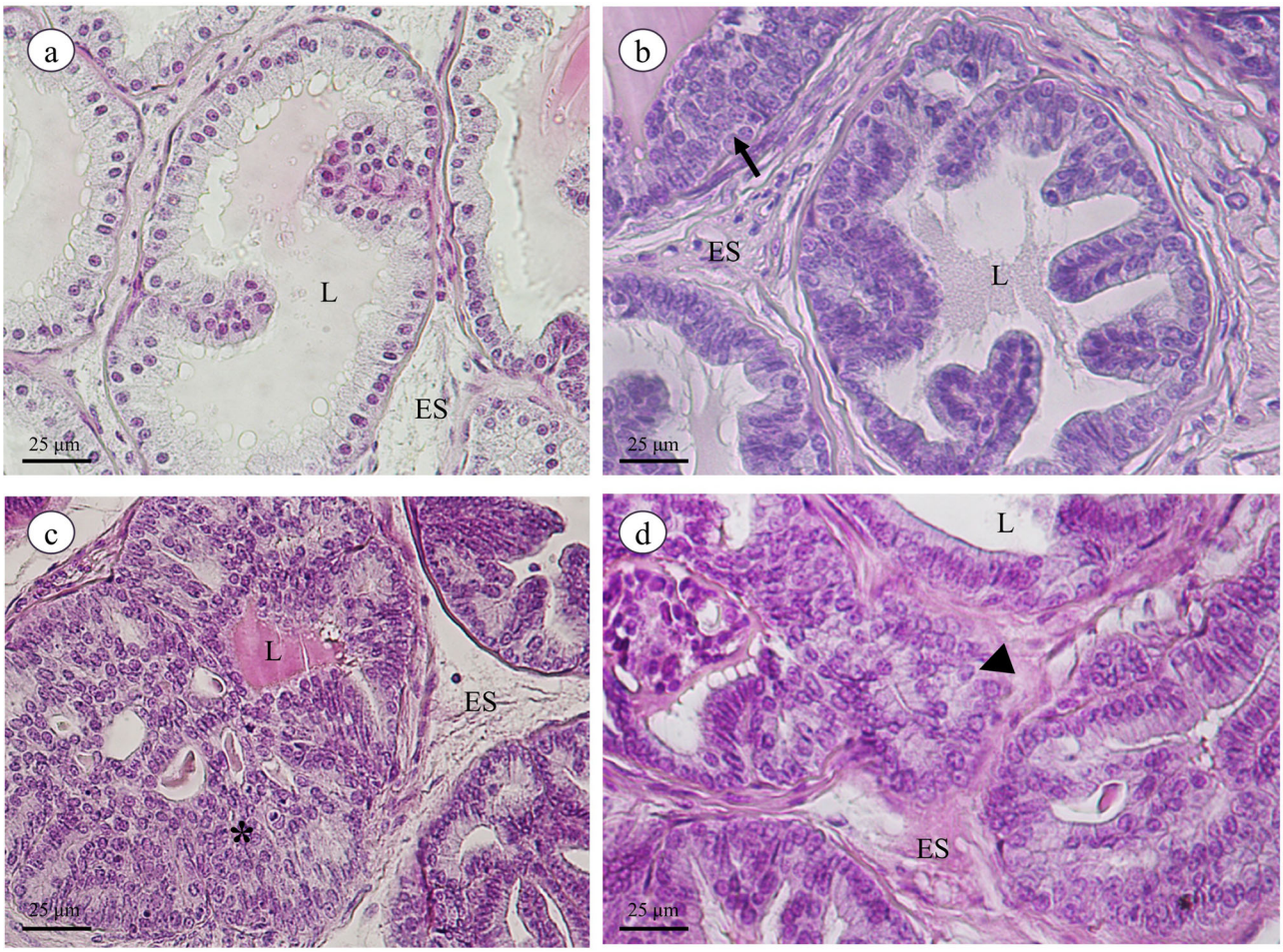

Figure 1. Photomicrographs of the prostate dorsolateral lobe from the TRAMP mice. (a) 8-week old mice, showing healthy histoarchitecture; (b) 12-week old mice with low-grade PIN (arrow); (c) 16-week old mice with high-grade PIN (asterisk); (d) 16-week old mice with well-differentiated adenocarcinoma (arrow head). ES, stroma; L, lumen. Hematoxilin-Eosin.

Table 1. Composition of the AIN 93 M modified diets (Modified: Goena et al. (29); Reeves et al. (28)).

Ingredients (g/kg)	Standard diet	High-fat diet
Casein (83.5% protein)	143.7	143.7
Maize starch	462	152
Maltodextrin	155	155
Sucrose	100	100
Fiber (cellulose)	50	50
Soybean oil	40	40
Lard		310
Mineral mixture (AIN93M)	35	35
Vitamin mixture (AIN93VX)	10	10
L-cystine	1.8	1.8
Choline bitartrate	2.5	2.5
Tert-butylhydroquinone	0.008	0.008
Total	1000.008	1000.008
Energy density (Kcal/g)	3.8	5.35

paper (30). Likewise, the PJE phenolic nature, composition and in vitro antioxidant activity has already been performed in detail (30).

After the experimental weeks, the mice were anesthetized with 2% xylazine hydrochloride (5 mg/kg; Konig, São Paulo, Brazil) and 10% ketamine hydrochloride (60 mg/kg; Fort Dodge, Iowa, USA) and euthanized by increasing the anesthetic level. Liver samples were collected for morphology evaluation ($n = 5$), lipid profile ($n = 5$) and western blotting analysis ($n = 4$). Blood was collected in tubes with EDTA and the plasma obtained was used for lipid profile and glucose levels analysis ($n = 5$).

2.2. Body weight gain and energy intake

The animals were weighed at the beginning and at the end of the experiment. The body weight gain (WG) was evaluated considering the initial body weight (WI) and the final body weight (WF) as WG = WF–WI. The energy intake was performed according to the method previously described (30).

2.3. Insulin tolerance test (ITT), glucose tolerance test (GTT) and fasting glucose levels

The ITT and GTT were measured with 4- and 12-h-fasted mice, respectively (modified from Ref. (31).

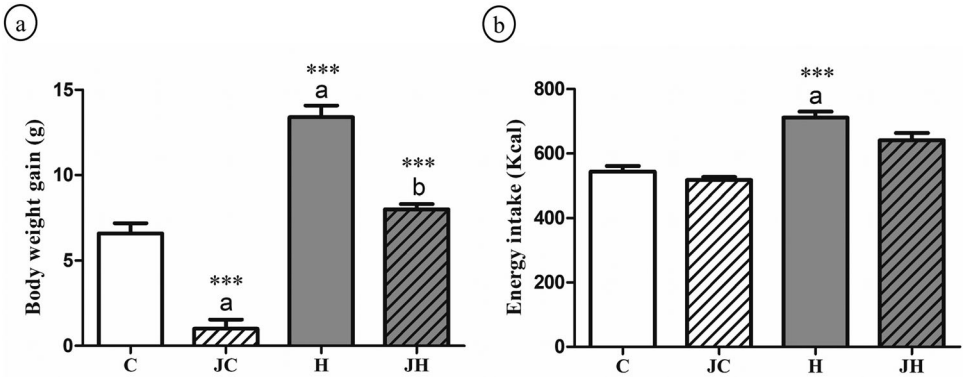

Figure 2. Body weight gain (a) and energy intake evaluation (b). C: group fed a normolipid diet; JC: group fed a normolipid diet and treated with PJE; H: group fed a HFD, JH: group fed a HFD and treated with PJE. ANOVA followed by Tukey test: indication of statistical difference as $^{*}p \leq 0.5$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$. (a) Significant $\neq$ C and (b) significant $\neq$ H.

Blood glucose levels were obtained by caudal incision and the glucose measurements were performed using a glucometer (Breeze^{TM} – Bayer$^{®}$). Before insulin or glucose administration, the blood glucose level was measured. Then, mice received intraperitoneal insulin (0.05 U/kg) or glucose (1.5 g/kg) by gavage and the blood glucose levels were verified at 10, 15, 30 and 60 min periods (ITT test) or 10, 30, 60 and 90 min periods (GTT test) (modified from Ref. (31). The ITT and GTT results were expressed considering the area under the curve (AUC) in the glucose concentration vs. the time graph. The plasma fasting glucose levels were performed in 12-h-fasted mice using a kit from Wiener Lab (Glycemic enzimatic AA, Rosario, Argentina) following the manufacturer's instructions and the results were expressed as mg/dL.

2.4. Lipid profile

The lipid profile in both plasma and the liver was evaluated in 12-h-fasted mice. Lipids were extracted from liver fragments (100 mg) frozen at –80 °C by means of the Folch method (32), and the total lipid was calculated and diluted in isopropanol for the following analyses. The content of triglycerides, total cholesterol and HDL-cholesterol were performed using kits from Wiener Lab (Rosario, Argentina), following the manufacturer instructions in both the liver and plasma. The results were expressed as mg/g (liver) or mg/dL (plasma).

2.5. Light microscopy

Liver samples were collected and fixed in Bouin's solution for 24 h. Then, the tissues were washed in 70% ethanol until the fix solution was removed, dehydrated in an increasing alcohol series, diaphanized in xylene and embedded in plastic polymers (Paraplast Plus, St. Louis, USA). Samples were cut into 5-µm-thick sections using the Hyrax M60 microtome (Zeiss, Munich, Germany) and were stained with hematoxylin–eosin (33). After that, 10 random images were captured using a 40× objective from a Nikon Eclipse E-400 photomicroscope (Nikon, Tokyo, Japan). Using Image the Pro Plus program, a grid with 1800 points was placed on the images and the percentage of lipid hepatic content was determined (modified from Ref. (30).

2.6. Western blotting

Liver samples were stored at –80 °C and then were homogenized using a Polytron homogenizer (Kinematica Inc., Lucerne, Switzerland) in RIPA buffer (Millipore, Temecula, CA) containing protease inhibitor cocktail (Sigma-Aldrich). The homogenates were centrifuged at 18.659 g for 20 min at 4 °C, and the protein content was analyzed using the Bradford reagent (Bio-Rad Laboratories, Hercules, CA). Then, the supernatants were mixed (1:1) with 3× Laemmli buffer and transferred to a dry bath at 100 °C for 5 min. Protein samples (50 µg/µL) were separated by electrophoresis in SDS-PAGE gels. The resulting gel was transferred to a Hybond-ECL nitrocellulose membrane (Amersham, Pharmacia Biotech, Arlington Heights, IL) at 120 V for 90 min. The membranes were blocked with 3% BSA for 60 min. Then, they were incubated at 4 °C overnight with the following primary antibodies in a dilution range of 1:350–1:500 in 1% BSA: monoclonal mouse anti-tumor necrosis factor alpha (TNFα) ab8348 (Abcam-EUA); polyclonal goat sc-17196 tyrosine phosphorylated insulin receptor substrate 1 (pIRS1) and monoclonal mouse anti-peroxisome proliferator-activated receptor gamma (PPARγ) sc-7273 (Santa Cruz Biotechnology, CA). Subsequently,

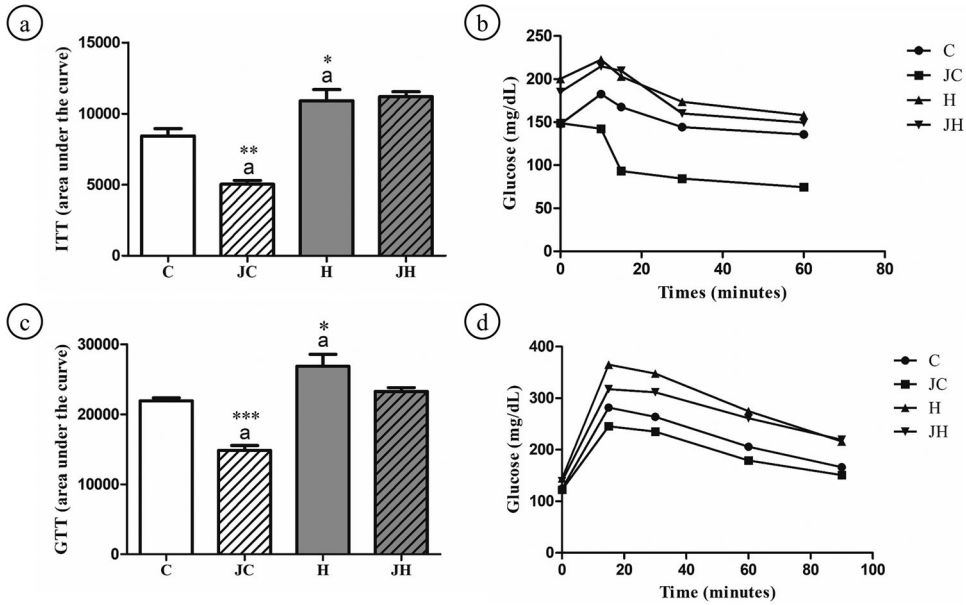

Figure 3. Insulin tolerance test (ITT) and glucose tolerance test (GTT). (a) Area under the curve and standard deviation of the ITT response. (b) Mean of blood glucose levels at 0 (before insulin administration), 10, 15, 30 and 60 min after insulin application. (c) Area under the curve and standard deviation of GTT response. (d) Mean blood glucose levels at 0 (before glucose administration), 10, 30, 60 and 90 min after the glucose solution administration. C: group fed a normolipid diet; JC: group fed a normolipid diet and treated with PJE; H: group fed a HFD, JH: group fed a HFD and treated with PJE. ANOVA followed by Tukey test: indication of statistical difference as $^{*}p \leq 0.5$; $^{***}p \leq 0.001$. (a) Significant $\neq$ C and (b) significant $\neq$ H.

the membranes were incubated for 2 h with the HRP-conjugated secondary antibodies diluted in a 1:2000–1:5000 range. Peroxidase activity was detected using chemiluminescent solution (Pierce Biotechnology, Rockford, IL) and captured using the Gene Gnome equipment and the Gene Sys image acquisition software (Syngene Bio Imaging, Cambridge, UK). Mouse monoclonal anti-β-actin, sc-81178 (Santa Cruz Biotechnology, CA) antibody was used as an endogen control. The intensity of antigen bands was determined using the Image J (Image Analysis and Processing in Java) software for image analyses.

2.7. Statistical analysis

The morphological, protein level, lipid and glucose profile parameters were analyzed statistically by analysis of variance (ANOVA) followed by the Tukey multiple range test. The significance level was considered to be 5%. The results were expressed as mean and standard deviation (34).

3. Results

3.1. Body weight gain and energy intake

The H group showed a body weight gain increase (Fig. 2a) and high-energy intake when compared to the C group (Fig. 2b). The PJE treatment reduced body weight gain in the JC and JH groups.

3.2. Insulin tolerance test and glucose tolerance test

The ITT and GTT showed significant differences among the experimental groups. The H group showed an increase in glucose intolerance and IR compared with the C group. On other hand, PJE administration reduced IR and glucose intolerance in the JC group in relation to the C group. However, there was no significant difference regarding the JH group (Fig. 3).

3.3. Lipid and glucose plasma levels

The HFD intake increased the fasting-blood glucose levels in the H group in relation to the C group (Fig. 4a). Meanwhile, PJE treatment reduced fasting-glycemia in the JH group compared with the H group (Fig. 4a). Moreover, a HDL-cholesterol increase was observed in the JH group compared to the H group. There were no significant differences among the experimental groups in the total cholesterol and triglyceride levels (Fig. 4c,d).

3.4. Liver morphological and lipid evaluation

Liver morphology was similar among the experimental groups (Fig. 5). The liver showed a typical hepatic

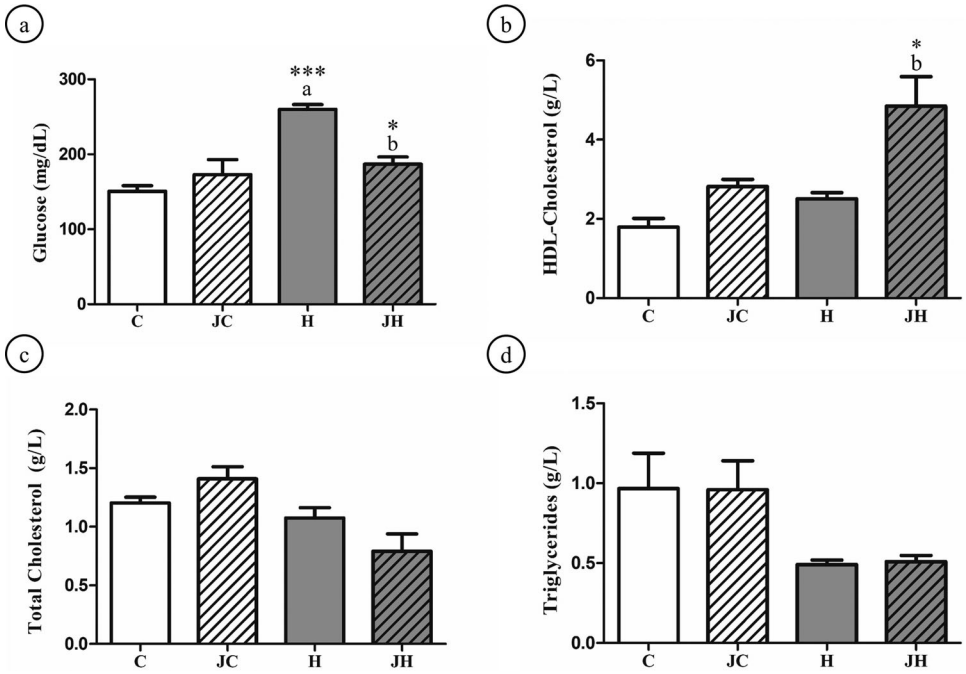

Figure 4. Lipid profile and fasting glycemia. (a) Blood glucose levels. (b) HDL-cholesterol concentrations. (c) Total cholesterol concentrations. (d) Triglycerides concentrations. C: group fed with the normolipid diet; JC: group fed a normolipid diet and treated with PJE; H: group fed a HFD, JH: group fed a HFD and treated with PJE. ANOVA followed by Tukey test: indication of statistical difference as $^{*}p \leq 0.5$; $^{**}p \leq 0.01$. (a) Significant $\neq$ C and (b) significant $\neq$ H.

histoarchitecture with hexagonal arrangement of the hepatocyte strands radiating from the centrilobular vein (CV) (Fig. 5a–d). Increased lipid frequency was observed in the H group compared to the C group (Fig. 5a,b,e). The PJE treatment decreased the hepatic fat accumulation in both experimental groups (JC and JH) in relation to their respective controls (Fig. 5c–e). There were no frequent inflammatory infiltrates in all experimental groups (Fig. 5a–d).

In the biochemical lipid quantification, there is no significant difference in the total liver cholesterol levels, HDL-cholesterol and triglyceride levels among the experimental groups (Fig. 5f,g,h).

3.5. Western blotting evaluation

The PPARγ protein levels did not change in the H group compared to the C group (Fig. 6a). However, these protein levels decreased significantly in both PJE treated groups (JC and JH) in relation to their respective control groups (C and H) (Fig. 6a). The pIRS1 levels showed a statistical difference in the H group in relation to the C group, and also, decreased pIRS1 was seen after PJE treatment in the JH group compared to the H group (Fig. 6b).

The TNFα protein level increased in the H group in relation to the C group (Fig. 6c). TNFα dropped, significantly, only in the JH group in relation to the H group (Fig. 6c).

4. Discussion

This study showed that PJE improved the metabolic disturbance caused by overweight, besides leading to hepatoprotection in the PCa model fed with a HFD. These results are in agreement with previous studies which demonstrated overweight prevention after jaboticaba peel extract intake by HFD fed aged mice (30).

Previous studies have demonstrated jaboticaba peel potential as a preventive approach against metabolic disorders, particularly those associated with HFD intake. Thus, different forms of jaboticaba peel administration were evaluated, such as freeze-dried peel added to the diet, and also flour (11, 35) and tea, replacing water (36,37) or even an extract, administered by gavage (30, 38). Apart from the different forms of jaboticaba peel, the health benefits of this fruit peel have been demonstrated (30, 38). In addition, some differences observed among these studies could be due to the amount of bioactive compounds consumed in the different experimental models, mainly related to polyphenols, soluble and insoluble fibers (39). Also, it is known that the dietary fiber content in extracts is lower when compared to the

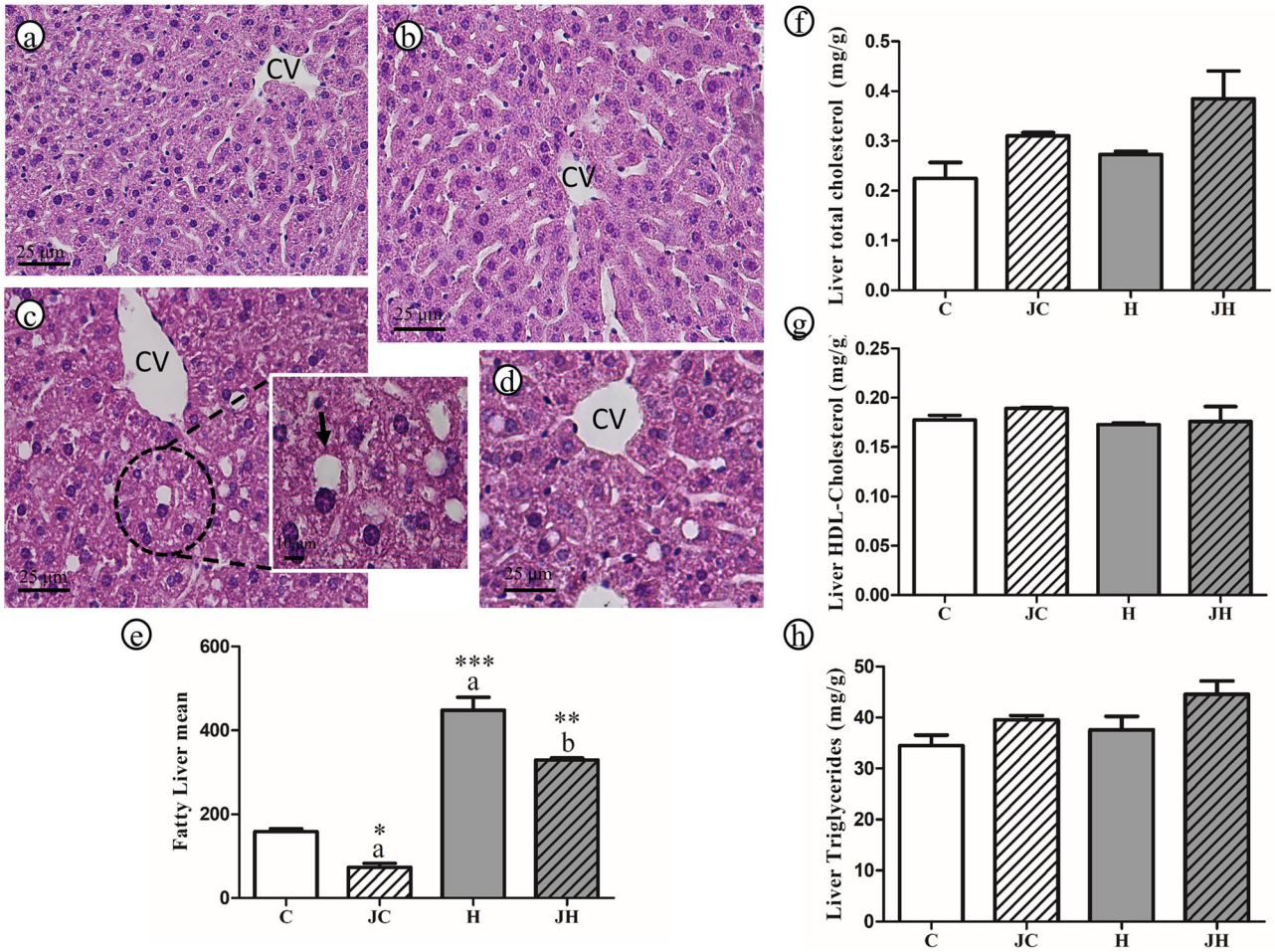

Figure 5. Photomicrographs and lipid profile of the liver in the experimental groups after the treatment. (a) C: group fed a normolipid diet. (b) JC: group fed with a normolipid diet and treated with PJE. (c) H: group fed a HFD showing lipid in the cytoplasm (arrow in the inset). (d) JH: group fed a HFD and treated with PJE. (e) Fat liver quantification. (f) Total cholesterol liver concentrations. (g) HDL-cholesterol liver concentrations. (h) Triglyceride liver concentrations. ANOVA followed by Tukey test: indication of statistical difference as $^{*}p \leq 0.5$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$. (a) Significant $\neq$ C and (b) significant $\neq$ H. CV: centrilobular vein. Hematoxilin-Eosin (a–d).

flour format, due to the smaller amount of insoluble fiber recovered in the extracts (39).

The phenolic compounds conjugated with glucosides or linked to the hydroxyl (OH) group, such as flavonoids, are frequently weakly binded to the plant cell walls, which are mainly made up of insoluble fiber. The weak binding helps these flavonoids to be easily extracted by hydrophilic solvents producing a free form (39). Thus, the extract use allows the higher bio accessibility of phenolic compounds from the matrix (39), and also the possibility of verifying a dose-dependent response in different experimental models, as long as the daily amount of extract administered is controlled (30, 38).

Thus, correlating the results of the present study with the specialized literature, we could show that mice received approximately 85 µg/kg per day of specific anthocyanins – cyanidin and delphinidin-3-O-glucoside, as per work by Ref. (38). And also, based on Ref. (30), 130 µg/kg/day of specific phenolics – ellagic acid, gallic acid and rutin could be quantified. In addition, the quantity of bioactive compounds consumed by the treated TRAMP mice in the present results was lower than that reported by different authors, using freeze-dried jaboticaba flour supplemented in the diet (35,36). This is true, considering that the quantity of Brazilian berry peel flour found in the supplemented diet was greater than that used in the present study as an extract.

However, the final concentration of cyanidin-3-O-glucoside (930 µg/mL) described in the extract used in this study was higher than that verified in aqueous jaboticaba peel extract reported by Ref. (36) and Ref. (40), showing concentration of 685.2 and 261.5 µg/mL, respectively. Finally, it is interesting to say that the use of ethanol–water mixtures is a useful method to intensify the extraction of phenolic compounds, as

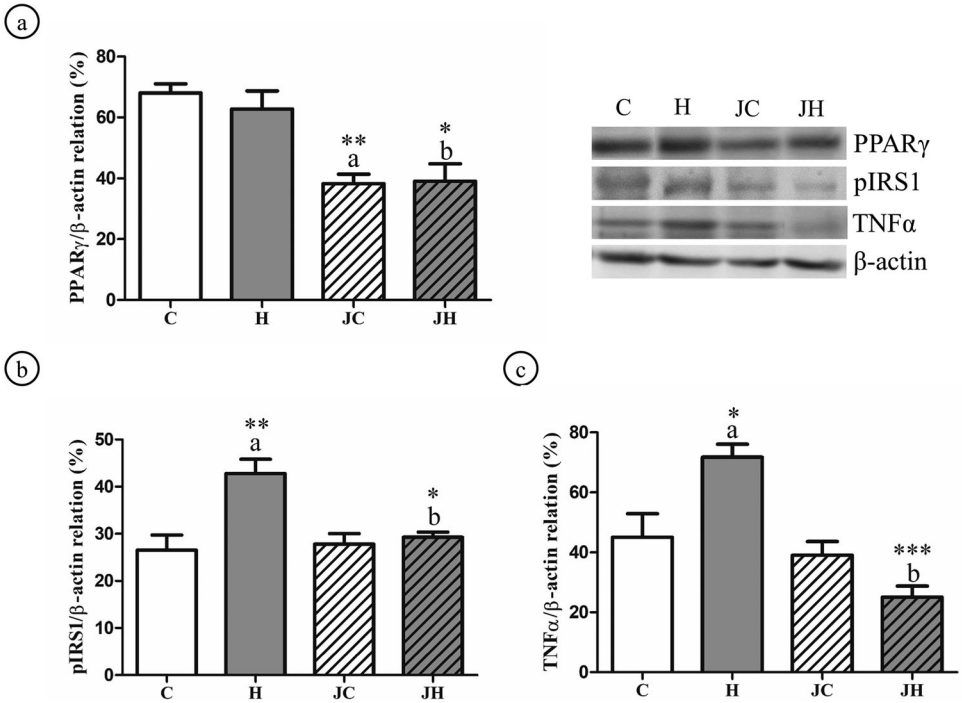

Figure 6. Western blotting analysis. (a) PPARγ protein level evaluation. (b) pIRS1 protein level evaluation. (c) TNFα protein level evaluation. C: group fed a normolipid diet; H: group fed a HFD, JC: group fed a normolipid diet and treated with PJE; JH: group fed a HFD and treated with PJE. ANOVA followed by Tukey test: indication of statistical difference as $^{*}p \leq 0.5$; $^{**}p \leq 0.01$; $^{***}p \leq 0.001$. (a) Significant $\neq$ C and (b) significant $\neq$ H. The results are shown as mean percentage $\pm$ standard deviation in relation to the endogenous control β-actin.

the ethanol improves the solubility of phenolic compounds and the water enhances the solute desorption (41). Our study showed that PJE promoted a lower weight gain which is in agreement with a previous study by Lamas et al. in aging mice. In addition, Baseggio and collaborators verified that jaboticaba peel extract administrated for 6 weeks, from 13 weeks of age, decreased body weight but not food intake in C57BL/6 mice (38). However, it should be taken into account that the administration period of the jaboticaba extract was carried out on older animals than those observed in the present study and the experimental model did not present PCa. Also, different studies showed that the 1%, 2% and 4% of the freeze-dried jaboticaba peel, added to the diet for 10 weeks, was not able to reduce weight gain and or food intake in the high-fat fed rats (11,12, 19). Thus, our findings suggest that PJE has the potential to be used as a therapeutic agent for prevention and treatment of overweight under PCa conditions.

Jaboticaba peel is pointed out as being an anthocyanin source, such as cyanidin-3-O-glucoside, and several studies have evaluated the effects of anthocyanin rich fruit ingestion on obesity comorbidities (11, 16, 42). Interestingly, IR and glucose intolerance improvement, after PJE intake, only occurred in mice that consumed a normolipid diet. On the other hand, fasting-plasma glucose levels decreased in the HFD-fed mice. Lamas and collaborators also observed that PJE treatment improved glucose intolerance and IR both in aged and in high-fat-fed aged mice, but that the metabolic response was more effective when severe damages were seen in the HFD group, where the higher PJE dose (30) was administered. It is important to consider that IR is related to several important mechanisms involved in cancer progression such as inflammation and cell growth (43). For example, high-fat fed TRAMP mice exhibited a serum insulin growth factor 1 (IGF-1) higher than in the normolipid diet fed TRAMP mice (44). Considering this fact, we suggest that the high-fat-fed PCa model presents alterations in which the PJE dose was not able to show beneficial effects for glucose intolerance and IR. Nevertheless, our results suggested that PJE could interfere in a good way in the insulin metabolism in the PCa model.

Although there is no sign of dyslipidemia in this study, the PJE increased the HDL-cholesterol plasma levels in HFD fed mice. Our results are in agreement with previous studies that demonstrated an HDL-cholesterol increase in HFD fed rodents after freeze-dried jaboticaba peel or jaboticaba peel extract intake (11,

30). Clinical studies reported that anthocyanin intake increased HDL-cholesterol without any effect on total cholesterol in dyslypidemic patients (45). Furthermore, epidemiological analyses revealed that the high circulating cholesterol levels have been associated with PCa risk diagnosis and progression (7, 46). Due to the fact that, high HDL-cholesterol serum levels have been linked to lower risk of PCa in smokers between 50 and 69 years old, we suggest that the increase of HDL-cholesterol, which was observed in the present study, is an important preventive effect of the PJE against this disease (47).

It is known that the liver plays a central role in lipid metabolism, acting in the circulating lipoprotein synthesis and secretion (48). In the present study, we observed that HFD increased the hepatocellular lipid accumulation. And also, PJE treatment decreased lipid accumulation in the liver. In addition, we verified an insulin receptor substrate 1 (pIRS1) and TNFα increase after HFD intake and a reduction of these molecules was verified after PJE treatment, particularly after the hyperlipidic diet intake by TRAMP mice. And also, a peroxisome proliferator-activated (PPARγ) decrease after PJE treatment. PPARs are a superfamily of nuclear hormones that can modulate the gene expression involved in lipid and glucose metabolism (49). It is know that PPARα promotes fat utilization and the PPARγ activation promotes its storage (50). Under normal conditions, the liver has low PPARγ concentrations and high hepatic levels of PPARs are related to increased lipogenesis in obese patients with nonalcoholic fatty liver (NAFLD) (51,52). The PPARγ liver levels did not differ between normolipidic and HFD groups. This could be due to the fact that we did not observe a dyslipidemia or NAFLD in the TRAMP model after HFD consumption.

According to our results, the PJE treatment improves the liver fat metabolism by decreasing PPARγ which is in agreement with Batista and collaborators (35) where Swiss mice fed with HFD showed small triglyceride droplets and a high expression of the PPARα mRNA in the liver. Despite this, these authors also observed that freeze-dried jaboticaba peel treatment decreased these parameters and increased serum HDL-cholesterol. In addition, anthocyanin-rich purple-corn extract reduced the lipid accumulation, TNFα and PPARγ protein levels in assays using adipocytes (18). On the other hand, a previous study from our group demonstrated that PPARγ increased in high-fat-fed aged mice after PJE administration (30).

The insulin receptor 1 (IRS-1) plays a critical role in the activation of liver insulin signaling together with insulin receptor 2 (IRS-2) (53). IRS tyrosine-phosphorylation is necessary for insulin signaling propagation in the cell and its decrease is correlated with IR. Recently, the literature demonstrated that polyphenols could increase the pIRS-1 protein levels in the liver in high-fat-fed mice (54). However, Honma and collaborators studied the IRS-1 and IRS-2 expression in the liver of patients with NAFLD and steatosis (55) and demonstrated that there were no changes in IRS-1 mRNA, in contrast to IRS-2, which demonstrated a significant decrease (55). In addition, the literature showed that IRS1 and IRS2 roles in insulin signaling could be tissue specific (53). According to these authors, mice with IRS1 mutation presented a systemic IR, interfering in the insulin signaling in the liver, skeletal muscle and adipose tissue (53). However, in the IRS2 mutation mice, IR occurred primarily in the liver through IRS2 signaling, which demonstrated its importance in this pathway (56). Thus, taking into consideration the results above-mentioned, we suggest that systemic IR improvement after PJE treatment could not be linked just to pIRS1 liver levels and lipid accumulation decreased which were observed in this study, but could be linked to other insulin receptors. However, more studies are required regarding IR signaling in the liver, particularly in mice, which have PCa.

It is well established that anti-inflammatory cytokines produced by adipose tissue in obesity contribute to systemic and hepatic TNFα increase (30, 50). Previous authors observed that high TNFα plasma levels are related to a high degree of liver fibrosis in obese patients with NAFLD (50). The TNFα cytokine has been reported to be an important IR regulator, because of its ability to induce IRS-1 serine phosphorylation and decrease its affinity for the insulin receptor (57). In addition, a study by our research group showed the PJE anti-inflammatory effect, also reducing liver TNFα, is an important aspect associated to its capacity to prevent hepatic steatosis in high-fat-fed aged mice. In agreement with our results, HFD consumption led to an important inflammatory marker increase in the liver and PJE administration was able to prevent this HFD deleterious effect, possibly preventing lipid and insulin metabolism alterations.

5. Conclusion

Considering the relationship between overweight and PCa progression, our results highlighted diet interference in the systemic balance of the organism in the PCa model. Thus, our results emphasized that PJE

treatment demonstrated protective effects in weight gain and IR also in the TRAMP mice model. In conclusion, PJE treatment promoted protective effects in the insulin and glucose metabolism and liver imbalance caused by HFD intake in the PCa model, suggesting that it may be a good alternative against metabolic disorders, considering the association of overweight and prostatic cancer.

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