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ORIGINAL ARTICLE

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Brazilian berry extract (*Myrciaria jaboticaba*): A promising therapy to minimize prostatic inflammation and oxidative stress

Celina A. Lamas PhD[1] | Larissa A. Kido PhD[1,2] | Túlio A. Hermes PhD[3] | Ellen Nogueira-Lima MD[1] | Elaine Minatel PhD[1] | Carla B. Collares-Buzato PhD[4] | Mário R. Maróstica Jr. PhD[2] | Valéria H. A. Cagnon PhD[1]

[1]Department of Structural and Functional Biology, Institute of Biology, University of Campinas, São Paulo, Brazil

[2]Department of Food and Nutrition, School of Food Engineering, University of Campinas, São Paulo, Brazil

[3]Department of Morphology and Physiology, ABC Medical School, Santo Andre, São Paulo, Brazil

[4]Department of Biochemistry and Tissue Biology, Institute of Biology, University of Campinas, São Paulo, Brazil

Correspondence
Valéria H. A. Cagnon, PhD, Department of Structural and Functional Biology, Institute of Biology, University of Campinas (UNICAMP), P.O. Box 6109, Campinas, São Paulo 13083-65, Brazil.
Email: quitete@unicamp.br

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Abstract

Background: Brazilian berry is a fruit popularly known as "Jaboticaba," rich in bioactive compounds with antioxidant and anti-inflammatory properties. Senescence and overweight are increasing worldwide and are considered risk factors to prostatic pathogenesis mainly due to oxidative and inflammatory processes induction. Thus, this study aimed to evaluate the effect of two increasing doses of the patented jaboticaba peel extract (PJE) on oxidative-stress and inflammation in the prostate of aging or high-fat-fed aging mice.

Methods: PJE and/or high-fat diet (HFD) treatments started with 11-month-old mice and lasted 60 days. The levels or the immunoexpression of different inflammatory (nuclear factor κB [NFκB], CD3+, cyclooxygenase 2 [COX-2], toll-like receptor 4 [TLR4], phosphorylated signal transducers and activators of transcription 3 [pSTAT-3], tumor necrosis factor α [TNF-α], interleukin 6 [IL-6], and IL-1β) and oxidative-stress (catalase, superoxide dismutase 2 [SOD2], glutathione reductase [GSR], reduced glutathione, and glutathione peroxidase 3 [GPx3]) related molecules were analyzed by western-blotting, immunohistochemistry, and enzyme-linked immunosorbent assays.

Results: Both PJE doses reduced the levels of oxidative-stress-related molecules (GPx3, GSR, catalase), lipid peroxidation (4-hydroxynonenal), inflammatory mediators (COX-2, TNF-α, and pSTAT-3) and CD3+ T cells number, which were associated with the maintenance of the glandular morphological integrity in aging and HFD-fed-aging mice. Nevertheless, only the high PJE dose reduced the NFκB and TLR4 levels in aging mice; and SOD2, IL-6, and IL-1β levels in HFD-aging mice. Aging itself promoted an oxidative inflammation in the prostate, interfering in the levels of the different oxidative-stress, lipid peroxidation, and inflammatory mediators evaluated, in association with high incidence of prostate epithelial and stromal damages. The HFD intake intensified aging alterations, showing an unfavorable prostatic microenvironment prone to oxidative and inflammatory damages.

Conclusions: PJE exerted a dose-dependent effect controlling inflammation and oxidative-stress in aging and HFD-fed aging mice prostate. This fact contributed to prostate microenvironment balance recovery, preserving the tissue architecture of this gland. Thus, the PJE emerges as a potential therapy to prevent inflammation and oxidative stress in the prostate.

KEYWORDS

aging, bioactive compounds, obesity, overweight, polyphenols

1 | INTRODUCTION

Aging is inherent process in human beings, being one of the major risk factors for prostate cancer.[1,2] The number of elderly people has increased in the world as well as the incidence of senescence consequences for human health.[3,4] Associated with this, a tendency to increase obesity during late life has been registered, making these important topics a concern.[3] Both aging and obesity seem to be directly involved in prostate pathogenesis, mainly by inducing oxidative stress, reactive oxygen species (ROS) accumulation, DNA damages, and inflammatory processes.[5-9] Richie et al[7] described alterations in glutathione cycle molecules during aging, and Colado-Velazquez et al[10] have observed that obesity modified total superoxide dismutase (SOD) and catalase activities in rodent prostate. These alterations predisposed the gland to an oncogenic phenotype by interfering with proliferative, inflammatory, and apoptotic pathways, which suggests they play an important role in prostate injury and malignance.[7,9-11]

The term oxidative inflammation has emerged to explain the cross talk between these processes and their influences in age- or obesity-related disorders.[4,12] The nuclear factor κB (NFκB) has been highlighted as a key mediator of chronic oxidative inflammation in the prostate.[6,8] This molecule is involved in the expression of genes related to both oxidative and inflammatory pathways, stimulating the expression of downstream proinflammatory mediators such as interleukins (IL), toll like receptors (TLR), and cyclooxygenase 2 (COX-2).[13-15] Thus, controlling the NFκB action could be a relevant target to reduce inflammatory oxidative damages caused by aging or obesity in this gland.

In this context, antioxidant protection seems to be a promissory alternative to increase longevity and provide better life quality to the elderly. The Brazilian Berry (*Myrciaria jaboticaba* (Vell.) Berg) is a typical fruit from the country, popularly known as "Jaboticaba."[16] Studies demonstrated that jaboticaba peel has bioactive compounds that exert in vivo and in vitro antioxidant effects, besides a positive effect on metabolic parameters altered by obesity.[16-21] A recent study by our research group, regarding the development of the patented jaboticaba peel extract (PJE), revealed it has a broader spectrum of bioactive compounds and a high in vitro antioxidant activity compared to other natural extracts previously reported.[22] Among the bioactive molecules found in the PJE, we highlight the presence of gallic acid, epicatechin, anthocyanins (cyanidine and delfinidine), kaempferol hexoside, chlorogenic acid, ellagic acid, quercetin, naringenim, rutin, and ascorbic acid.[22,23] The anti-inflammatory and antiobesity actions of the PJE have been previously described in aging or high-fat-fed-aging mice. In these experimental models, this extract reduced the weight gain, improved insulin sensibility, prevented hepatic steatosis, besides reducing the hepatic levels of COX-2 and tumor necrosis factor α (TNF-α).[22] Recently, Lamas et al[24] showed the PJE capacity to interfere in angiogenesis and hormonal pathways in the prostate, reducing the frequency of prostatic intraepithelial neoplasia (PIN) and well-differentiated adenocarcinoma foci in aging or high-fat-fed aging mice. Interestingly, in this study the PJE treatment also reduced the frequency of inflammatory infiltrates in the prostate, prompting us to investigate further the possible effect of this extract on inflammatory mediators.

Therefore, the aim of this study was to evaluate the PJE effect on inflammation and oxidative stress in the prostate of aging or high-fat-fed aging mice. To accomplish this, we quantified the level of the endogenous antioxidant molecules and lipid peroxidation, besides the levels of NFκB and other inflammatory molecules related to its signaling pathway in the prostate of aging and high-fat-fed aging mice.

2 | MATERIAL AND METHODS

2.1 | Patented jaboticaba peel extract

The protocol used to prepare the PJE was patented.[25] The PJE method of preparation consisted of the mixture of freeze-dried *Myrciaria cauliflora* (Vell. Berg) peel in an ethanol, followed by the solvent removal.[22,25] Previous studies by our research group, focusing on the development and characterization of the PJE, verified that PJE has a total phenolic content of 121.0 mg GAE/g, a total monomeric anthocyanins of 1381 mg cyd 3-glu 100 g^{-1}, a total flavonoids content of 24.5 mg CAT g^{-1}, 13.3 mg/g cyanidin-3-*O*-glucoside, 1.4 mg/g delphinidin-3-*O*-glucoside, 0.2 mg/g ellagic acid, 0.02 mg/g rutin, and 0.02 mg/g gallic acid.[22-24] In addition, the following bioactive compounds were also identified in PJE, considering the fragmentation pattern of the analytical standard: HHDP-galloylglucose, bis-HHDP-glucose (casuariin), bis-HHDP-glucose isomer (pedunculagin), HHDPgalloylglucose isomer, (−)-epicatechin, galloyl-bis-HHDPglucose (casuarinin), galloyl-bis-HHDP-glucose (casuarictin), HHDP-digalloylglucose (tellimagrandin I), kaempferol hexoside, chlorogenic acid, HHDP-trigalloylglucose (tellimagrandin II), pentagalloyl hexose, myricetin-rhamnoside, quercetin-3-rhamnoside

(quercitrin), quercetin, and naringenin.[22,24] Furthermore, the PJE demonstrated a high in vitro antioxidant capacity, representing 4250 μMTE^{-1} measured by ORAC and 6834.5 μMTE^{-1} measured by ABTS.[22]

The experimental doses used in this study were based on previous studies by our group, which confirmed the safety of its administration to mice, considering the PJE dose-dependent effect in blood and liver parameters related to the metabolism of mice.[22] Also, the experimental doses administered herein presented smaller amounts of phenolic compounds, almost 10 times less than those usually used in the literature.[16,20,22] The lowest PJE dose (2.9 g/kg) used herein has 4.65 mg cyanidin-3-*O*-glucoside, 0.5 mg delphinidin-3-*O*-glucoside, 0.07 mg ellagic acid, 0.007 mg rutin, and 0.006 mg gallic acid; and the highest PJE dose (5.8 g/kg) used herein showed twice the content of phenolic compounds described above for the lowest dose.[22,24]

2.2 | Animals and experimental procedures

A total of 70 male FVB mice were obtained from the Multidisciplinary Center for Biological Investigation on Laboratory Animal Science at the University of Campinas. All the experimental procedures were submitted to the Ethics Committee on Animal Use of the University of Campinas (Protocol: #3421-1). The animals were distributed into seven experimental groups (n = 10 mice/group) according to a specific experimental protocol (Figure 1). The mice were housed one per cage, under controlled lighting conditions (12 hours light-dark cycle), with food and drinking water ad libitum, during 60 days. The experimental period and the diets used in the present study were based on previous data by our group.[22,24]

At the end of the treatments, the animals were weighed on a semianalytical scale (Marte AS 5500; Marte, São Paulo, Brazil), anesthetized with xylazine hydrochloride (5 mg/kg intramuscular [IM]; König, Sao Paulo, Brazil) and ketamine hydrochloride (60 mg/kg IM; Fort Dodge, Iowa; EUA). The euthanasia was performed by increasing the anesthetic level and ventral prostate samples were removed. Subsequently, blood samples were collected by left ventricle puncture in tubes containing EDTA. The data regarding the mouse weight gain and food intake was previous described in Lamas et al.[22]

2.3 | Western blot analysis

Five ventral prostate samples per group were frozen at −80°C and used in this analysis. The prostate samples were homogenized in radioimmunoprecipitation assay (RIPA) buffer and the lysates were centrifuged at 14 000 rpm 4°C for 20 minutes. Subsequently the supernatants were collected and the protein amount was quantified by the Bradford assay reagent (Bio-Rad Laboratories, Hercules, CA). The same amount of protein (50 μg) per sample was separated by electrophoresis on sodium dodecyl sulfate polyacrylamide gels and electroblotted on nitrocellulose membranes. The nonspecific sites were blocked with bovine serum albumin solution (1%-5%) for 1 hour at room temperature. Then, the membranes were overnight incubated at 4°C with the following primary antibodies for the detection of catalase (C0979; Sigma-Aldrich), glutathione peroxidase 3 (GPx3; ab-104448; Abcam), glutathione reductase (GSR; SAB100980; Sigma-Aldrich), SOD2 (HPA001814; Sigma-Aldrich), 4-hydroxynonenal (4HNE; ab 20953; Abcam), COX-2 (sc-376861; Santa Cruz Biotechnology), transcription factor κB (NFκB; ab 13594; Abcam), phosphorylated signal transducers and activators of transcription 3 (pSTAT-3; 3E2; Cell Signaling Technology), toll-like receptor 4 (TLR4; sc-16240; Santa Cruz Biotechnology), TNF-α (ab 8348; Abcam), and β-actin (sc-81178; Santa Cruz Biotechnology). The membranes were subsequently incubated for 2 hours with a specific horseradish peroxidase-conjugated

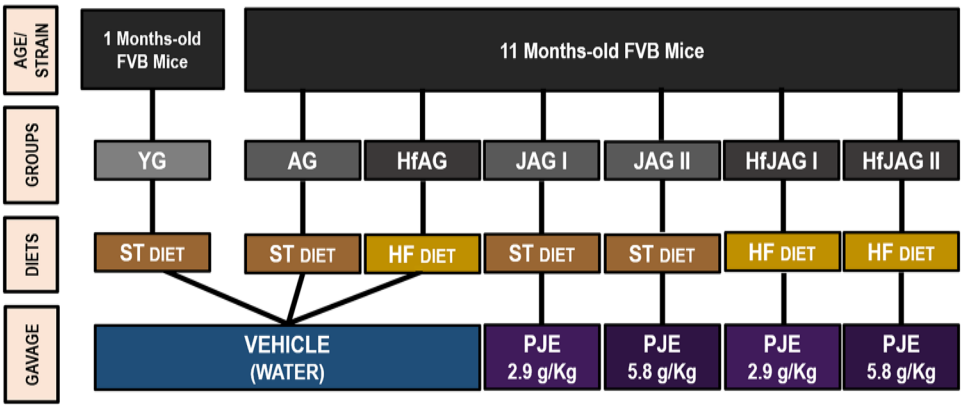

FIGURE 1 Distribution of the FVB mice in the different experimental groups. Experimental groups (n = 10 *per* group): YG, AG, HfAG, JAG I, JAG II, HfJAG I, and HfJAG II. Diets: ST (Nuvital CR1, Colombo, Parana, Brazil/composition: 22 g % protein, 53 g % carbohydrate, 4.5 g % lipid, and 2.9 kcal/g); HF (composition: 20 g % protein, 50 g % carbohydrate, 21 g % lipid, and 4.5 kcal/g). The PJE and the vehicle (water) were administered daily by gavage. All the experimental treatments lasted 60 days. AG, aging group; HF, high-fat diet; HfAG, high-fat diet and aging group; HfJAG I, high-fat diet, PJE dose I and aging mice; HfJAG II, high-fat diet, PJE dose II and aging mice; JAG I, aging and PJE dose I group; JAG II, aging and PJE dose II group; PJE, patented jaboticaba extract; ST, standard diet; YG, young group [Color figure can be viewed at wileyonlinelibrary.com]

secondary antibody: anti-mouse IgG (W4021; Promega Corporation), anti-rabbit IgG (W4018; Promega Corporation), or anti-goat IgG (14-14-06; KPL). Detection of the antigen-bound antibody was carried out for 5 minutes with SuperSignal West Pico Chemiluminescent Substrate (Thermo Fisher Scientific, Rockford, IL), followed by image capture using the G-Box Chemi associated with the GeneSnap (Syngene, Cambridge, UK) image acquisition software. The band quantification was performed by densitometry using the Image J software. The results were expressed as means of the ratio with β-actin band intensity.

2.4 | Total reduced glutathione (GSH) assay

The same lysates prepared for the Western blot analysis analysis were used in this assay. The total GSH content was evaluated according to the Ellman reaction using 5050-dithio-bis-2-nitrobenzoic acid, described by Anderson.[26] The intensity of the yellow color was read at 412 nm. The results were expressed as nmol/μg of protein.

2.5 | Morphology and immunohistochemistry analysis

Samples from the ventral prostate of five mice *per* group were fixed in Bouin for 24 hours. The prostate samples were rinsed with ethanol (70%), dehydrated, diaphanized, and embedded in plastic polymers (Paraplast Plus; St. Louis, MO; EUA). The samples were sectioned into 5 μm thick slices using a micrometer (Hyrax M60; Zeiss, Germany), placed on silanized slides and stained with hematoxylin and eosin or submitted to immunohistochemistry.

The COX-2 and CD3 immunohistochemistry was done in prostate sections, based on the on the study of Kido et al.[27] COX-2 mouse monoclonal primary antibody (sc-376861; Santa Cruz Biotechnology) or CD3 rabbit monoclonal primary antibody (ab16669; Abcam) were used, followed by counter-staining with Harris' hematoxylin. This analysis was also performed in sections where the incubation step with the primary antibody was omitted (negative controls).

The slides staining with hematoxylin and eosin were scanned to provide a broader visualization of the morphological pattern observed in the different experimental groups, especially in the regions of COX-2 and CD3+ immunoreactivity. The prostatic features which were observed, such as the PIN, well-differentiated adenocarcinoma, and epithelium atrophy were based on previous studies from our group and others.[24,27-29]

To evaluate COX-2 immunoreactivity, 10 random images were captured (×40 magnification) *per* animal using a Nikon Eclipse E-400 microscope (Nikon, Tokyo, Japan) coupled to the NIS-Elements software. The COX-2 immunoreactivity was quantified, using a grid with 700 intersections over the captured imagens by means of the Image Pro Plus program (modified from Silva et al[30]). The intersections corresponding to the positive COX-2 immunoreactivity was counted and discriminated as epithelium or stroma COX-2 immunostaining. The total number of points, quantified in the epithelium or stroma, was divided by the total number of intersections on the prostatic acini. The results were expressed as a percentage of prostate epithelium or stroma positive COX-2 immunostaining. The percentage of COX-2 total immunostaining was obtained by adding the percentage of COX-2 positively immunostained to the epithelium and stroma. Also, the COX-2 staining was scored and the frequency was graded on a 0 to 3 scale, according to the total percentage of positive staining areas: 0 (absence of immunostaining) 0%; 1 (weak immunostaining) 1% to 33%; 2 (moderate immunostaining) 34% to 66%; and 3 (intense immunostaining) more than 66%.[31] The percentage of total COX-2 positive immunostaining (0-100) was multiplied by the COX-2 staining score (0-3), resulting in an *H* score value adapted for cytoplasmic staining (0-300) (*H* score = 3 × percentage of total intense immunostaining + 2 × percentage of total moderate immunostaining + 1 × percentage of total weak immunostaining) (modified from Beeghly-Fadiel et al,[32] Jalalabadi et al,[33] and Cass et al[34]).

The quantification of CD3+ T cells were performed by the counting of CD3+ T cells in 10 imagens with ×100 magnification *per* animal, captured by a Nikon Eclipse E-400 microscope (Nikon, Tokyo, Japan) coupled to the NIS-Elements software. The number expressed from the CD3+ T cells was displayed as the mean value and standard deviation *per* experimental group (modified from Carvalho et al[35]).

2.6 | Enzyme-linked immunosorbent assay (ELISA)

Blood samples were centrifuged at 3000 rpm, 4°C, for 10 minutes. The plasma obtained was used to determine IL-1β and IL-6 concentration using commercial kit reagents (Novex, Invitrogen). The absorbance of the samples were read using the Multi-Mode Microplate Reader Model Synergy H1M (Bio-Tek Instruments) with specific wavelength for each molecule as described in the respective protocol of each assay.

2.7 | Statistical analyses

The statistical analyses of the immunohistochemistry quantifications, Western blot analysis, total GSH assay, and ELISA data were carried out by analysis of variance (one-way analysis of variance) followed by Tukey's multiple range posttest or Student's *t* test when appropriate. A significance limit of $P < .05$ was considered. All these data were expressed as means ± standard deviation.[36] Pearson's correlation test was performed to obtain correlation values (*r*) between the number of CD3+ T cells and the total frequency of COX-2 immunostaining.

3 | RESULTS

3.1 | PJE reduced the oxidative stress by improving the prostatic antioxidant response in aging and high-fat diet (HFD)-fed aging mice

The AG group showed reduced GSH and GPx3 levels, and increased GSR, catalase, and SOD2 levels compared to the YG group. The GSR

and catalase levels were higher in the HfAG group than in the AG group. Both PJE doses increased the GPx3 level as well as reduced the GSR, catalase and SOD2 levels in JAGI and JAGII groups compared to the AG group. Nevertheless, only the highest PJE dose, administered in the JAGII group, improved the GSH level in relation to the AG group. The HfJAGI and HfJAGII groups displayed high GPx3, as well as low GSR and catalase protein levels, compared to the HfAG group. The dose-dependent effect of PJE was evidenced since only the high PJE dose intake, in HfJAGII group, led to increased GSH and reduced SOD2 levels compared to the HfAG group. Moreover, increased GPx3 and reduced catalase levels were observed in the HfJAGII group in relation to the HfJAGI group (Figure 2A-F).

3.2 | PJE led to a decrease of lipid peroxidation in the prostate

The lipid peroxidation evaluated through 4HNE level increased in the AG group compared to the YG group. The HfAG group showed a higher 4HNE level than the AG group. Both PJE doses reduced the lipid peroxidation in JAGI and JAGII groups compared to the AG group. Also, the HfJAGI and HfJAGII groups presented a reduction of the 4HNE level in relation to the HfAG group (Figure 2G,H).

3.3 | PJE treatment downregulated prostatic proinflammatory hallmarks dose-dependently

The AG group showed higher protein levels of COX-2, NFκB, TLR4, TNF-α, and pSTAT-3 than the YG group. The levels of these proinflammatory mediators increased even more in the HfAG group in relation to the AG group. Both PJE doses reduced the COX-2, TNF-α, and pSTAT-3 levels in JAGI and JAGII groups compared to the AG group. The effect of PJE treatment in aging mice was dose-dependent as evidenced by the fact that only the JAGII group showed a decrease in NFκB and TLR4 levels compared to the AG group, as well as resulted in even lower pSTAT-3 levels compared to the JAGI group. The HfJAGI and HfJAGII groups displayed a decrease in COX-2, NFκB, TLR4, TNF-α, and pSTAT-3 levels in comparison with the HfAG group. Moreover, the HfJAGII group showed even lower TNF-α levels compared to the HfJAGI group (Figure 3A-F).

3.4 | PJE treatment reduced the COX-2 immunoexpression in association with lower CD3+ T cells number

The positive COX-2 immunostaining and CD3+ T cells were observed in the prostate from the different experimental groups, varying in terms of immunostaining frequency and number, respectively. Pearson's coefficient showed a high and positive correlation ($r = .9872$; $P < .0001$) between the total COX-2 immunostaining frequency and the number of CD3+ T cells.

The YG group demonstrated a weak COX-2 epithelial immunostaining mainly in regions characterized by a simple folded epithelium with columnar cells and basal nuclei. The stroma presented fibromuscular features distributed concentrically around the acini and weak positive immunostaining for COX-2 in the YG group (Figure 4A-C and Table 1). Few CD3+ T cells were observed in the stroma, close to the acini fibromuscular layer (Figure 4D and Table 1).

In contrast, both the AG and HfAG groups showed intense COX-2 immunolabeling in both the prostate epithelium and stroma. The increased COX-2 immunoexpression was associated with PIN regions and with well-differentiated adenocarcinoma foci. The PIN regions showed intense cell proliferation and stratification, as well as nuclear atypia. The well-differentiated adenocarcinoma foci identified were characterized by the basement membrane rupture and stromal invasion by epithelial cells. COX-2 immunostaining was also verified in proliferative inflammatory atrophy regions, characterized by reduced epithelial cell cytoplasm, and inflammatory infiltrates distributed throughout the glandular stroma. The glandular stroma was hypertrophic and hyperplastic in these groups, presenting a thick fibromuscular layer around the acini, which was intensely COX-2 immunostained (Figures 4E-G and 4I-K; Table 1) In addition, both the AG and HfAG groups showed a higher number of CD3+ T cells, than the YG group. These cells were organized in periacinar clusters (Figure 4H,L and Table 1).

Both groups treated with the PJE low dose (JAGI and HfJAGI), showed a moderate COX-2 immunolabeling, and the groups treated with the PJE high dose (JAGII and HfJAGII) showed a weak COX-2 immunolabeling. Low COX-2 immunostaining was predominant in the healthy morphological prostate regions, similar to the YG group, especially in JAGII and HfJAGII groups. (Figure 4M-O,Q-S,U-W,Y-AA and Table 1) The CD3+ T cell number diminished in the different PJE treated groups. In the JAGI and HfJAGI groups, the CD3+ T cells were organized in small clusters close to the prostate acini. In the JAGII and HfJAGII groups, the CD3+ T cells were predominantly isolated in the prostatic stroma. (Figure 4P,T,Y,BB and Table 1) A dose-dependent effect of the PJE was verified, due to the fact that the HfJAGII group demonstrated a lower COX-2 immunostaining frequency and a smaller number of CD3+ T cells than the HfAGI group (Table 1).

3.5 | PJE led to decreased IL-6 and IL-1β plasma levels

The AG group showed high plasma levels of IL-6 and IL-1β compared to the YG group. The level of these molecules in the HfAG group was as high as in the AG group. Both PJE doses reduced the plasma levels of these ILs in JAGI and JAGII groups in relation to the AG group. The dose-dependent effect of PJE was verified in the HfJAGII group, since only the treatment with the PJE high dose reduced the IL-1β and IL-6 plasma level compared to the AG group. Moreover, the IL-6

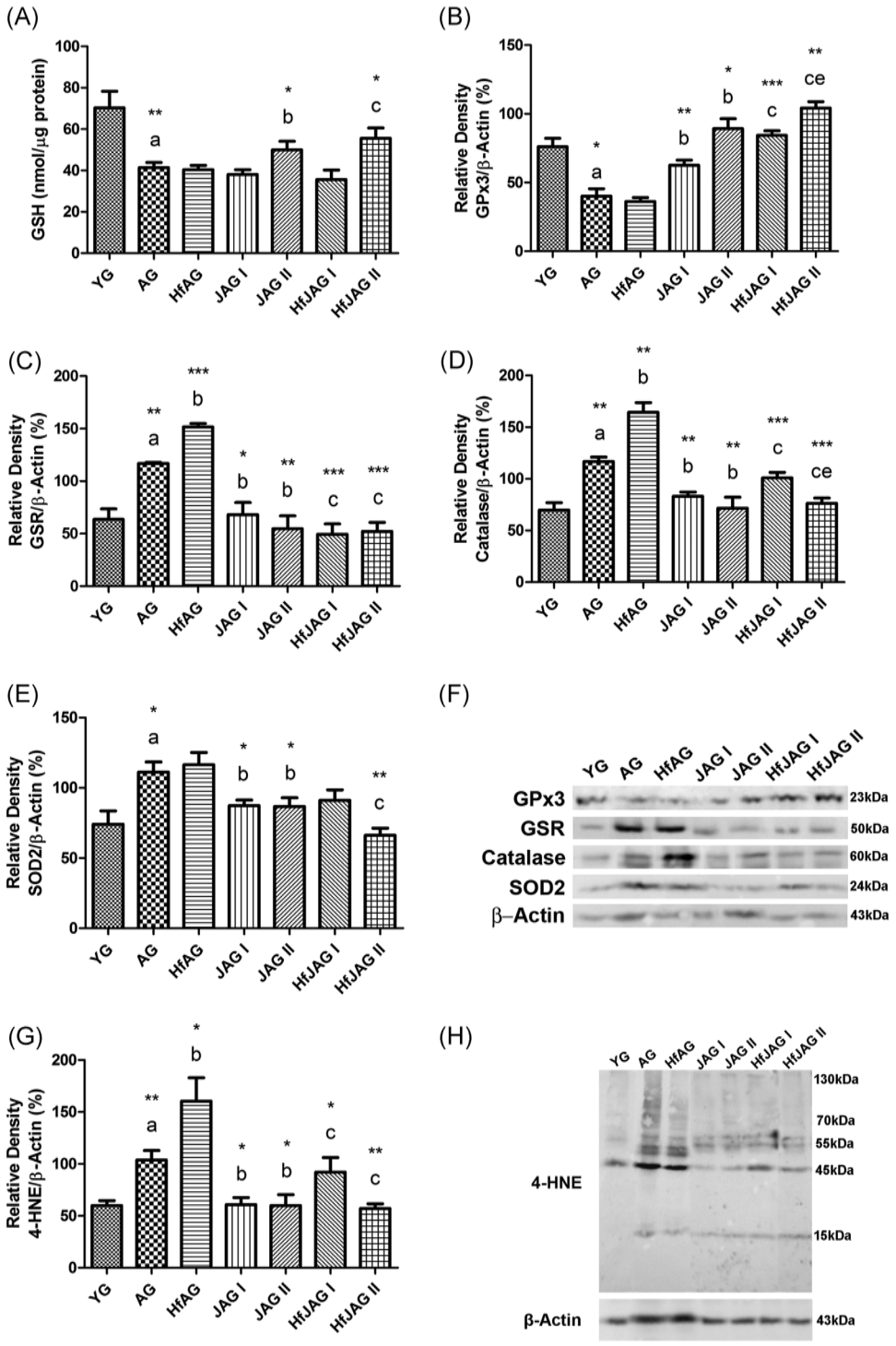

FIGURE 2 Evaluation of oxidative stress and lipid peroxidation biomarkers in the prostate ventral lobe. A, Determination of the GSH total level. B, Relative frequency of GPx3. C, Relative frequency of GSR. D, Relative frequency of catalase. E, Relative frequency of SOD2. F, Representation of GPx3, GSR, catalase, and SOD2 Western-Blotting analysis. G, Relative frequency of 4-HNE. H, Representation of 4-HNE Western-Blotting analysis. Significant differences: [a]relative to YG group; [b]relative to AG group; [c]relative to HfAG group; [e]relative to HfJAGI group. Considering: $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$. $n = 5$ mice *per* group. 4-HNE, 4-hydroxynonenal; GSH, reduced glutathione; GSR, glutathione reductase; GPx3, glutathione peroxidase 3; SOD2, superoxide dismutase 2

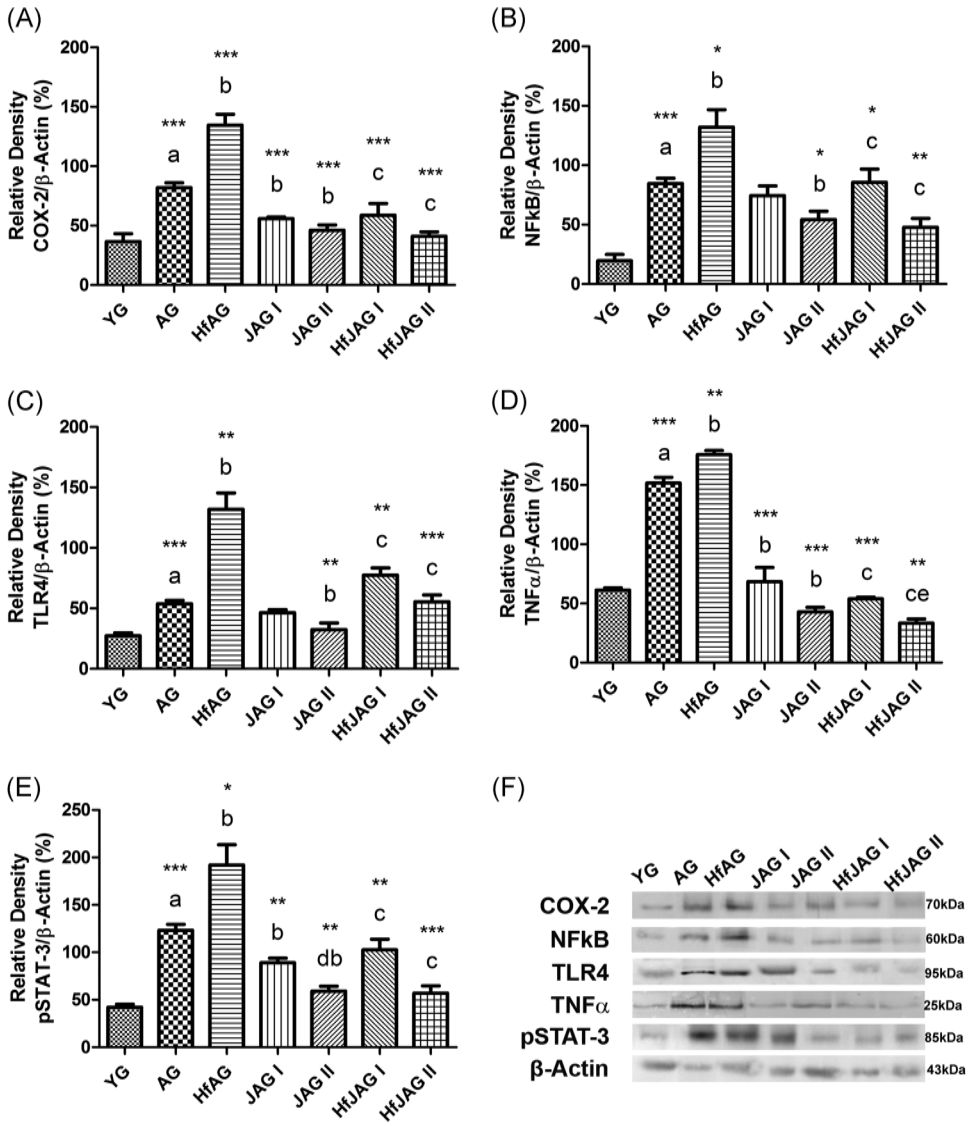

FIGURE 3 Western-blotting analysis of COX2, NFκB, TLR4, TNF-α, and pSTAT3 in homogenates of the prostate ventral lobe from mice of all experimental groups. Significant differences: [a]relative to YG group; [b]relative to AG group; [c]relative to HfAG group; [d]relative to JAGI group; [e]relative to HfJAGI group. Considering: $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$. $n = 5$ mice *per* group. COX2, cyclooxygenase-2; NFκB, nuclear transcription factor κB; pSTAT3, phosphorylated signal transducer and activator of transcription 3; TLR4, toll-like receptor 4; TNF-α, tumor necrosis factor α

plasma level was lower in the HfAGII group than in the HfJAGI group (Figure 5A,B).

4 | DISCUSSION

Oxidative stress is an essential factor for prostate cancer and pre-malignant lesion development, showing a direct association with the inflammatory process.[7,8,37] The present results indicated a dose-dependent positive effect of PJE treatment, diminishing a broad spectrum of inflammatory and oxidative molecules, contributing to prostate homeostasis and the morphological maintenance, which was altered by aging and/or HFD intake.

There are no studies in scientific literature regarding the antioxidant effect of jaboticaba on the prostate microenvironment. However, Batista et al[38] showed that the ingestion of HFD supplemented with jaboticaba peel for 10 weeks reduced lipid peroxidation and SOD activity, besides increasing GPx activity in the mouse liver. In addition, Lenquiste et al[16] observed the re-establishment of catalase and GSH hepatic levels after jaboticaba peel tea ingestion by rats which were fed with HFD for 12 weeks. Our findings showed a systemic effectiveness of PJE, interfering in antioxidant molecules and in lipid peroxidation levels in the prostate of high-fat-fed aging mice, even after a low PJE dose and a reduced period of treatment. Taking into consideration, particularly, the HFD-fed aging mice, the high PJE dose showed a strong

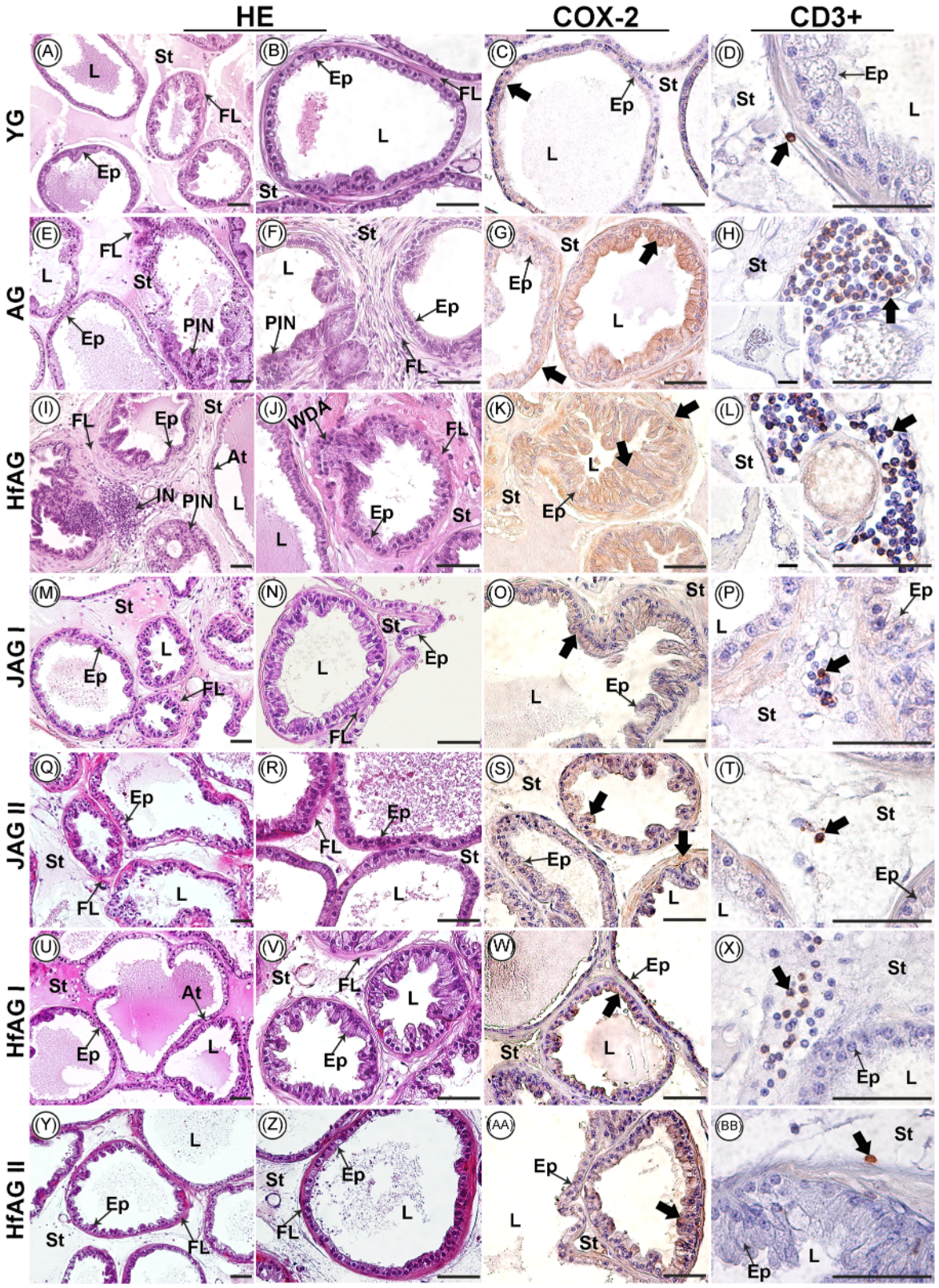

FIGURE 4 Photomicrographs of the prostate ventral lobe stained with HE, immunostained for COX2 or immunostained for CD3. Bar = 50 μm. Thick arrow, COX-2 or CD3+ immunostaining. The COX-2 and CD3+ immunostaining were quantified and/or classified according to the Table 1. *n* = 5 mice *per* group. At, epithelial atrophy; COX2, cyclooxygenase-2; Ep, epithelium; FL, fibromuscular layer; HE, hematoxylin and eosin; IN, inflammatory infiltrate; L, lumen; PIN, prostatic intraepithelial neoplasia; St, stroma; WDA, well-differentiated adenocarcinoma [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 1 COX-2 and CD3 positive immunoreactivity in the prostate from different experimental groups

Experimental groups	COX-2					CD3+
	Epithelium immunoreactivity, %	Stroma immunoreactivity, %	Total immunoreactivity, %	Score scale (0-3)	*H* score	Number of CD3+ T Cell
YG	21.9 ± 2.7	6.8 ± 1.8	28.7 ± 0.9	1	28.7	1 ± 0.7
AG	54.3 ± 14.3^{a,**}	28.9 ± 2.6^{a,***}	83.2 ± 13.5^{a,***}	3	249.9	11 ± 1.4^{a,***}
HfAG	62.5 ± 4.3^{a,**}	29.1 ± 4.1^{a,***}	91.6 ± 7.0^{a,***}	3	274.8	13 ± 2.1^{a,***}
JAG I	28.6 ± 2.3^{b,*}	11.5 ± 3.6^{b,***}	40.1 ± 5.9^{b,**}	2	82.2	4 ± 1.4^{b,***}
JAG II	20.3 ± 2.6^{b,**}	9.4 ± 2.4^{b,***}	29.7 ± 3.4^{b,***}	1	29.7	3 ± 1.4^{b,***}
HfJAG I	37.1 ± 5.0^{c,***}	14.2 ± 1.0^{c,***}	51.3 ± 6.0^{c,***}	2	102.6	7 ± 2.1^{c,***}
HfJAG II	22.6 ± 3.7^{c,e,***}	9.4 ± 4.3^{c,***}	32.0 ± 0.7^{c,e,***}	1	32.0	2 ± 1.2^{c,e,***}

Note: Score distribution according to predominant immunoreactivity range: 0 (0%), 1 (<33%), 2 (33%-66%), and 3 (>66%). The results of COX-2 epithelium, stroma and total immunoreactivity (%) and the CD3+ T cell number is displayed as mean values ± standard deviation. $n = 5$ mice *per* group.
Significant differences: [a]relative to YG group, [b]relative to AG group, [c]relative to HfAG group, [e]relative to HfJAGI group.
Considering: $^{*}p < .05$, $^{**}p < .01$, and $^{***}p < .001$.

interference, not only increasing GSH and GPx3 but also decreasing CAT and SOD2.

The amount of total phenolic and flavonoids, as well as the in vitro antioxidant activity of the PJE showed higher values than other jaboticaba extracts, utilizing different solvents for these the extraction and that of other purple fruits extracts.[16,38-41] Nogueira-Lima et al[21] suggested that ethanol-water solutions could be a good alternative for a solvent, considering it increases the phenolic compound solubility and the solute adsorption. In addition, the jaboticaba peel presents a great amount of flavonoids, and other phenolic compounds conjugated with glucosides or with a hydroxyl group, assisting in extraction using hydrophilic solvents.[21-23,42] Thus, the methodology applied in the PJE preparation provides high accessibility of phenolic compounds from the food matrix, leading to an extract with preserved amount and variety of bioactive compounds, which could have contributed, to the broad antioxidant and anti-inflammatory action.

Our results also confirmed that aging led to an increase in oxidative stress and consequently to high lipid peroxidation in the prostate, which further increased after the HFD intake. Prostate cancer cell cultures, treated with saturated fatty acid, showed increased catalase activity and lipid peroxidation levels, agreeing with the results observed herein during aging and HFD intake.[43] Catalase high levels are generally accompanied by high SOD levels, especially the manganese dependent SOD (MnSOD).[44] The MnSOD is the primary endogenous mitochondrial enzyme, encoded by SOD2, mainly related to upper levels of superoxide anions.[45,46] Studies regarding different SOD2 genotype have elucidated the fact that the Ala/Ala SOD2 genotype has considerable MnSOD activity, which is associated with reduced antioxidant status, increased DNA damage and the risk of aggressive prostate cancer.[45,46] A recent clinical trial on the effect of muscadine grape skin extract, which is rich in polyphenols, in men with biochemically recurrent prostate cancer, showed the supplementation increased the PSADT in Ala/Ala SOD2

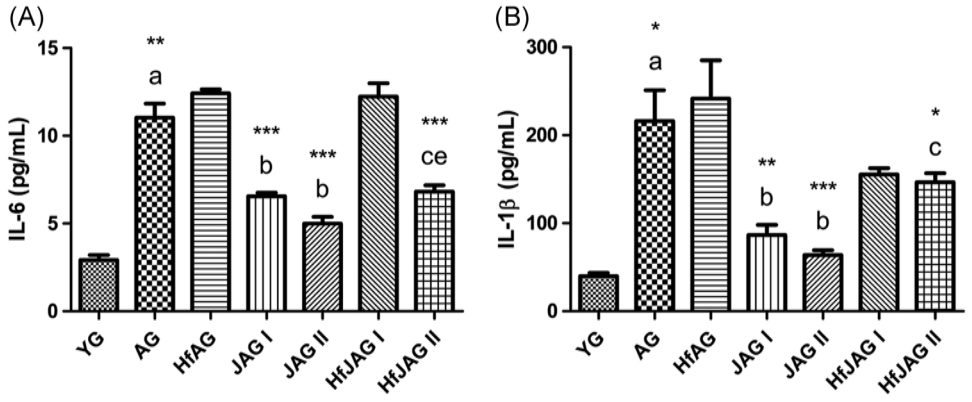

FIGURE 5 Enzyme-linked immunosorbent assay. A, Determination of IL-6 plasmatic concentration in the experimental groups. B, Determination of IL-1β plasmatic concentration in the experimental groups. Significant differences: [a]relative to YG group, [b]relative to AG group; [c]relative to HfAG group; [d]relative to JAGI group; [e]relative to HfJAGI group. Considering: $^{*}P < .05$, $^{**}P < .01$, and $^{***}P < .001$. $n = 5$ mice *per* group

genotype patients and reduced their basal oxidative state, suggesting effective therapy.[47] It is known polyphenols such as epicatechin, can inhibit the NADPH-oxidase enzyme, resulting in superoxide production reduction and oxidative stress decrease.[48] Also, the anthocyanin, present in a great majority in the PJE, has a phenolic structure with hydroxyl radical in specific locations which mediates and improves the scavenging of superoxide and singlet oxygen, inhibiting the peroxide formation and the free radical activity of these molecules.[49,50] Based on these data, we could suggest that the reduced SOD2, observed herein after the PJE treatment, could indicate that one of the PJE action pathways was the reduction of the production of superoxide anions in the prostate of aging or high-fat-fed aging mice. The low catalase levels after PJE treatment were expected as their action and tissue levels are associated with SOD level.

In contrast to the results herein, reduction in the total SOD and catalase activities was described in the prostate of obese rats after sucrose intake.[10] Nevertheless, the percentage of sucrose in the diet was high, providing a higher daily caloric amount than in the present study.[10] Pires et al[51] suggested that the increase or maintenance of the antioxidant enzyme activity is a process that occurs before its inhibition, as an attempt to reach the oxidative balance, varying according to the exposure time or type of factor/substance used to induce oxidative stress.[51] Thus, the use of more aggressive methods to trigger oxidative stress could lead to the inhibition of the endogenous antioxidant machinery, showing lower levels of the antioxidant molecules. Interestingly, Colado-Velazquez et al[10] verified the recovery of SOD and CAT activities and reduced prostate epithelium and stromal proliferation in aging animals that ingested a high-sucrose diet and a palm tree leaf extract, which is rich in bioactive compounds. Their results suggested that the antioxidant action of the bioactive compounds is not restrict to HFD intake. In fact, the ingestion of both sucrose and fat, or even aging, are associated with important hormonal alterations in the body, especially related to alterations in the testosterone levels, which is an important stimulator of ROS production, oxidative stress and prostatic damages.[10] Previous studies by our research group showed the PJE supplementation reduced the androgen and estrogen receptor levels, mainly through downregulation of aromatase, in the prostate of high-fat-fed aging mice. Thus, the PJE capacity to interfere in hormonal mediators could indicate a significant involvement in the modulation of the endogenous antioxidant machinery and in the oxidative stress reduction observed herein.

Moreover, we showed in the study herein that aging, associated or not with HFD intake, interfered with the glutathione antioxidant cycle. Sekine et al[52] verified that HFD intake induced GPx3 low levels in mouse prostate, which was especially related to the local increase of ROS levels and the amplification of oxidative-related cell/tissue damage. The relatively low GSH level is considered a consequence of its oxidation, suggesting oxidative stress onset, whereas high GSR levels during oxidative stress could be interpreted as a response to maintain the upper levels of the antioxidant glutathione, indicating an attempt to neutralize this stress.[53] Recently, Ishola et al[54] verified that the *Moringa oleifera* leaf extract, which is rich in quercetin and other flavonoids that are also present in the PJE, reduced the GSH and lipid peroxidation level in rats with benign prostatic hyperplasia, preserving the integrity of prostatic cells. The study of flavonoids showed they could modulate the g-glutamylcysteine synthetase and glutamate cysteine ligase enzymes which are directed related to the GSH synthesis.[51,55,56] These data contributed to our understanding that the improvement of GSH and GPx3 levels after the PJE treatment suggested the glutathione cycle was the main endogenous antioxidant tool stimulated in the prostate to promote hydrogen peroxide neutralization. The low GSR levels after the PJE treatment could be related to an attempt to reduce GSH and to recovery the natural oxidative levels in this gland. The PJE reducing lipid peroxidation in the prostate reinforced this premise since it represents an endpoint of oxidative injury.

Another novelty of our findings is that we indicated, for the first time in scientific literature that PJE reduced the chronic inflammatory process observed in the prostate of aging or HFD-fed aging mice, by means of reducing the levels of essentials inflammatory mediators. Previous studies have already demonstrated that the PJE or lyophilized jaboticaba peel reduced the IL-6, IL-1β, COX-2, and TNF-α levels in the liver of HFD-fed mice, showing that they had a relevant response reducing hepatic pathogenesis.[20,22] By and large, some authors believe that the antioxidant activity of bioactive compounds that lead to the reduction of oxidative stress, is one important action mechanisms in the downregulation of inflammatory mediators levels.[57,58] Reinforcing this fact, Henning et al[59] verified that the oxidative stress reduction, due to the polyphenol epigallocatechin ingestion, which was also identified in the PJE used herein, was associated with a decrease in the inflammatory process; angiogenesis; and tumor growth in a xenograft prostate cancer mouse model.

Also, several studies demonstrated that NFκB activation could either stimulate or be stimulated by ROS in obese animals, resulting in oxidative inflammation.[5,10,13,37] The NFκB acts as a key molecule considering the maintenance of inflammatory state, stimulating downstream inflammatory mediators, such as IL-1β, IL-6, and TNF-α, besides interfering in genes related to cell proliferation, survival, and invasion.[13] Liu et al[14] observed that COX-2 expression, regulated by NFκB binding, promoted a chronic inflammation via the TLR4-derived signaling pathway in prostate cancer cell culture treated with saturated fatty acids.[6,14,60,61] In addition, the NFκB and STAT-3 signaling crosstalk, keeps the NFκB permanently activated, sustaining prostatic chronic inflammation, the lymphocyte accumulation; and the neoplastic lesion development after HFD intake by adult mice.[6,13,62] Recently, Ignacio et al[63] proposed that reduced NFκB levels by muscadine grape skin extract supplementation were associated with cell-cycle arrest, reducing the tumor proliferative characteristics in a xenograft prostate cancer. According to Davalli et al[64] green tea-derived polyphenols reduced NFκB binding to DNA, decreasing the expression of its active subunit, as well as the activation of downstream molecules and proliferative processes in prostatic cancer cell culture. Moreover, the study of renal ischemia and melanoma models clarified that interfering in the TLR4/NFκB pathway is an important strategy to reduce tissue damages, thereby reducing IL-1β, IL-6, and TNF-α serum levels.[57,65]

A positive correlation of CD3 + T lymphocytes cell and COX-2 expression have been described in canine breast cancer, mainly due to prostaglandins secretion by lymphocytes.[35] According to these authors, increased CD3+ T cell/COX-2 were correlated with increased tumor aggressiveness, high rates of tumor inflammation and necrosis, as well as to metastasis development. Yang et al[66] suggested the number of CD3+ T cells and other lymphocytes could be used to evaluate the prostate cancer progress and prognosis, considering a lower expression of CD3+ T cells at the end of radiotherapy treatment of men with prostate cancer. The present results showed the decrease in CD3+ T lymphocytes after PJE treatment was also positively correlated with low COX-2 immunoexpression. These results suggest that the CD3+ T cell decrease could have contributed, at least in part, to reduce the inflammatory signaling mediator level.

Generally speaking, we believe the antioxidant properties of the PJE reduced the oxidative stress in the prostate of aging or high-fat-fed aging mice, negatively stimulated the intraprostatic inflammation in different signaling pathways, which was proportional to the oxidative damage. In addition, the PJE capacity to reduce NFκB represents a promissory step to minimize the generalized chronic prostatic oxidative inflammation due to the fact that NFκB can regulate the level of downstream inflammatory mediators and interfere in different inflammatory pathways in the prostate. Thus, we encourage the development of future studies focusing on specific PJE action mechanisms involved with NFκB to clarify this perspective.

The study of polyphenols absorption, excretion, and tissue accumulation could contribute toward the understanding of their therapeutic effect.[67] The anthocyanins, an important class of polyphenols present in the PJE, are rapidly absorbed in the small intestine, due to the presence of the β-glucosidase enzyme in the microbiota.[67,68] Also, they are easily degraded, reaching a maximal concentration in feces 24 hours postconsumption.[67,69,70] When anthocyanins reach the microbiota, occur their biotransformation into metabolites, which are essential for its absorption and biological activity.[67] Felgines et al[71] verified that the anthocyanins from blackberry, in the form of native anthocyanins or as anthocyanin metabolites (cyaniding monoglucuronide, methylated forms of cyanidin 3-glucoside and cyanidin monoglucuronide, and aglycones) can be found in the plasma and in the prostate, in greater amounts than in testis, heart and adipose tissue. Also, one of the ellagic acid metabolites, urolithin-A, was identified in the mouse prostate after Pomegranate extract supplementation.[72] It is unclear how these natural polyphenols or metabolites concentrate in the prostate, however these data suggests this event could be related to their positive action in this gland.

5 | CONCLUSION

This study brings significant implications about the jaboticaba (Brazilian berry) extract effect on prostatic oxidative inflammation. The antioxidant and anti-inflammatory actions of this extract represents a new promissory adjutant approach to reestablish the prostatic microenvironment balance altered by aging associated or not with HFD intake, suggesting the maintenance of a normal crosstalk between the prostatic epithelium and stroma. The jaboticaba extract antioxidant and anti-inflammatory effects were dose-dependent; especially considering the tissue responses in a drastically damaged prostatic microenvironment after the HFD intake, suggesting its effectiveness could be related to the tissue injury level.

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CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest.

ORCID

Valéria H. A. Cagnon http://orcid.org/0000-0001-5331-7376

REFERENCES

1. Li J, Djenaba JA, Soman A, Rim SH, Master VA. Recent trends in prostate cancer incidence by age, cancer stage, and grade, the United States, 2001-2007. *Prostate Cancer*. 2012;2012:691380.
2. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394-424.
3. WHO. Global health and aging. 2011. pp. 1-32.
4. De la Fuente M, Miquel J. An update of the oxidation-inflammation theory of aging: the involvement of the immune system in oxi-inflamm-aging. *Curr Pharm Des*. 2009;15(26):3003-3026.
5. Vykhovanets EV, Shankar E, Vykhovanets OV, Shukla S, Gupta S. High-fat diet increases NF-kappaB signaling in the prostate of reporter mice. *Prostate*. 2011;71(2):147-156.
6. Shankar E, Bhaskaran N, MacLennan GT, Liu G, Daneshgari F, Gupta S. Inflammatory signaling involved in high-fat diet induced prostate diseases. *J Urol Res*. 2015;2(1):1018.
7. Richie JP Jr., Das A, Calcagnotto AM, Aliaga CA, El-Bayoumy K. Age related changes in selenium and glutathione levels in different lobes of the rat prostate. *Exp Geront*. 2012;47(3):223-228.
8. Kido LA, Montico F, Vendramini-Costa DB, Pilli RA, Cagnon VHA. Goniothalamin and celecoxib effects during aging: targeting pro-inflammatory mediators in chemoprevention of prostatic disorders. *Prostate*. 2017;77(8):838-848.
9. Ahmed Amar SA, Eryilmaz R, Demir H, Aykan S, Demir C. Determination of oxidative stress levels and some antioxidant enzyme activities in prostate cancer. *The Aging Male*. 2019;22(3):198-206.
10. Colado-Velazquez J III, Mailloux-Salinas P, Medina-Contreras J, Cruz-Robles D, Bravo G. Effect of *Serenoa Repens* on oxidative stress, inflammatory and growth factors in obese wistar rats with benign prostatic hyperplasia. *Phytotherapy research: PTR*. 2015;29(10):1525-1531.
11. Vital P, Castro P, Ittmann M. Oxidative stress promotes benign prostatic hyperplasia. *Prostate*. 2016;76(1):58-67.

12. Garrido A, Cruces J, Ceprian N. Oxidative-inflammatory stress in immune cells from adult mice with premature aging. *Int J Mol Sci*. 2019;20(3):769-792.
13. Shankar E, Vykhovanets EV, Vykhovanets OV, et al. High-fat diet activates pro-inflammatory response in the prostate through association of Stat-3 and NF-kappaB. *Prostate*. 2012;72(3):233-243.
14. Liu J, Hu S, Cui Y, et al. Saturated fatty acids up-regulate COX-2 expression in prostate epithelial cells via toll-like receptor 4/NF-kappaB signaling. *Inflammation*. 2014;37(2):467-477.
15. Pasparakis M. Regulation of tissue homeostasis by NF-kappaB signalling: implications for inflammatory diseases. *Nat Rev Immunol*. 2009;9(11):778-788.
16. Lenquiste SA, Marineli RdS, Moraes ÉA, Dionísio AP, Brito ESd, Maróstica MR. Jaboticaba peel and jaboticaba peel aqueous extract shows in vitro and in vivo antioxidant properties in obesity model. *Food Res Int*. 2015;77:162-170.
17. Lenquiste SA, de Almeida Lamas C, da Silva Marineli R, et al. Jaboticaba peel powder and jaboticaba peel aqueous extract reduces obesity, insulin resistance and hepatic fat accumulation in rats. *Food Res Int*. 2018;120:880-887.
18. Leite-Legatti AV, Batista ÂG, Dragano NRV, et al. Jaboticaba peel: antioxidant compounds, antiproliferative and antimutagenic activities. *Food Res Int*. 2012;49(1):596-603.
19. Lenquiste SA, Batista ÂG, Marineli RdS, Dragano NRV, Maróstica MR. Freeze-dried jaboticaba peel added to high-fat diet increases HDL-cholesterol and improves insulin resistance in obese rats. *Food Res Int*. 2012;49(1):153-160.
20. Dragano NRV, Marques AC, Cintra DEC, et al. Freeze-dried jaboticaba peel powder improves insulin sensitivity in high-fat-fed mice. *Br J Nutr*. 2013;110(3):447-455.
21. Nogueira-Lima E, Lamas CA, Baseggio AM, do Vale JSF, Marostica Junior MR, Cagnon VHA. High-fat diet effects on the prostatic adenocarcinoma model and jaboticaba peel extract intake: protective response in metabolic disorders and liver histopathology. *Nutr Cancer*. 2019;7:1-12.
22. Lamas CA, Lenquiste SA, Baseggio AM, et al. Jaboticaba extract prevents prediabetes and liver steatosis in high-fat-fed aging mice. *J Funct Foods*. 2018;47:434-446.
23. Baseggio AM, Nuñez CEC, Dragano NRV, et al. Jaboticaba peel extract decrease autophagy in white adipose tissue and prevents metabolic disorders in mice fed with a high-fat diet. *PharmaNutrition*. 2018;6(4):147-156.
24. Lamas CA, Kido LA, Montico F, Collares-Buzato CB, Maróstica MRJ, Cagnon VHA. A jaboticaba extract prevents prostatic damage associated with aging and high-fat diet intake. *Food & function*. 2020; 26(11):1547-1559.
25. Maróstica Junior MR, Quitete VHAC, Lamas CA, et al. Instituto Nacional da Propriedade Industrial. Composition comprising jabuticaba extract, and its use. BR 10201700546242017.
26. Anderson ME. Determination of glutathione and glutathione disulfide in biological samples. *Methods Enzymol*. 1985;113:548-555.
27. Kido LA, Montico F, Sauce R, et al. Anti-inflammatory therapies in TRAMP mice: delay in PCa progression. *Endocr Relat Cancer*. 2016; 23(4):235-250.
28. De Marzo AM, Marchi VL, Epstein JI, Nelson WG. Proliferative inflammatory atrophy of the prostate: implications for prostatic carcinogenesis. *Am J Pathol*. 1999;155(6):1985-1992.
29. Roy-Burman P, Wu H, Powell WC, Hagenkord J, Cohen MB. Genetically defined mouse models that mimic natural aspects of human prostate cancer development. *Endocr Relat Cancer*. 2004;11(2): 225-254.
30. Silva RF, Nogueira-Pangrazi E, Kido LA, et al. Nintedanib antiangiogenic inhibitor effectiveness in delaying adenocarcinoma progression in Transgenic Adenocarcinoma of the Mouse Prostate (TRAMP). *J Biomed Sci*. 2017;24(1):31.
31. Kido LA, Hetzl AC, Candido EM, Montico F, Lorencini RM, Cagnon VH. Antiangiogenic and finasteride therapies: responses of the prostate microenvironment in elderly mice. *Life Sci*. 2014;106(1-2):58-70.
32. Beeghly-Fadiel A, Wilson AJ, Keene S, et al. Differential cyclooxygenase expression levels and survival associations in type I and type II ovarian tumors. *J Ovarian Res*. 2018;11(1):17.
33. Jalalabadi Y, Shirazi A, Ghavam-Nasiri MR, et al. Evaluating the expression of cyclooxygenase-2 enzyme by immunohistochemistry in normal and tumoral tissue before and after neoadjuvant chemoradiotherapy in patients with esophageal cancer in Khorasan Province. *J Cancer Res Ther*. 2018;14(3):509-515.
34. Cass JD, Varma S, Day AG, et al. Automated quantitative analysis of p53, Cyclin D1, Ki67 and pERK expression in breast carcinoma does not differ from expert pathologist scoring and correlates with clinicopathological characteristics. *Cancers*. 2012;4(3):725-742.
35. Carvalho MI, Pires I, Prada J, et al. High COX-2 expression is associated with increased angiogenesis, proliferation and tumoural inflammatory infiltrate in canine malignant mammary tumours: a multivariate survival study. *Veterinary and comparative oncology*. 2017;15(2):619-631.
36. Zar J. *Biostatistical Analysis*. New Jersey: Prentice Hall; 1999.
37. Valko M, Leibfritz D, Moncol J, Cronin MT, Mazur M, Telser J. Free radicals and antioxidants in normal physiological functions and human disease. *Int J Biochem Cell Biol*. 2007;39(1):44-84.
38. Batista ÂG, Lenquiste SA, Cazarin CBB, et al. Intake of jaboticaba peel attenuates oxidative stress in tissues and reduces circulating saturated lipids of rats with high-fat diet-induced obesity. *J Funct Foods*. 2014;6:450-461.
39. Ky I, Teissedre PL. Characterisation of Mediterranean grape pomace seed and skin extracts: polyphenolic content and antioxidant activity. *Molecules*. 2015;20(2):2190-2207.
40. Wang Y, Xiang L, Wang C, Tang C, He X. Antidiabetic and antioxidant effects and phytochemicals of mulberry fruit (*Morus alba* L.) polyphenol enhanced extract. *PLOS One*. 2013;8(7):e71144.
41. Mezni A, Aoua H, Khazri O, Limam F, Aouani E. Lithium induced oxidative damage and inflammation in the rat's heart: Protective effect of grape seed and skin extract. *Biomed Pharmacother*. 2017;95:1103-1111.
42. Beres C, Freitas SP, Godoy RLdO, et al. Antioxidant dietary fibre from grape pomace flour or extract: does it make any difference on the nutritional and functional value? *J Funct Foods*. 2019;56:276-285.
43. Rezende LP, Galheigo MRU, Landim BC, et al. Effect of glucose and palmitate environment on proliferation and migration of PC3-prostate cancer cells. *Cell Biol Int*. 2019;43(4):373-383.
44. Day BJ. Catalase and glutathione peroxidase mimics. *Biochem Pharmacol*. 2009;77(3):285-296.
45. Sutton A, Khoury H, Prip-Buus C, Cepanec C, Pessayre D, Degoul F. The Ala16Val genetic dimorphism modulates the import of human manganese superoxide dismutase into rat liver mitochondria. *Pharmacogenetics*. 2003;13(3):145-157.
46. Li H, Kantoff PW, Giovannucci E, et al. Manganese superoxide dismutase polymorphism, prediagnostic antioxidant status, and risk of clinical significant prostate cancer. *Cancer Res*. 2005;65(6):2498-2504.
47. Paller CJ, Zhou XC, Heath EI, et al. Muscadine grape skin extract (MPX) in men with biochemically recurrent prostate cancer: a randomized, multicenter, placebo-controlled clinical trial. *Clin Cancer Res*. 2018;24(2):306-315.
48. Fraga CG, Galleano M, Verstraeten SV, Oteiza PI. Basic biochemical mechanisms behind the health benefits of polyphenols. *Mol Aspects Med*. 2010;31(6):435-445.
49. Jang H, Ha US, Kim SJ, et al. Anthocyanin extracted from black soybean reduces prostate weight and promotes apoptosis in the prostatic hyperplasia-induced rat model. *J Agric Food Chem*. 2010;58(24): 12686-12691.
50. Wang SY, Jiao H. Scavenging capacity of berry crops on superoxide radicals, hydrogen peroxide, hydroxyl radicals, and singlet oxygen. *J Agric Food Chem*. 2000;48(11):5677-5684.

51. Pires VC, Gollücke AP, Ribeiro DA, Lungato L, D'Almeida V, Aguiar O Jr. Grape juice concentrate protects reproductive parameters of male rats against cadmium-induced damage: a chronic assay. *Br J Nutr*. 2013;110(11):2020-2029.
52. Sekine Y, Osei-Hwedieh D, Matsuda K, et al. High fat diet reduces the expression of glutathione peroxidase 3 in mouse prostate. *Prostate*. 2011;71(14):1499-1509.
53. Townsend DM, Tew KD, Tapiero H. The importance of glutathione in human disease. *Biomed Pharmacother*. 2003;57(3):145-155.
54. Ishola IO, Yemitan KO, Afolayan OO, Anunobi CC, Durojaiye TE. Potential of *Moringa oleifera* in the treatment of benign prostate hyperplasia: role of antioxidant defence systems. *Med Principles Prac*. 2018;27(1):15-22.
55. Cheng YT, Wu CH, Ho CY, Yen GC. Catechin protects against ketoprofen-induced oxidative damage of the gastric mucosa by up-regulating Nrf2 in vitro and in vivo. *J Nutr Biochem*. 2013;24(2):475-483.
56. Yang YC, Lii CK, Lin AH, et al. Induction of glutathione synthesis and heme oxygenase 1 by the flavonoids butein and phloretin is mediated through the ERK/Nrf2 pathway and protects against oxidative stress. *Free Radic Biol Med*. 2011;51(11):2073-2081.
57. Li Y-F, Tang L-P, He R-R, et al. Anthocyanins extract from bilberry enhances the therapeutic effect of pollen of *Brassica napus* L. on stress-provoked benign prostatic hyperplasia in restrained mice. *J Funct Foods*. 2013;5(3):1357-1365.
58. Qian Y, Chen Y, Wang L, Tou J. Effects of baicalin on inflammatory reaction, oxidative stress and PKDl and NF-kB protein expressions in rats with severe acute pancreatitis1. *Acta cirurgica brasileira*. 2018;33(7):556-564.
59. Henning SM, Wang P, Said J, et al. Polyphenols in brewed green tea inhibit prostate tumor xenograft growth by localizing to the tumor and decreasing oxidative stress and angiogenesis. *J Nutr Biochem*. 2012;23(11):1537-1542.
60. Sugar LM. Inflammation and prostate cancer. *Can J Urol*. 2006;13(1):46-47.
61. Zhang Q, Peng J, Zhang XH, Guo YL, Jin J. Study of effect and mechanism of cyclooxygenase-2 on the prostatic hyperplasia in rats. *Chinese J Urol*. 2005;7:468-471.
62. Darnell JE Jr., Kerr IM, Stark GR. Jak-STAT pathways and transcriptional activation in response to IFNs and other extracellular signaling proteins. *Science (New York, NY)*. 1994;264(5164):1415-1421.
63. Ignacio DN, Mason KD, Hackett-Morton EC, et al. Muscadine grape skin extract inhibits prostate cancer cells by inducing cell-cycle arrest, and decreasing migration through heat shock protein 40. *Heliyon*. 2019;5(1):e01128.
64. Davalli P, Rizzi F, Caporali A, et al. Anticancer activity of green tea polyphenols in prostate gland. *Oxid Med Cell Longevity*. 2012;2012:984219.
65. Chen X, Chang L, Qu Y, Liang J, Jin W, Xia X. Tea polyphenols inhibit the proliferation, migration, and invasion of melanoma cells through the down-regulation of TLR4. *Int J Immunopathol Pharmacol*. 2018;32:394632017739531.
66. Yang Z-R, Zhao N, Meng J, et al. Peripheral lymphocyte subset variation predicts prostate cancer carbon ion radiotherapy outcomes. *Oncotarget*. 2016;7(18):26422-26435.
67. Faria A, Fernandes I, Norberto S, Mateus N, Calhau C. Interplay between anthocyanins and gut microbiota. *J Agric Food Chem*. 2014;62(29):6898-6902.
68. Aura AM, Martin-Lopez P, O'Leary KA, et al. In vitro metabolism of anthocyanins by human gut microflora. *Eur J Nutr*. 2005;44(3):133-142.
69. Sánchez-Patán F, Cueva C, Monagas M, et al. In vitro fermentation of a red wine extract by human gut microbiota: changes in microbial groups and formation of phenolic metabolites. *J Agric Food Chem*. 2012;60(9):2136-2147.
70. Czank C, Cassidy A, Zhang Q, et al. Human metabolism and elimination of the anthocyanin, cyanidin-3-glucoside: a (13)C-tracer study. *Am J Clin Nutr*. 2013;97(5):995-1003.
71. Felgines C, Texier O, Garcin P, Besson C, Lamaison JL, Scalbert A. Tissue distribution of anthocyanins in rats fed a blackberry anthocyanin-enriched diet. *Mol Nutr Food Res*. 2009;53(9):1098-1103.
72. Seeram NP, Aronson WJ, Zhang Y, et al. Pomegranate ellagitannin-derived metabolites inhibit prostate cancer growth and localize to the mouse prostate gland. *J Agric Food Chem*. 2007;55(19):7732-7737.

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