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# Brazilian Berry Extract Chemopreventive Action: Hormone Receptors as a Target to Mitigate Aging Prostatic Disorders.

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**HIGHLIGHTS**

- Brazilian berry mitigate the disorder onset in prostate of senile/high-fat mice.
- Brazilian berry reduced AR/ERα/IGFR-1 labeling in prostate of senile/high-fat mice.
- Brazilian berry preventive effect increases according to the prostatic damages rise.

**Abstract:** The Brazilian berry, also known as jaboticaba, has a great antioxidant and anti-inflammatory potential, besides demonstrating positive effects on hormonal regulation and weight loss. Nowadays, both aging and overweight are considered public health issues, promoting metabolic and hormonal changes that have a substantial role in prostate injury. We demonstrated herein that a low dose of jaboticaba peel extract (PJE) is enough to limit the onset of damages and hormone receptor alterations on the anterior prostate in the senile or high-fat diet (HFD) groups. The senile mice (11-months old) received the PJE and/or a HFD for 60 days. The anterior prostates were collected for histopathological, immunohistochemistry and western-blotting analysis. The PJE treatment reduced the epithelium atrophy and inflammatory infiltrate frequencies besides decreasing the androgen receptor (AR); estrogen receptor alpha (ERα); and insulin-like growth factor 1 receptor (IGFR-1) immunoexpression; in the anterior prostate of both senile and HFD-senile mice. However, low prostatic intraepithelial neoplasia (PIN) frequency, reduced immunoexpression of stromal AR and epithelium IGFR-1 were only observed in the anterior prostate of the PJE and HFD-treated groups. HFD intake intensified the aging-induced histopathological and hormonal alterations by further increasing the AR, ERα and IGFR-1 immunoexpression, as well as the PIN lesion incidence in the anterior prostate.

Thus, the PJE was able to interfere in the hormonal signaling mediators, protecting the anterior prostate microenvironment and preventing lesion development due to aging associated or not with HFD intake.

**Keywords:** Jaboticaba; High-fat diet; Prostate; Androgen receptor; Estrogen receptor; Insulin-like growth factor.

## INTRODUCTION

Senescence is a period of life associated with significant hormonal changes in the body as well as with increased invasive cancer incidence [1-3]. The “Global Cancer Statistics 2020” showed the prostate cancer was the second most incident cancer among men worldwide, describing aging as one of the most important risk factor to the development of this disease [4]. Along with this, the expected 22% rise in the aged global population until 2050 [5], highlight the importance of studying aging consequences in this gland and proposing new approaches to prevent prostatic alterations.

The mouse prostate is divided into four pairs of lobes, considering their position around the urethra: the ventral, lateral, dorsal and anterior [6]. Prostate cancer studies indicated that these lobes display different hormonal responses and lesion severity [7]. The ventral and lateral lobes develop severe prostatic lesions, such as high-grade prostatic intraepithelial neoplasia (PIN) and well-differentiated adenocarcinoma, faster and more aggressively than in the anterior lobe [6]. The anterior lobe, specifically, has been used in chemopreventive assays, mainly due to the slow progression of its lesions [8]. Thus, the study of prostate damage development, considering the different prostatic lobe responses, allow the evaluation of different tissue injury levels [7].

A progressive decline in circulating testosterone levels accompanied by an increase in estrogen levels can occur during late life [1, 9]. The reduction of testosterone levels and androgen receptor (AR) expression leads to the prostate growth regression, as well as the involution of prostatic epithelial cells [10-12]. Meanwhile, the increase of estrogen level is associated with the stimulation of the estrogen receptor α (ERα), involved in cell proliferation and migration processes [11, 13, 14]. The insulin-like growth factor 1 (IGF-1) is another molecule that plays an important role in the prostatic microenvironment, where its signaling is mediated mainly by the IGF-1 receptor (IGFR-1) [15]. The upregulation of both IGF-1 and IGFR-1 during late life has been linked to increased cell proliferation and reduced apoptosis in the prostate, which is considered an important event in the progression towards a lethal disease [9, 15, 16].

Studies have pointed that diet or eating habits are associated with the etiology of prostate disorders [11, 17-19]. High adiposity levels have been related to changes in serum levels of different hormones, such as testosterone, estrogen and insulin; which are linked directly or indirectly to the development of prostate cancer [17, 20, 21]. A recent research by our group showed that the ingestion of a high-fat diet (HFD) promoted weight gain and insulin resistance, besides increasing the level of premalignant lesions in the ventral prostate mainly by means of increasing AR and ERα immunoexpression, and inflammatory infiltration in this gland [11, 22].

Natural extracts have been studies as potential mitigators of prostatic disorders, showing anticancer, antiproliferative, antiapoptotic properties [23, 24]. The Jaboticaba (MYRTACEAE *Myrciaria jaboticaba* (Vell.) O. Berg) [25], a Brazilian berry belonging to the Myrtaceae family, occurs spontaneously in a great part of the country. The jaboticaba peel contains high levels of minerals and dietary fiber, besides high levels of bioactive compounds [26]. The major bioactive compounds present in the jaboticaba peel are anthocyanins, especially the cyanidin-3-O-glucoside [27]. Ha and coauthors [28] showed increased apoptosis and reduction of PSA and AR expression in prostate cancer cells treated with anthocyanin. In addition, a recent study by our research group demonstrated that the jaboticaba peel extract treatment prevented the pre-malignant lesion development in the ventral prostate of senile or HFD-senile mice, mainly through its interference in hormonal and angiogenesis mediators [11]. According to these authors, a high dose of jaboticaba peel extract downregulated the AR level which was associated with low aromatase and low ERα levels, besides reducing the vascular endothelial growth factor (VEGF) level, possibly limiting the nutrient supply for cell proliferation. Other functional properties have been described regarding the jaboticaba bioactive compounds that are involved in its protective effect of the prostate, such as its antioxidant and anti-inflammatory capacity [18, 29, 30]. Their antioxidant mechanism in the prostate involves the modulation of endogenous antioxidant machinery, downregulating the catalase and SOD2 levels and increasing the GPx3 levels [18]. The authors verified that the antioxidant properties further led to the downregulation of NFkB signaling pathway, which is mainly activated by oxygen reactive species [18].

All these data prompted us to investigate the influence of HFD ingestion, during late life, on the anterior prostate, considering slower and less severe lesion progression in this glandular lobe. Also, we aimed to

verify if low dose jaboticaba peel extract treatment could be effective in the prevention of the prostatic pathological and hormonal features on the anterior prostate of senile or HFD-senile mice.

## MATERIAL AND METHODS

### Animals and Experimental Procedures

The present study used fifty FVB mice that were purchased from the Multidisciplinary Center for Biological Investigation on Laboratory Animal Science of the Brazil. The Research Ethics Committee of University of Campinas approved the experimental procedures performed herein (Protocol nº 4178-1).

The FVB mice were randomly distributed into five experimental groups (n=10 mice *per* group): **Young group (Yg).** Untreated 3-month old mice. **Senile group (Se).** Mice with 11-month-old that gotten daily water' gavage (vehicle), and the standard diet, composed by 22 g% protein; 53 g% carbohydrate; 4.5 g% lipid and 2.9 kcal/g (Nuvital CR1, Colombo, Paraná, Brazil). **Senile and HFD group (SeHf).** Mice with 11-month-old that gotten daily water' gavage (vehicle), and the HFD, composed by 20 g% protein; 50 g% carbohydrate; 21 g% lipid and 4.5 kcal/g. **Senile and PJE group (SeJ).** Mice with 11-month-old that gotten daily PJE gavage at the dosage of 2.9 g/Kg, water and standard diet. **Senile, HFD and PJE group (SeHfJ).** Mice with 11-month-old that gotten daily PJE gavage at the dosage of 2.9 g/Kg, water and HFD.

The experimental treatments were carried on for 60 days, according to our previous studies [11, 22]. The mice were allocated one per cage under a cycle of 12h light-dark. Food and water were offered *ad libitum.* The PJE dose used herein was chosen based on previous studies by our research group that showed this dose was the lowest dose that exerted positive preventive effects against hepatic and prostatic injury [11, 22]. The dose of 2.9 g PJE/Kg body weight/day contains the following amount of bioactive compounds: cyanidin-3-O-glucoside (4.65 mg/Kg), delphinidin-3-O-glucoside (0.5 mg/Kg), ellagic acid (0.07 mg/Kg), rutin (0.007 mg/Kg) and gallic acid (0.006 mg/Kg) [11, 22, 27]. The complete PJE method of preparation, bioactive composition and *in vitro* antioxidant activity were detailed by Lamas and coauthors [22]. After the experiments conclusion, the FVB mice were weighed (Marte AS 5500, São Paulo, Brazil) and then euthanized by increasing the anesthetic level (Xylazine Hydrochloride 2%, König, São Paulo, Brazil and Ketamine Hydrochloride 10%, Fort Dodge, Iowa, USA). The anterior lobe prostate samples were removed and frozen at -80ºC for western blotting analysis or fixed in Bouin' solution for light microscopy and immunohistochemistry. The anterior prostate was investigated in this study, due to the fact of it shows slow lesion progression which is interesting to evaluate in preventive assays [8].

### Histopathological Analysis

The histological protocol for preparation of five anterior prostate samples *per* group as well as the Masson's Trichrome staining procedures, followed the descriptions of [Lamas and coauthors [11], Lamas and coauthors [18]].

The quantification of prostate histopathological features was developed by means of a photomicroscope (Nikon Eclipse E-400 - Nikon, Tokyo, Japan) and the following software's: NIS-Elements/Image and Image Pro-Plus. A grid having 432 intersections was applied over 10 random captured images with 400x magnification, for five mice *per* experimental group. A total of 4320 points *per* animal were quantified and classified into the following categories: healthy epithelium, PIN, atrophic epithelium, acini lumen and fibromuscular layer around the acini, according to the studies of De Marzo and coauthors [12], Roy-Burman and coauthors [2], Silva and coauthors [8] and Alves and coauthors [31]. The total number of intersections were used to calculate the relative percentage of each predetermined feature for the different experimental groups [11]. The inflammatory infiltrate foci were counted in 10 random photomicrographs *per* animal at 400x magnification (modified from Lamas and coauthors [11]).

### Immunohistochemistry Evaluation

The same five anterior prostate samples *per* experimental group used for the morphological analysis were used for the immunohistochemistry evaluation. The anterior prostate were sectioned (5 µm thick) using a micrometer (Hyrax M60, Zeiss, Germany) and disposed on silanized slides. The immunostaining protocol was previously described by Lamas and coauthors [11]. The antibodies used for antigen detection were: rabbit polyclonal AR (sc-816 - Santa Cruz Biotechnology, Santa Cruz, CA), mouse monoclonal ERα (sc-71064 - Santa Cruz Biotechnology, Santa Cruz, CA), mouse monoclonal IGFR-1 (sc-271606 - Santa Cruz Biotechnology, Santa Cruz, CA). The anterior prostate sections were incubated (2 hours) with secondary antibodies (HRP-conjugated): goat anti-mouse IgG (W4021, Promega Corporation, Madison, WI, EUA) or

goat anti-rabbit IgG (W4018, Promega Corporation, Madison, WI, EUA). The peroxidase-conjugated with the secondary antibodies reacted with the 3,3-diaminobenzidine (Sigma–Aldrich) which led to the formation of a brown precipitate. The counter-staining were performed with Harris' hematoxylin.

The immunolabeling evaluation was developed using a photomicroscope (Nikon Eclipse E-400 - Nikon, Tokyo, Japan) and the NIS Elements and Image Pro-Plus software. A grid with 300 intersections was applied over 10 random images with 400x magnification, in the 5 animals from each experimental group. The immunolabeling relative percentage was calculated dividing the number of intersections that corresponded to the brown staining region, by the total number of intersections in the grid (300) [31]. The results were described as positive immunolabeling relative frequency for the epithelium or the stroma in all the experimental groups [31].

### Western Blotting Evaluation

The western blot experiments were performed using the five anterior prostate samples *per* experimental group that were frozen (-80ºC). The western blot protocol used in the study herein can be found in Kido and coauthors [13]. The following primary antibodies were used: mouse monoclonal PCNA (ab29 – Abcam, Cambridge, MA), mouse monoclonal IGFR-1 (sc271606, Santa Cruz Biotechnology, California, USA) or mouse monoclonal β-actin (sc-517582, Santa Cruz Biotechnology, California, USA). The following secondary antibodies were used: goat anti-mouse IgG (W4021, Promega Corporation, Madison, WI, USA). The western blot bands were obtained by chemiluminescence detection according to the manufactures' instructions (Pierce Biotechnology Western Blotting). The software GeneSnap (Syngene, Cambridge, UK) associated with the equipment GeneSnap (Syngene, Cambridge, UK) were used to capture the bands' images. The ImageJ software was used to quantify the immunoreactivity' bands intensity. The results were obtained as a percentage by correlating the specific molecules imunorreactive band intensity and its respective β-actin imunorreactive band intensity.

### Statistical analysis

The results obtained in the morphological, immunohistochemistry, and western blotting analyses were statistically analyzed by the analysis of variance (one-way ANOVA) followed by Tukey's multiple range post-test. The analysis was performed considering a significance limit of 5%. All the results were displayed as mean ± standard deviation. The correlation values (r) between the PIN frequency and the PCNA levels were obtained by using the Pearson's correlation test. [11, 32]

## RESULTS

### Histopathological Analysis

**Young group (Yg).** The anterior prostatic lobe presented acini with simple epithelium, showing cubic cells with centrally located nuclei. The prostatic mucosa exhibited an intense folding, which is a characteristic feature of the anterior prostate epithelium. Also, the frequent presence of basal cells located between secretory epithelial cells was verified. A fibromuscular layer with collagenous and muscular fibers surrounding the glandular acini was observed, in addition to blood capillaries and few inflammatory cells in the stroma. (Figure 1; Figure 2A-C)

**Senile group (Se).** One of the main alterations observed in the anterior prostate of the Se group was the reduction of healthy epithelium frequency and loss of mucosa folding in comparison to the Yg group. Increased PIN occurrence was also observed in relation to the Yg group. The PIN regions were characterized by an intense proliferative activity showing epithelial cell stratification. Another important tissue finding in the Se group was epithelium atrophy. The atrophic acinar regions showed smaller epithelium volume than that found in healthy acinar regions. Presence of inflammatory infiltrates close to the atrophic regions was identified in this group, characterizing a proliferative inflammatory atrophy of the prostate. A higher number of inflammatory foci was verified in the prostatic stroma in the Se group than that found in the Yg group. (Figure 1; Figure 2D-F)

**Senile and HFD group (SeHf).** There was a significant reduction of healthy epithelium areas and increased PIN frequency in the SeHf group when compared to the Se group. Atrophic epithelium regions and inflammatory foci was also observed in the SeHf group with a similar frequency to the Se group. Also, some acini in the SeHf group showed irregular and reduced mucosa folding, besides a reduction in lumen area in relation to the Yg and Se groups. (Figure 1; Figure 2G-I)

**Senile and PJE (SeJ).** After the PJE treatment, senile mice showed a reduction in epithelium atrophy compared to the Se group. Thus, glandular acini with folded mucosa were observed in these animals, exhibiting a simple columnar epithelium with occasional atrophic foci. The prostatic stroma showed thinner fibromuscular layer around acini and lower number of inflammatory foci than those found into the Se group. (Figure 1; Figure 2J-L)

**Senile, HFD and PJE (SeHfJ).** The anterior lobe of SeHfJ group had a significant increase in the healthy prostatic epithelium frequency, as well as a significant PIN and atrophy frequencies reduction in relation to the SeHf group. Healthy epithelium maintenance led to a high lumen area, compared to the SeHf group. A relatively low number of inflammatory foci and a thin fibromuscular layer surrounding the acini in the prostatic stroma were also observed. In general, the PJE treatment in the SeHfJ group led to folded acini, with less proliferative areas and inflammation foci, demonstrating a typical anterior prostate morphology. (Figure 1; Figure 2M-O)

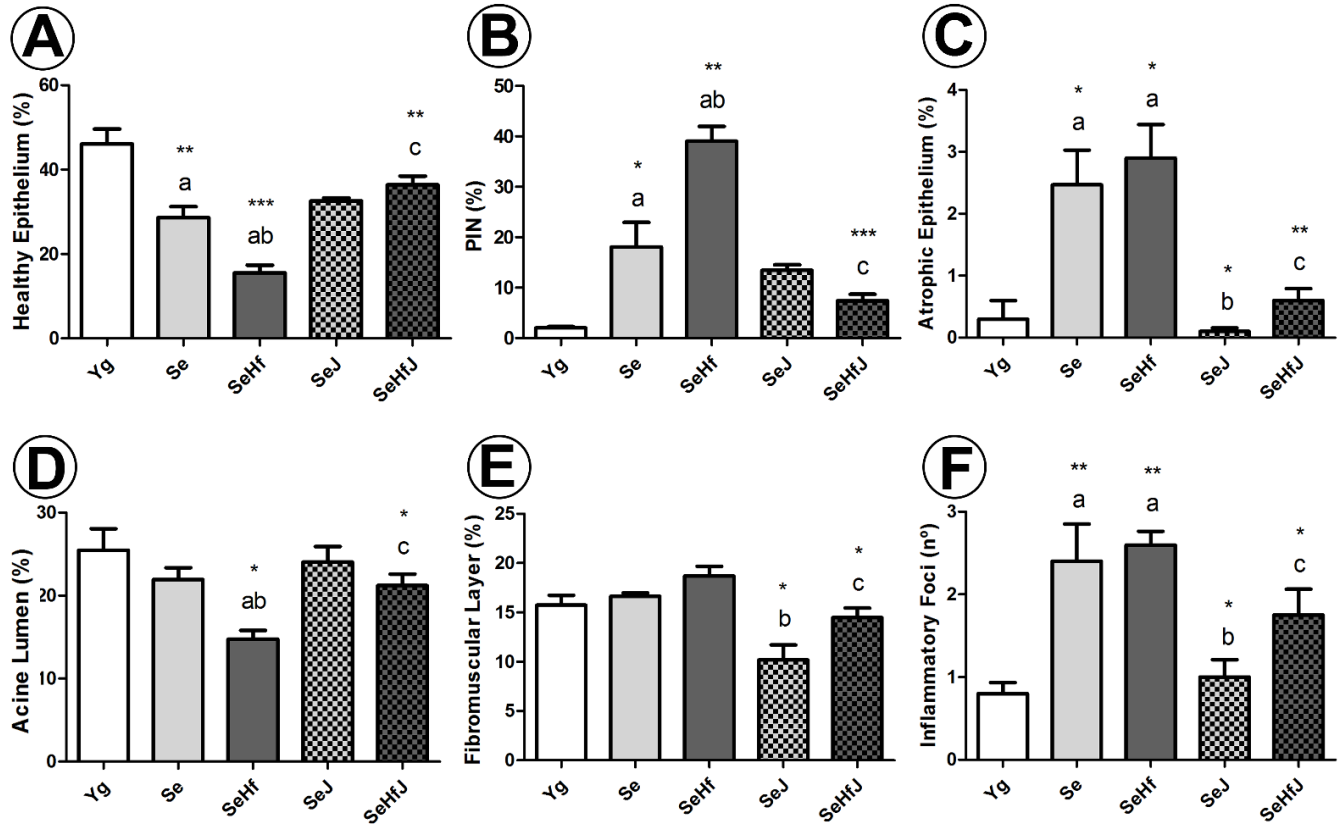


**Figure 1.** Quantification of the morphological features evaluated in the anterior prostate of mice from different experimental groups. (A) Healthy Epithelium (%). (B) Prostatic Intraepithelial Neoplasia (%). (C) Atrophic Epithelium (%). (D) Acini Lumen (%). (E) Fibromuscular Layer Around Acini (%). (F) Number of Inflammatory Infiltrate Foci (nº). Significant differences: [a] relative to the Yg group; [b] relative to the Se group. [c] relative to the SeHf group. Considering: *p < 0.05; **p < 0.01 and *** p < 0.001

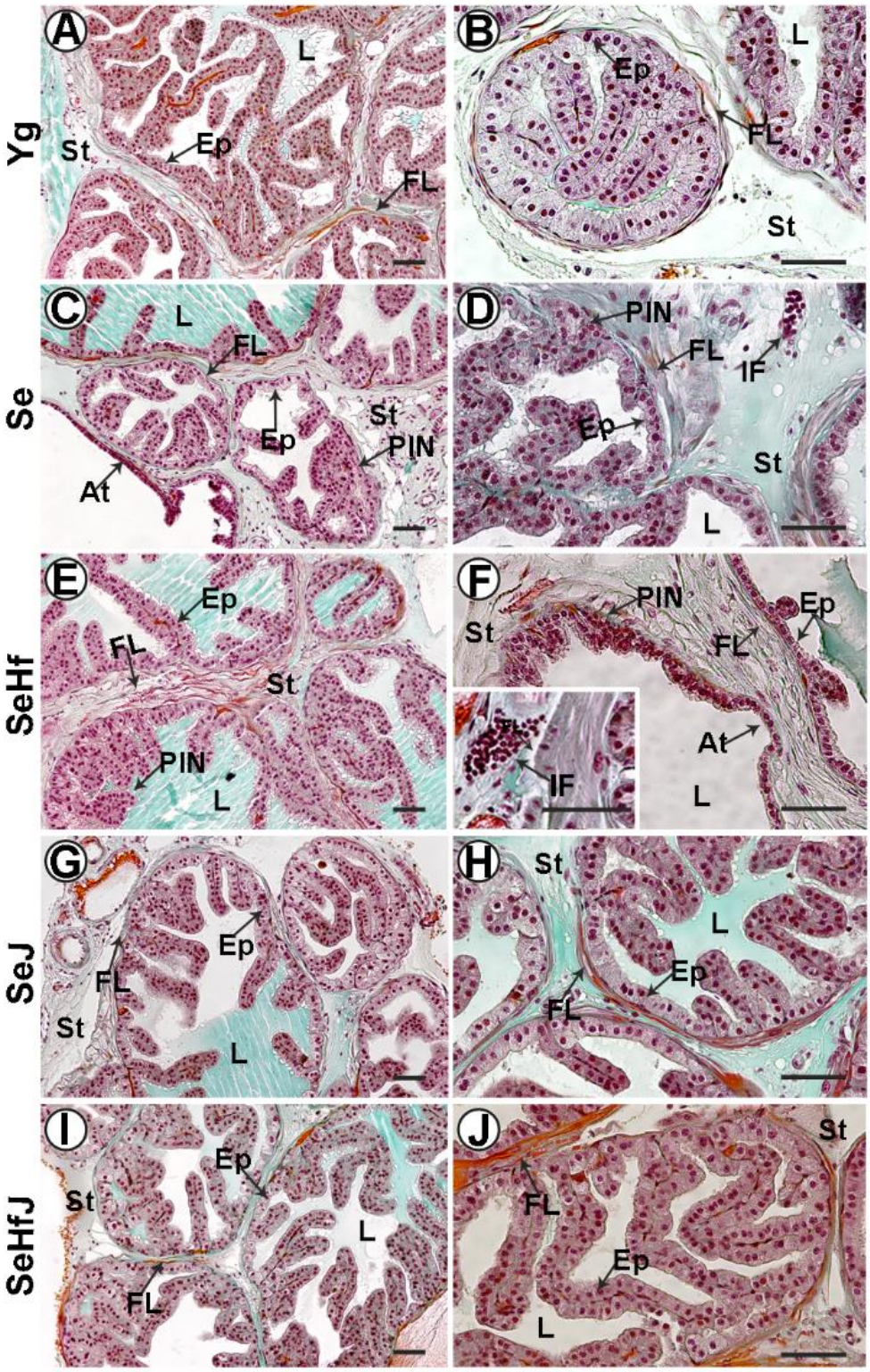


**Figure 2.** Photomicrographs of the anterior prostate morphology in the different experimental groups. (A-B) Yg group. (C-D) Se group. (E-F) SeHf group. (G-H) SeJ group. (I-J) SeHfJ group. Scale Bar=50µm. (At): atrophic epithelium; (Ep): epithelium; (FL): fibromuscular layer; (IF): inflammatory infiltrate; (L): lumen; (PIN): prostatic intraepithelial neoplasia; (St): stroma. Sections stained with Masson's Trichrome.

## Cell proliferation evaluation

The Se group showed increased PCNA level compared to the Yg group. The SeHf group further increased the PCNA level in relation to the Se group. The PJE intake reduced the PCNA level only in the SeHfJ group compared to the SeHf group. The correlation analyses (Pearson's coefficient) between the PIN frequency and the PCNA levels showed a high and positive correlation (r=0.9436; p=0.0159). (Figure. 3A)

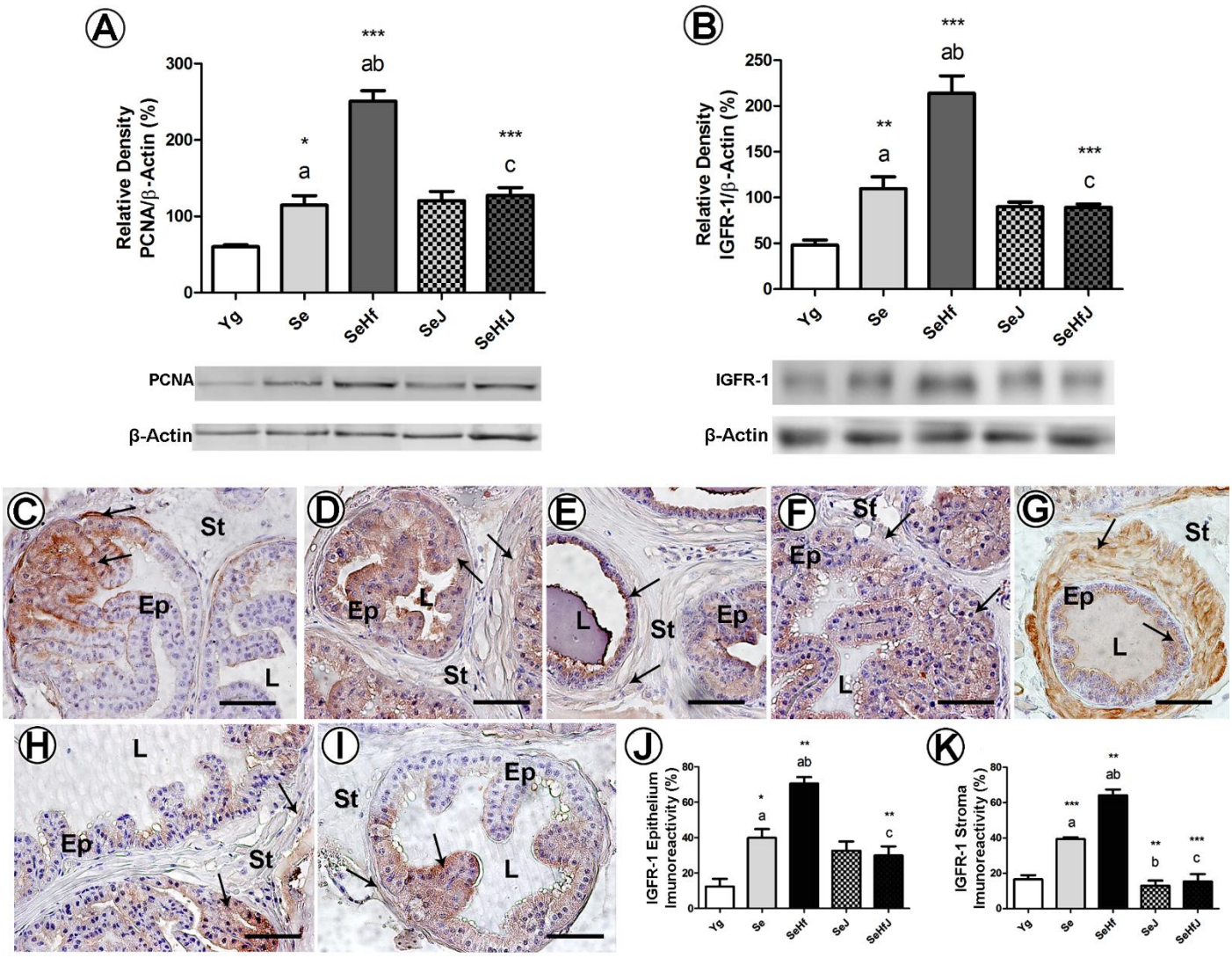


**Figure 3.** Western blotting analysis in the anterior prostate of the experimental groups: (A) Proliferating cell nuclear antigen (PCNA). (B) Insulin-like growth factor receptor 1 (IGFR1). Photomicrographs of IGFR-1 immunoreactivity in the different experimental groups: (C) Yg group. (D-E) Se group. (F-G) SeHf group. (H) SeJ group. (I) SeHfJ group. Frequency of IGFR-1 immunoreactivity in the different experimental groups: (J) Relative frequency of epithelium IGFR-1 immunoreactivity (%). (K) Relative frequency of stroma IGFR-1 immunoreactivity. Significant differences:[a] relative to the Yg group; [b] relative to the Se group. [c] relative to the SeHf group. Considering: *p < 0.05; **p < 0.01 and *** p < 0.001.

## Hormone Receptors

**Insulin-like Growth Factor 1 Receptor (IGFR-1).** The IGFR-1 protein level increased in Se group compared to the Yg group. HFD ingestion further increased the IGFR-1 protein level in the SeHf group when compared to the Se group. The PJE intake led to decreased IGFR-1 protein level in SeHfJ group in relation to the SeHf group. (Figure 3B)

The IGFR-1 immunolabeling was observed in both epithelial and stromal compartment in all the experimental groups. In agreement to the Western blotting results, senescence itself increased the IGFR-1 epithelial and stromal immunolabeling in the Se group when compared to the Yg group. The HFD ingestion enhanced the IGFR-1 epithelial and stromal immunolabeling in the senescence condition. Interestingly, the PJE treatment was able to reduce IGFR-1 immunolabeling in senile mice, associated or not with the HFD ingestion. (Figure 3C-K).

**Androgen Receptor (AR).** AR immunostaining was observed in the epithelium and stroma of the prostatic anterior lobe in all the experimental groups.

Senescence itself decreased the AR epithelial and stromal immunolabeling when compared to the Yg group. HFD ingestion increased the epithelial AR in the SeHf group, not altering the stromal staining for the

receptor. The PJE treatment effectively reduced the AR immunolabeling for the senile mice, associated or not with HFD ingestion (Figure 4)

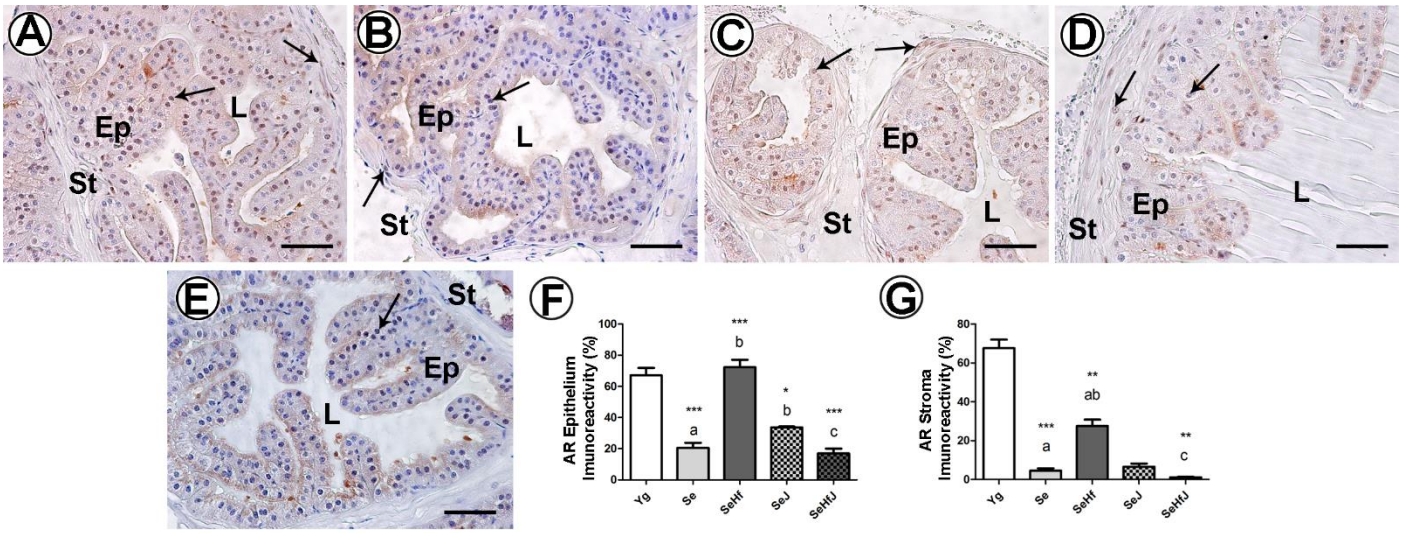


**Figure 4.** Photomicrographs of AR immunoreactivity in the different experimental groups: (A) Yg group. (B) Se group. (C) SeHf group. (D) SeJ group. (E) SeHfJ group. Frequency of AR immunoreactivity in the different experimental groups: (F) Relative frequency of epithelium AR immunoreactivity (%). (G) Relative frequency of stroma AR immunoreactivity. Significant differences: [a] relative to the Yg group; [b] relative to the Se group. [c] relative to the SeHf group. Considering: *p < 0.05; **p < 0.01 and *** p < 0.001.

**Estrogen Receptor Alpha (ERα).** Epithelial and stromal ERα immunolabeling were observed in all the experimental groups. Senescence itself increased the ERα epithelial immunolabeling when compared to the Yg group and HFD ingestion further increased the ERα epithelial immunolabeling in the SeHf group. PJE treatment reduced ERα epithelial and stromal immunostaining associated or not with HFD ingestion. (Figure 5).

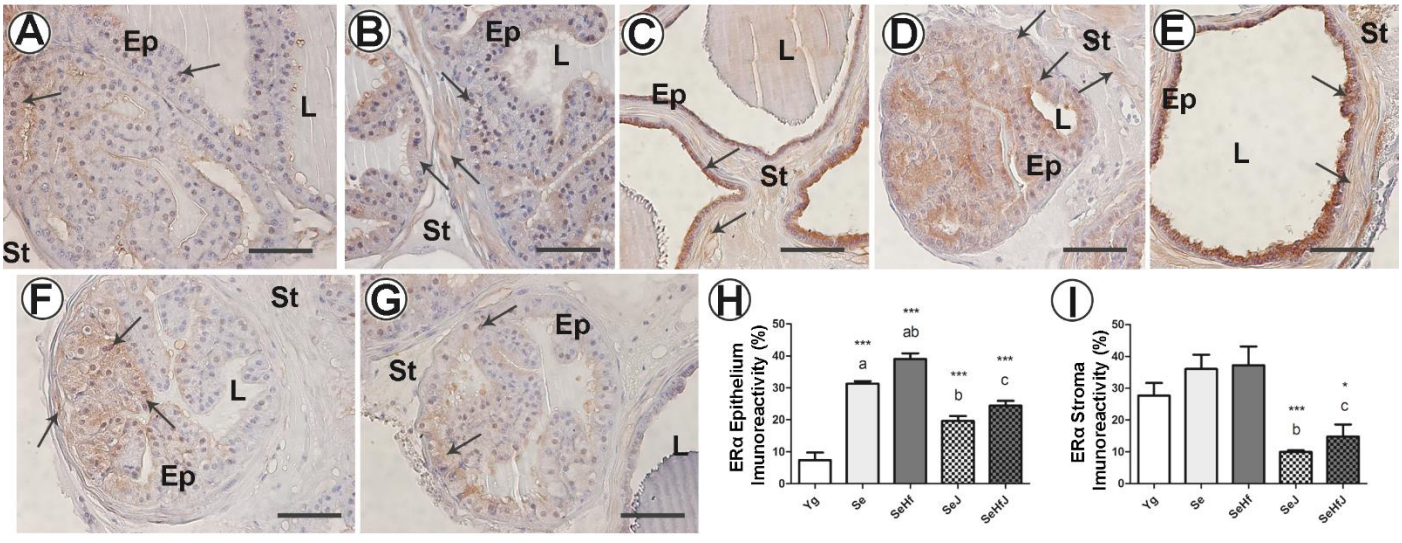


**Figure 5.** Photomicrographs of ERα immunoreactivity in the different experimental groups: (A) Yg group. (B-C) Se group. (D-E) SeHf group. (F) SeJ group. (G) SeHfJ group. Frequency of AR immunoreactivity in the different experimental groups: (H) Relative frequency of epithelium ERα immunoreactivity (%). (I) Relative frequency of stroma ERα immunoreactivity. Significant differences: [a] relative to the Yg group; [b] relative to the Se group. [c] relative to the SeHf group. Considering: *p < 0.05 and *** p < 0.001

## DISCUSSION

The present results showed that a PJE low dose was effective to mitigate the onset of disorders of the prostatic anterior lobe, a glandular region characterized by slow lesion progression, in senile or HFD-senile mice. Previous studies demonstrated that the PJE has a large range of bioactive compounds, such as anthocyanins, quercetin, epicatechin, ellagic acid, rutin, among others [22]. The synergistic effect of these bioactive compounds as well as the increased phenolic compounds solubility by the use of organic solvent for the extraction, pointed to the PJE as a promising alternative to prevent prostatic lesion development, compared to other extracts derived from purple fruits described in literature [22, 33]. The study of PJE dose-dependent effect on hepatic and prostatic lesions induced by aging and/or HFD intake, showed the PJE preventive action were associated with the tissue injury level [11, 18]. We could verify herein that the positive PJE action was different considering the senile or the HFD-senile prostatic microenvironment, showing a particularly broad effect in the anterior prostate damaged by both aging and HFD intake.

A low PJE dose was effective to decrease the AR and ERα immunolabeling in the anterior prostate of the HFD-fed senile and senile mice. Regarding the PJE effect in the prostate of the senile mice, the AR immunolabeling reduction was restricted to the epithelium cells, corroborating previous research which reported that the epithelium AR expression is more sensitive to prostatic microenvironment alterations than the stroma [34]. In a previous study, we observed that a low PJE dose reduced the AR and ERα immunolabeling in the ventral prostate of the HFD-fed senile mice, whereas just the high PJE dose alone was effective in decreasing the AR immunolabeling in the ventral prostate of the senile mice [11]. Thus, the results described herein reinforce the idea that the greater the tissue damage, the better the PJE effect on the prostatic microenvironment.

The study of AR or ERα knockout rodent models have confirmed that the reduction of the sex steroid receptor expression is associated with a low proliferative index and a low incidence of poorly differentiated adenocarcinoma [35, 36]. The main action mechanisms described for the PJE bioactive compounds towards AR reduction is associated with the interference in upstream pathways related to inflammation and oxidative stress, which usually stimulate the AR synthesis [11, 37, 38]. On the other hand, Wang and coauthors [39] verified that the bioactive compound resveratrol downregulated the estrogen signaling pathway by reducing the aromatase levels in breast cancer cells. Similarly, Lamas and coauthors [11] demonstrated that the PJE action reducing the ERα level is associated with low aromatase levels in the ventral prostate, which was involved in the reduction of cell proliferation. Therefore, the PJE capacity to reduce these receptors could represent a pathway towards the reduction of inflammatory and proliferative prostatic lesions. Due to the well-known association of ERα and cell proliferation mechanism, the PJE capacity to reduce this receptor could be considered an important step in the prevention of PIN lesions. Interestingly, our results herein show that the PJE action on AR and ERα extends to a less damaged prostatic microenvironment, which gives a good perspective about the PJE administration, considering the prevention of aging consequences to the prostate and systemic hormone signaling.

Another novelty of our findings is that we showed for the first time that a low PJE dose significantly reduced the IGFR-1 immunolabeling in the anterior prostate of senile and HFD-fed senile mice, giving insights of a possible new hormone-related molecule associated with the PJE positive effect against prostatic injury. Interestingly, considering the aging microenvironment, the PJE reducing action on the IGFR-1 immunolabeling was restricted to the stroma where the IGF-1 is synthesized [40], suggesting that the PJE exerted its primary effect on the source of this molecule.

There are few studies in literature investigating the effect of isolated bioactive compounds or other natural extracts on IGFR-1 signaling in prostate cancer cells or prostate cancer xerographic models [41-43]. These data demonstrated that the action of bioactive compounds on the IGFR-1 is associated with a decrease of Akt phosphorylation, inhibiting the IGFR-1/Akt pathways and consequently the downstream targets [41, 42].

Koyama and coauthors [43] showed that pomegranate extract, rich in bioactive compounds, prevented the IGF-1-induced cell proliferation and stimulated apoptosis in human prostate cancer cells mainly by increasing JNK phosphorylation and decreasing Akt and mTOR activation. Recently, pharmacological approaches have focused on IGFR-1 blockage as a target for prostate cancer treatment. The Cixutumumab is an example of monoclonal antibody that blocks IGFR-1 and have been tested extensively for prostate cancer therapy, showing that low IGFR-1 can reduce tumor cell proliferation[44].

In addition, studies have pointed to the interplay between IGFR-1 and AR signaling as a key aspect in prostate cancer progression, since IGFR-1 expression is directly regulated by androgen. In addition, upper levels of both molecules are associated with the proliferative process and poor clinical outcomes [45]. Montico and coauthors [46] reported that androgen ablation reduced the IGFR-1 expression in the dorsolateral prostate in senile mice, reinforcing that the interplay between IGFR-1 and AR signaling pathways is not

restricted to the prostate cancer environment [44, 47]. Thus, studies have suggested that co-targeting these two molecules possibly improves the therapeutic effect [45]. IGFR-1 involvement in interconnected pathways associated with cell survival, cell cycle progression, and apoptosis blockade, makes this molecule a strategic target to prevent prostatic malignancy [40, 46]. The PJE capacity to reduce IGFR-1 level represents a possible action mechanism to reduce proliferative lesion in senile or HFD-senile anterior prostate. The low IGFR-1 was associated with low AR after PJE treatment observed in the study herein, which could indicate a significant involvement of AR in IGFR-1 reduction as well as pointing to PJE as promising therapeutics. Nevertheless, future research focusing on the PJE effect on AR and IGFR-1 crosstalk, as well as on IGFR-1 signaling pathway, is needed to clarify this perspective.

Finally, our study brings new detailed information regarding the incidence of prostatic lesions in the anterior prostate during late life associated or not with HFD intake. The senescence process promoted the development of proliferative lesions in the anterior prostate [11, 13]. These alterations become more severe in the anterior prostate of the HFD-fed senile group, especially considering high PIN frequency, which contributed to the reduction of lumen frequency due to its invasion by epithelium proliferation. The high levels of PCNA in the anterior prostate of HFD-fed senile mice are in agreement with the increased cell proliferation in this group in relation to senile mice. However, the loss of healthy epithelium occurred in the senile and the HFD-senile mice, suggesting that in both cases the incidence of epithelium lesion contributed to altering, at some point, the functionality of prostatic epithelial cells.

We believe that the high AR, ERα and IGFR-1 immunolabeling showed that the HFD intake during late life improved the hormonal imbalance caused by aging, leading to the anterior prostate microenvironment to be more prone to damage. Recently, a clinical trial indicated a strong association between IGFR-1 immunoexpression and increased risk of lethal prostate cancer [15]. In addition, AR and ERα have been shown to be risk factors involved in prostate cancer development [8, 48, 49]. Silva and coauthors [8] demonstrated that elevated AR and ERα immunolabeling persist in the advanced stages of cancer progression in the anterior lobe, highlighting the significant involvement of these molecules in prostatic cancer with slow progression. Thus, our results reinforce the importance of preventing HFD intake consequences during late life, in order to promote healthy prostatic aging, even in a microenvironment with slow lesion progression.

## CONCLUSION

PJE low dose protected the senile anterior prostate microenvironment, interfering at some point on hormone receptors and especially preventing early lesion such as PIN. Nevertheless, the PJE treatment was more effective in minimizing the negative effect of HFD intake on the anterior prostate of senile mice by stabilizing the hormone signaling pathways and the epithelial-stromal interaction. The PJE preventive effect on a microenvironment showing a slow lesion progression, contributed to reinforce this extract as a promising therapy, which could be administered even before or at the initial steps of prostatic injury.

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