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Efeitos do óleo de coco na hiperplasia prostática benigna induzida pela testosterona em gerbilos da Mongólia (*Meriones unguiculatus*)

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DOUTORADO

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Câmpus de São José do Rio Preto

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Aos meus pais, que me permitiram vir a esse mundo e forneceram todas as condições para que eu chegasse até aqui.

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“ Estou entre aqueles que acham que a ciência tem uma grande beleza. ”

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RESUMO

A hiperplasia prostática benigna (HPB) é o crescimento benigno e não controlado da próstata e alguns fatores que contribuem para essa condição são as alterações dos hormônios esteroides e a variação na expressão dos seus receptores, assim como a inflamação crônica. Essa doença acomete mais que 40% dos homens acima de 40 anos, causando diversos sintomas que afetam a qualidade de vida dos pacientes. Os medicamentos para HPB causam diversos efeitos colaterais e por isso, compostos naturais são investigados como tratamentos alternativos. O óleo de coco (CO) possui em sua composição, ácidos graxos como ácido láurico e cáprico, que apresentaram efeitos benéficos para o tratamento da HPB. No entanto, os efeitos desse óleo sobre a próstata ainda são pouco conhecidos. O gerbilo da Mongólia (*Meriones unguiculatus*) é um roedor muito utilizado em estudos da próstata e a administração de testosterona é capaz de induzir a HPB, o que favorece a utilização dessa espécie como modelo para estudos dessa natureza. Assim, esse trabalho investigou os efeitos do CO sobre a hiperplasia prostática induzida pela testosterona em gerbilos adultos. Dividimos os animais em três grupos experimentais: controle intacto (IC) formado por animais que não receberam nenhum tratamento; hiperplasia induzida (HI), animais que receberam injeções subcutâneas de testosterona (3mg/Kg) em dias alternados por 30 dias e o grupo hiperplasia mais óleo de coco (HCO) em que os animais receberam injeções subcutâneas de testosterona em dias alternados por 30 dias e em seguida CO (Copra) (1 ml/Kg) via gavagem, diariamente, por 30 dias. Verificamos que o tratamento com CO na próstata hiperplásica não alterou o peso da próstata ventral em comparação ao grupo hiperplásico, porém causou alterações na morfologia, reduzindo a altura e a frequência do epitélio e do estroma muscular, a área nuclear, assim como a frequência das fibras colágenas. Observamos redução da imunomarcação dos receptores de andrógeno (ARs) e de estrógeno (ERα e ERβ) e observamos a mesma tendência no western blot, embora não significativo. A proliferação celular foi maior no grupo HCO, porém também verificamos aumento da morte celular por apoptose nesse grupo. Houve a redução da expressão das enzimas 5 α redutase, ciclooxigenase 2 (COX-2), metaloproteinases de matriz 2 e 9 (MMP2 e MMP9). Observamos a redução dos focos inflamatórios subepiteliais e

periductais, como também a redução de células positivas para marcadores de macrófagos F4/80 no estroma e CD68 e CD163 no epitélio do grupo HCO. O nível sérico de testosterona aumentou nos grupos HI e HCO. Esses resultados demonstram que o óleo de coco possui efeito pro-apoptótico e anti-inflamatório, como também altera a expressão dos ARs e ERs, minimizando os efeitos hiperplásicos, indicando que este óleo pode ser benéfico e auxiliar no tratamento da HPB.
Palavras–chave: Óleo de coco. Hiperplasia. Testosterona. Próstata. Gerbilos.

ABSTRACT

Benign prostatic hyperplasia (BPH) is the benign and uncontrolled growth of the prostate, and some factors that contribute to this condition are changes in steroid hormones and variation in the expression of their receptors, as well as chronic inflammation. This disease affects more than 40% of men over the age of 40, causing various symptoms that affect the quality of life of patients. Medications for BPH cause several side effects and therefore natural compounds are investigated as alternative treatments. Coconut oil (CO) has in its composition, fatty acids such as lauric and capric acid that have shown beneficial effects for the treatment of BPH. However, the effects of this oil on the prostate are still poorly known. The Mongolian gerbil (*Meriones unguiculatus*) is a rodent widely used in studies of the prostate and the administration of testosterone can induce BPH, which favors the use of this species as a model for studies of this nature. Thus, this work investigated the effects of CO on testosterone-induced prostatic hyperplasia in adult gerbils. We divided the animals into three experimental groups: intact control (IC) formed by animals that received no treatment; induced hyperplasia (HI), animals that received subcutaneous injections of testosterone (3mg/Kg) every other day for 30 days and the group hyperplasia plus coconut oil (HCO) in which the animals received subcutaneous injections of testosterone every other day for 30 days and then CO (Copra) (1 ml/Kg) via gavage, daily, for 30 days. We found that treatment with CO in the hyperplastic prostate did not change the weight of the ventral prostate compared to the hyperplastic group, but caused changes in morphology, reducing the height and frequency of the epithelium and muscle stroma, nuclear area, as well as the frequency of collagen fibers. We observed reduced immunolabeling of androgen receptors (ARs) and estrogen receptors (ERα and ERβ) and observed the same trend in western blot, although not significant. Cell proliferation was higher in the HCO group, but we also observed increased cell death by apoptosis in this group. There was a reduction in the expression of enzymes 5 α reductase, cyclooxygenase 2 (COX-2), matrix metalloproteinases 2 and 9 (MMP2 and MMP9). We observed a reduction in subepithelial and periductal inflammatory foci, as well as a reduction in positive cells for macrophage markers F4/80 in the stroma and CD68 and CD163 in the epithelium of the CO group. The serum testosterone level increased

in the HI and HCO groups. These results show that coconut oil has pro-apoptotic and anti-inflammatory effects, as well as changes the expression of ARs and ERs, minimizing hyperplastic effects, indicating that this oil may be beneficial and assist in the treatment of BPH.
Keywords: Coconut oil. Hyperplasia. Testosterone. Prostate. Gerbil.

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LISTA DE ABREVIATURAS E SIGLAS

5αR 5 α-redutase
ACTH hormônio adrenocorticotrófico
AR receptor de andrógenos
BL bexiga urinária (do inglês *bladder*)
CML células musculares lisas
CO óleo de coco (do inglês *coconut oil*)
DHT di-hidrotestosterona
DL lobo dorsal (do inglês *dorsal lobe*)
DLL lobo dorsolateral (do inglês *dorsolateral lobe*)
EGF fator de crescimento epidérmico
ER-α receptor de estrógeno tipo α
ER-β receptor de estrógeno tipo β
CG glândula coaguladora ou lobo anterior (do inglês *coagulating gland*)
HPB hiperplasia prostática benigna
IGFs fatores de crescimento tipo insulina
KGF fator de crescimento de queratinócitos
LH hormônio luteinizante
LHRH hormônio luteinizante liberador de hormônio
LUTS sintomas do trato urinário inferior (do inglês *lower urinary tract symptoms*)
MEC matriz extracelular
PIN neoplasia intraepitelial (do inglês *prostatic intraepithelial neoplasia*)
PSA antígeno prostático específico
PUFA ácidos graxos poli-insaturados (do inglês *polyunsaturated fatty acids*)
SV vesícula seminal (do inglês *seminal vesicle*)
T testosterona
TGF-β fator de crescimento de transformação β
UR uretra
VL lobo ventral (do inglês *ventral lobe)*

SUMÁRIO

1. INTRODUÇÃO

1.1. Próstata

A próstata é a maior glândula acessória do aparelho genital masculino e está localizada na base da bexiga, rodeando a porção proximal da uretra (ALSHAHRANI; MCGILL; AGARWAL, 2013). Ela produz um fluido alcalino que compõe a maior parte do líquido seminal e tem a função de proteger e nutrir os espermatozoides (ALSHAHRANI; MCGILL; AGARWAL, 2013; VERZE; CAI; LORENZETTI, 2016).

Ilustrações anatômicas da próstata foram publicadas desde meados do século XVI, quando Andreas Vesalius, em 1543, publicou as suas observações sobre as glândulas acessórias masculinas (AARON; FRANCO; HAYWARD, 2016; SAUNDERS; O'MALLEY, 2013). Em 1912, Lowsley utilizou o termo "lóbulos" para descrever as regiões da próstata, que foram designados o lóbulo médio, dois laterais, posterior, e lóbulos ventrais. Em humanos adultos, os lóbulos que descreveu são fundidos e não podem ser separados (HUTCH; RAMBO, 1970; TISSELL; SALANDER, 1984). A nomenclatura mais utilizada para descrever a estrutura da próstata humana é a de McNeal, que dividiu a próstata em três áreas principais que são histologicamente distintas e anatomicamente separados em zonas central, de transição, periférica e fibromuscular (MCNEAL, 1984).

Assim como nos humanos, mamíferos como o cão, o gato e alguns morcegos, a organização multilobar não é observada nos adultos e a próstata constitui um órgão compacto (PRICE, 1963; ZHU et al., 2004; MCNEAL, 1983). Porém, na maioria dos outros animais, incluindo outros primatas e roedores, os vários lobos prostáticos são separados em vários graus sob um ponto de vista anatômico, histológico e fisiológico (AARON; FRANCO; HAYWARD, 2016).

Em roedores, a próstata é formada por quatro lobos distintos que circundam a uretra na base da bexiga, designados como o lobo ventral, lobo lateral, lobo dorsal e lobo anterior ou glândula de coagulação que se encontram associadas as vesículas seminais (JESIK; HOLLAND; LEE, 1982; PRICE, 1963). Embora a presença de lobos seja uma característica comum da maioria dos roedores investigados até o momento, algumas variações foram observadas. Em ratos, o lobo lateral e o lobo dorsal são frequentemente dissecados e analisados em conjunto, como o lobo dorsolateral, pois estes componentes exibem uma origem ductal da mesma região (CASTRO et al.,

2021; JESIK; HOLLAND; LEE, 1982; PRICE, 1963). O lobo ventral dessas espécies é o componente mais proeminente e exibe uma resposta epitelial mais rápida à ablação androgênica, portanto é o principal modelo experimental utilizado para compreender a biologia da próstata (JESIK; HOLLAND; LEE, 1982; SHAPPELL et al., 2004; SUGIMURA; CUNHA; DONJACOUR, 1986).

Histologicamente, a próstata de roedores é composta um tecido epitelial secretor, que forma as unidades tubulares, e um estroma rico em células musculares lisas (PRICE, 1963; SUGIMURA; CUNHA; DONJACOUR, 1986). O epitélio prostático apresenta tipos variados de células (basais, secretoras luminais, intermediárias e neuroendócrinas) dependentes de hormônios esteroides, que reagem diferentemente a cada um deles (RISBRIDGER; TAYLOR, 2006; RUMPOLD et al., 2002). Nesse compartimento também podem ser encontrados outros tipos celulares, como os macrófagos e os linfócitos (CHATTERJEE, 2003; SFANOS et al., 2018).

O estroma é composto por células musculares lisas (CML) que atuam na contração durante a ejaculação, sendo o tipo celular mais frequente nesse compartimento. Também são encontradas células envolvidas na resposta inflamatória, na manutenção e na organização do tecido como fibroblastos, células endoteliais, células nervosas, telócitos e células do sistema imune como os linfócitos, macrófagos e mastócitos, imersos em uma matriz extracelular (MEC) (CHATTERJEE, 2003; CORRADI et al., 2013; SFANOS et al., 2018). Uma intensa sinalização ocorre entre os compartimentos prostáticos e pode influenciar células epiteliais, responsáveis pela renovação do compartimento epitelial e secreção parcial do fluido seminal (ROCHEL et al., 2007).

Comparações do desenvolvimento da próstata nos seres humanos e nos roedores mostraram que a morfogênese ocorre de forma análoga (PRICE, 1963; TIMMS; MOHS; DIDIO, 1994). No entanto, ainda existe muita controvérsia em relação à homologia entre os lobos prostáticos de roedores e as zonas prostáticas dos humanos. Apesar de não existir uma homologia definida, roedores de laboratório têm sido amplamente utilizados para investigações relativas à histofisiologia e à patologia da próstata, sendo a maioria dos estudos concentrados no lobo ventral (SANTOS et al., 2017; VENÂNCIO et al., 2012; VILAMAIOR et al., 2000; VILAMAIOR; TABOGA; CARVALHO, 2006).

Esta ênfase no lobo ventral deve-se à sua maior sensibilidade a andrógenos, e portanto, maior incidência de hiperplasia e neoplasia (BANERJEE et al., 1998;

CORDEIRO et al., 2008; SHAPPELL et al., 2004; VILAMAIOR et al., 2000) essas características também tem sido observadas no gerbilo da Mongólia (CORRADI et al., 2004; OLIVEIRA et al., 2007; PEGORIN DE CAMPOS et al., 2006).

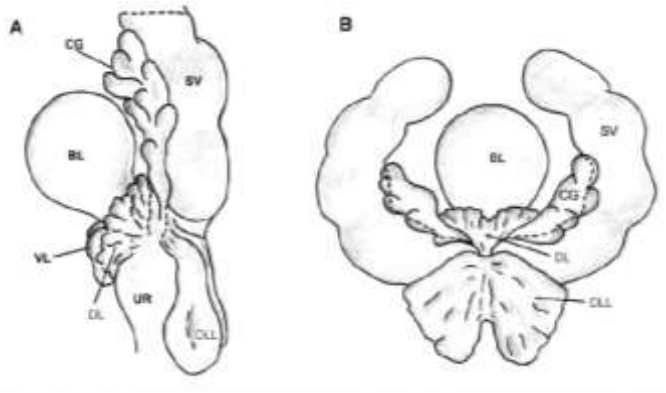
1.2. Modelo experimental

O gerbilo da Mongólia (*Meriones unguiculatus)*, também conhecido como esquilo da Mongólia ou gerbilo de laboratório, é um pequeno roedor da família Muridae, subfamília Gerbillinae. Originário das regiões áridas da China e da Mongólia, foi introduzido nas Américas como nova proposta de animal experimental (SCHWENTKER, 1963) por ser um animal de fácil manipulação e apresentar comportamento dócil (BATCHELDER et al., 2012; RICH, 1968). Esse pequeno roedor tem sido utilizado em diversos estudos, tais como fisiologia (NOLAN; BROWN; CAVANAGH, 1990), e morfologia (CORRADI et al., 2004; DOS SANTOS et al., 2003; PINHEIRO et al., 2003; ROCHEL et al., 2007) e vem sendo largamente utilizado em estudos da próstata (CAMPOS et al., 2008, 2011; CASTRO et al., 2021; CORRADI et al., 2013, 2017; DE JESUS et al., 2015; GUERRA et al., 2019; SANCHES et al., 2014; TABOGA; VILAMAIOR; GÓES, 2009).

A próstata do gerbilo é constituída pelos lobos ventral (VL), anterior ou glândula coaguladora (CG), dorsal (DL) e dorsolateral (DLL) que estão associados à uretra (UR) (Figura 1). O LV distingue-se dos outros apresentando uma organização mais frouxa do tecido do estroma e células musculares mais finas e lisas, o compartimento subepitelial é mais espesso e contém duas a quatro camadas de fibroblastos rodeadas por fibras de colágeno e material amorfo (ROCHEL et al., 2007) e o epitélio é composto por duas linhagens celulares, células secretoras e células basais.

Estudos mostram que as características histológicas, histoquímicas e ultra-estruturais da próstata dos gerbilos adultos são comparáveis com a próstata humana (PEGORIN DE CAMPOS et al., 2006), sendo possível a aplicação desse modelo animal em estudos experimentais que permitam uma maior compreensão das doenças da próstata (SCARANO; VILAMAIOR; TABOGA, 2006). Além disso, a aplicação de testosterona exógena nos gerbilos adultos, causam efeitos proliferativos na próstata, sugerindo que possam ser utilizados como modelo para o estudos dessa natureza (CASTRO et al., 2021; SCARANO; VILAMAIOR; TABOGA, 2006).

Figura 1. Representação esquemática do complexo prostático do gerbilo da Mongólia. (A). Vista lateral; (B) Vista dorsal. Bexiga urinária (BL); glândula coaguladora ou lobo anterior (CG); lobo dorsal (DL); lobo dorsolateral (DLL); vesícula seminal (SV); uretra pélvica e músculo uretral (UR); lobo ventral (VL).



FONTE: Extraído e adaptado de ROCHEL et al., 2007.

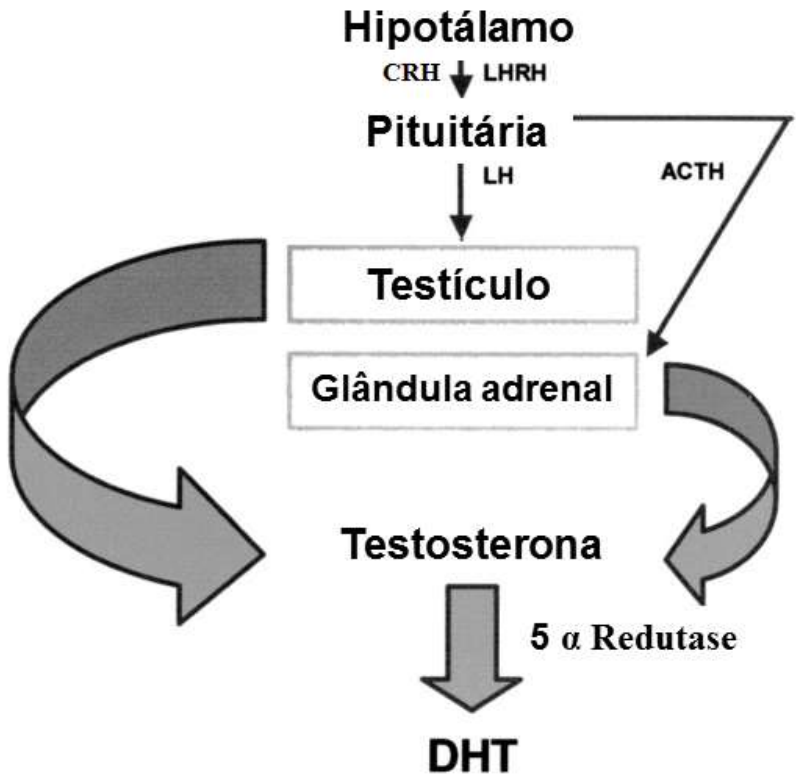
1.3. Fisiologia da próstata

A próstata é dependente de hormônios esteroides tanto para o desenvolvimento e diferenciação durante a embriogênese, como também na manutenção durante a vida adulta (THOMSON; CUNHA; MARKER, 2008; VICKMAN et al., 2020).

A produção de andrógenos é regulada pelo eixo hipotalâmico-hipofisário-gonadal (CUNHA et al., 2001) e a testosterona (T) é o principal andrógeno circulante no organismo masculino, sendo a maior parte produzida nos testículos pelas células de Leydig e uma pequena proporção pelas glândulas adrenais (HO; HABIB, 2011; ROEHRBORN, 2008; WILSON, 2011). Na próstata a T é convertida pela enzima 5 α redutase (5αR) em di-hidrotestosterona (DHT), sua forma hidroxilada (ASADA et al., 2001) (Figura 2). A DHT é o andrógeno predominante na próstata e o de maior atuação na glândula, devido à alta afinidade com os receptores de andrógeno (AR).

Os andrógenos são imprescindíveis para os órgãos sexuais masculinos e atuam nas células alvo da próstata através da interação com seus receptores (ARs) (MARKER et al., 2003), estimulando atividades específicas nas células prostáticas, como a proliferação das células epiteliais e estromais (TABOGA; VILAMAIOR; GÓES, 2009; WANG et al., 2001). Devido à manutenção da homeostasia prostática, assim como o crescimento serem dependentes desses andrógenos, estudos mostram que os andrógenos possuem um papel importante no desenvolvimento de lesões malignas e hiperplasia prostática benigna em animais de laboratório (CASTRO et al., 2021; SCARANO; VILAMAIOR; TABOGA, 2006; SHIRAI et al., 2000).

Figura 2. Esquema do eixo hipotálamo-hipófise-testicular-adrenal, produção e conversão da testosterona (T) em di-hidrotestosterona (DHT). Hormônio luteinizante liberador de hormônio (LHRH); Hormônio luteinizante (LH); Hormônio adrenocorticotropina (ACTH), Dihidrotestosterona (DHT) e CRH (hormônio liberador de corticotropina).

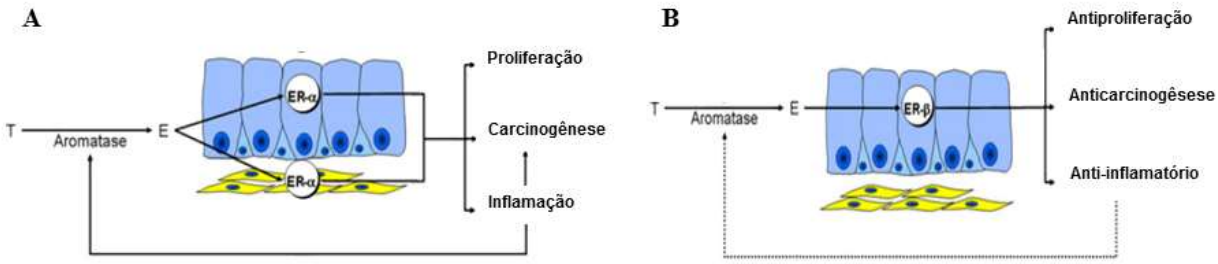


FONTE: Extraído e adaptado de CARSON & RITTMASTER, 2003.

Embora o desenvolvimento e a manutenção dessa glândula sejam dirigidos por andrógenos, os estrógenos também influenciam na homeostase prostática, sendo produzidos localmente a partir da conversão de testosterona em estradiol pela atividade da enzima aromatase (CYP19) (GRINDSTAD et al., 2016). Os estrógenos agem via receptores de estrógenos do tipo alfa (ERα) e do tipo beta (ERβ)(GRINDSTAD et al., 2016; HORVATH et al., 2001; MCPHERSON et al., 2007). Estes receptores estão localizados em ambos compartimentos prostáticos, porém o ER-α pode ser mais expresso no estroma e o ER-β no epitélio, estimulando a proliferação das células epiteliais e estromais (CUNHA et al., 1987; MCPHERSON et al., 2010)(Figura 3). O ERα é considerado oncogênico, promovendo proliferação e inflamação (ATTIA; EDERVEEN, 2012; MCPHERSON et al., 2001; RISBRIDGER et al., 2007 WARNER et al., 2020), enquanto o ERβ é predominantemente protetor,

sendo considerado anticancerígeno, anti-proliferativo, anti-inflamatório e pró-apoptótico (ATTIA; EDERVEEN, 2012; HORVATH et al., 2001; MCPHERSON et al., 2001; ZHU et al., 2004) (Figura 3). Dessa forma, a variação na expressão dos receptores ERα e ERβ tem sido considerada um dos fatores hormonais de risco associados ao desenvolvimento de hiperplasia prostática benigna (HPB) (CHOI et al., 2016).

Figura 3. Variação da expressão dos receptores de estrógeno. (A). Receptor de estrógeno tipo α. (B). Receptor de estrógeno tipo β.



FONTE: Extraído e adaptado de ELLEM & RISBRIDGER, 2009.

1.4. Hiperplasia Prostática Benigna

A hiperplasia prostática benigna (HPB) consiste no aumento da glândula resultante da hiperplasia estromal e epitelial (NOA et al., 2005). Esse aumento da glândula pode comprimir o canal da uretra, provocando a interrupção parcial ou total desta, interferindo no fluxo normal da urina e causando outros sintomas como dificuldade de urinar, incontinência e gotejamento terminal que afetam a qualidade de vida dos pacientes (CARRERO-LÓPEZ & MI, 2016).

É uma das patologias mais comuns nos homens a partir da quinta década de vida, podendo associar-se a sintomas do trato urinário inferior (LUTS) (BHARGAVA; CANDA; CHAPPLE, 2004; LANGAN, 2019; THORPE; NEAL, 2003; UNTERGASSER et al., 2005). Apesar da sua alta prevalência, a etiologia da HPB ainda é pouco compreendida (MADERSBACHER; SAMPSON; CULIG, 2019; NICHOLSON; RICKE, 2011; ROEHRBORN, 2008 LANGAN, 2019; MADERSBACHER; SAMPSON; CULIG, 2019). Alguns fatores que contribuem para essa condição foram observados como alterações hormonais e desbalanço dos seus receptores, assim como a inflamação

(EOM et al., 2017a; KRUŠLIN et al., 2017; SCARANO; VILAMAIOR; TABOGA, 2006) (Figura 4).

O aumento dos níveis de testosterona e de seus receptores na próstata exercem papel fundamental no desenvolvimento da HPB, sendo que o aumento da expressão desses receptores está associada com o aumento da proliferação celular, inibição da apoptose e recrutamento de células inflamatórias (BELLO et al., 1997; VICKMAN et al., 2020; WANG et al., 2012). Dessa forma, a T e outros análogos androgênicos são utilizados na indução da HPB através de suplementação exógena em modelos experimentais, na tentativa de ampliar o conhecimento sobre essa doença e testar novos tratamentos (ABDEL-AZIZ et al., 2020; CASTRO et al., 2021; KIRIYA et al., 2019; SCARANO; VILAMAIOR; TABOGA, 2006; SHIRAI et al., 2000).

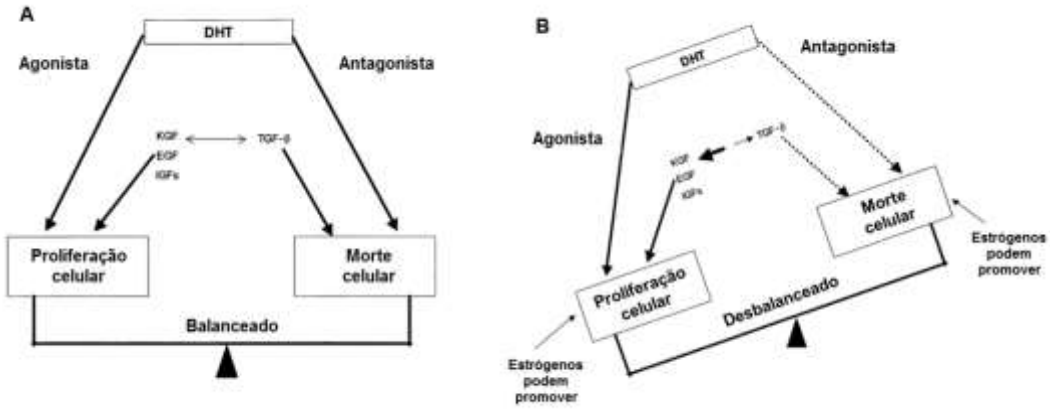
Os estrógenos também possuem relação com a HPB (TABOGA; VILAMAIOR; GÓES, 2009). Os estrógenos desempenham papéis importantes na regulação do crescimento da próstata em todas as fases da vida (ELLEM; RISBRIDGER, 2009) e através da associação com seus receptores (ER) estimulam a diferenciação e a proliferação celular (RISBRIDGER et al., 2001). Especificamente, a ativação de ER-α leva a uma proliferação aberrante, inflamação, e ao desenvolvimento de lesões pré-malignas, enquanto, a ativação de ER-β tem efeitos que equilibram a ação proliferativa dos andrógenos sobre o epitélio (ELLEM; RISBRIDGER, 2009).

Estudos demonstram que o estrógeno leva a distúrbios proliferativos no epitélio prostático, como a hiperplasia de células basais e as neoplasias intraepiteliais (PIN), bem como a displasias. As características da remodelação estromal na situação de distúrbio estrogênico não apenas confirmou sua ação sobre a proliferação e hipertrofia das células musculares, como também o aumento de constituintes estromais como as fibras elásticas e colágenas (SCARANO et al., 2005, 2008).

A inflamação pode ser um meio através do qual o estrógeno promove o desenvolvimento da malignidade, e seu desenvolvimento é uma resposta direta da próstata aos estrógenos, sendo independente dos andrógenos (ELLEM; RISBRIDGER, 2009). Observou-se que a inflamação crônica coexiste com alterações histológicas de HPB, sugerindo que a inflamação desempenha um papel no desenvolvimento desta doença através do aumento de infiltrados inflamatórios como linfócitos e macrófagos, assim como da secreção de citocinas, quimiocinas e fatores de crescimento envolvidos na resposta inflamatória, que causam o crescimento da próstata (DE NUNZIO; PRESICCE; TUBARO, 2016; SOLER et al., 2013).

Sabe-se também que a hiperlipidemia é estreitamente associado à obesidade, índice de massa corporal (IMC) mais elevado, e estes parâmetros mostram uma correlação positiva com o BPH (PARSONS et al., 2009). Cai et al., forneceu as primeiras provas dos efeitos promotores do crescimento prostático de gordura dietética em ratos (CAI et al., 2001). Da mesma forma, Rahman et al., observaram o alargamento do próstata ventral e aumento da expressão dos receptores alfa-adrenérgicos em ratos hiperlipidêmicos (RAHMAN et al., 2007). Além disso, a inclusão da gordura animal saturada (banha) na dieta induziu o aumento da próstata e mudou a expressão do receptor de andrógeno (ESCOBAR; GOMES-MARCONDES; CARVALHO, 2009). Sendo assim, os lipídeos também estão relacionados a etiologia da HPB.

Figura 4. Representação esquemática dos fatores envolvidos na manutenção da homeostase (A) e desequilíbrio (B) entre a proliferação e morte celular na próstata. Dihidrotestosterona (DHT); Fator de crescimento epidérmico (EGF); Fatores de crescimento tipo insulina (IGFs); Fator de crescimento de queratinócitos (KGF); Fator de crescimento transformador (TGF).



FONTE: Extraído e adaptado de CARSON & RITTMASTER, 2003.

1.5. Tratamentos para HPB

Diversos medicamentos são utilizados com o intuito de aliviar os sintomas da HPB e melhorar a qualidade de vida dos pacientes, bem como uma tentativa de prevenir a progressão da doença clínica e o desenvolvimento de complicações. Para pacientes com sintomas moderados a severos de HPB, ou suaves que sejam considerados incômodos, podem ser oferecidos tratamentos farmacológicos. As 2 principais classes de medicamentos utilizados são bloqueadores alfa-adrenérgicos e

inibidores de 5αR (ALCÁNTARA MONTERO; MÜLLER-ARTEAGA, 2019; LAM et al., 2003; LANGAN, 2019; SANDHU; REPORTS; 2004).

Os bloqueadores alfa-adrenérgicos bloqueiam as fibras nervosas simpáticas na próstata e na bexiga, reduzindo a contração do músculo liso e a obstrução urinária. Enquanto o bloqueio da ação de 5αR reduz a estimulação do tecido prostático através da redução dos níveis de DHT, preservando dos efeitos androgênicos da testosterona, eles são capazes de reduzir o tamanho da próstata em até 25% (CARSON; RITTMASTER, 2003; LANGAN, 2019; MADERSBACHER; SAMPSON; CULIG, 2019; VICKMAN et al., 2020).

Porém, esses benefícios necessitam ser equilibrados contra os potenciais efeitos secundários desses tratamentos, como tontura, miopatia, redução da libido, descontrole da ejaculação, disfunção erétil e ginecomastia (PATEL; CHAPPLE, 2006; SANDHU; REPORTS; 2004; SHUKLA et al., 2010). Devido a esses efeitos colaterais indesejáveis, muitos homens se interessam na utilização de terapias alternativas para tratar os sintomas e a procura de remédios fitoterápicos que fossem capazes de inibir a HPB aumentou ao longo dos anos.

Estudos com diversos produtos naturais demonstram melhorar os sintomas da HPB como os extratos de bagas de *Serenoa repens*, de semente de *Curcurbita pepo* (abóbora), de raiz de *Urtica dioica* (urtiga picante), de flor de *Opuntia* (cacto), de *Hypoxis rooperi* (erva estrela sul-africana), de *Pygeum africanum* (ameixoeira africana), assim como o óleo de coco (ARRUZAZABALA et al., 2007; CICERO et al., 2019; EOM et al., 2017a; HABIB et al., 2005; PATEL; CHAPPLE, 2006).

1.6. Óleo de coco

O óleo de coco (CO) é obtido da espécie *Cocos nucifera* a partir da polpa de coco fresco, leite de coco ou de resíduos de leite de coco. Há séculos é utilizado como planta medicinal na medicina tradicional tailandesa e na medicina Ayurveda, como também é usado como ingrediente nos medicamentos indígenas (DEBMANDAL; MANDAL, 2011; INTAHPHUAK; KHONSUNG; PANTHONG, 2010; WALLACE, 2019).

Recentemente vem sendo utilizado em larga escala nos países ocidentais, como um "super alimento" e elevado a um status de alimento funcional que oferece vários benefícios tais como a perda de peso, saúde cardíaca, melhora da imunidade,

cicatrização de feridas, melhoria da memória e higiene oral (SANKARARAMAN; SFERRA, 2018).

Devido esse aumento exponencial no seu consumo, suas propriedades biológicas têm sido investigadas (KABARA, 2013; SHEELA et al., 2019; TENG et al., 2020; WALLACE, 2019; YEAP et al., 2014). Há estudos recentes sobre os diversos efeitos do CO na saúde (SACKS, 2020; WALLACE, 2019), e seu papel nas doenças cardiovasculares são os mais estudados (JAYAWARDENA et al., 2020; SACKS, 2020; TENG et al., 2020).

Sua composição apresenta ácidos graxos saturados (cerca de 90%), sendo o ácido láurico o mais abundante, seguido dos ácidos mirístico, palmítico, caprílico, cáprico e esteárico (AKPAN et al., 2006; HUI, 1996). O ácido láurico mostrou-se capaz de inibir a enzima 5αR (HABIB et al., 2005; LIANG; LIAO, 1992; NIEDERPRÜM et al., 1994; RAYNAUD et al., 2002) e juntamente com o ácido cáprico foram importantes na atuação da resposta anti-inflamatória através da redução da IL 6 e IL 8, como também através da inibição da ativação da NF-kB e da fosforilação das MAP quinases. (HUANG et al., 2014; PEREIRA; DA SILVA; LANGONE, 2004; WITCHER; NOVICK; SCHLIEVERT, 1996).

Na porção insaturada (cerca de 9%), encontram-se os ácidos graxos poli-insaturados (PUFA) como os ácidos oleico e linoleico que desempenham um papel fundamental nos processos inflamatórios (BENNETT; GILROY, 2016; CALDER, 2008; FANG et al., 2007; ISHIHARA; YOSHIDA; ARITA, 2019; MARION-LETELLIER; SAVOYE; GHOSH, 2015). O ácido oleico demonstrou inibir a enzima 5αR (RAYNAUD et al., 2002) e ambos podem reduzir a proliferação celular e a viabilidade do câncer de próstata (ASTORG, 2004; HAGEN; RHODES; LADOMERY, 2013; HUERTA-YÉPEZ; TIRADO-RODRIGUEZ; HANKINSON, 2016). Também afetam nos níveis de estresse oxidativo e na remodelação do tecido prostático, possuindo relação direta com a HPB (VANELLA et al., 2014; YANG et al., 1999).

Estudos mostram a eficácia do CO em ações analgésicas e antipiréticas (INTAHPHUAK; KHONSUNG; PANTHONG, 2010), ação antioxidante (YEAP et al., 2014), além de promover a resposta do sistema imunológico nas reações anti-inflamatórias, eliminando completamente as respostas do fator imunitário à endotoxina, diminuindo a produção de citocinas pró-inflamatórias *in vivo* (RATHEESH et al., 2017; WAN; GRIMBLE, 1987), fatos que estão intimamente ligados à HPB. ARRUZAZABALA et. al. (2007) mostraram através das análises do peso e tamanho

da próstata, que o CO foi capaz de reduzir essas características hiperplásicas em ratos Sprague-Dawley adultos com HPB induzida pela testosterona (ARRUZAZABALA et al., 2007).

2. JUSTIFICATIVA

Estudos sobre a ação do CO na próstata são escassos na literatura e os efeitos morfofisiológicos de sua suplementação após a indução da HPB pela testosterona são pouco conhecidos. O gerbilo da Mongólia vem sendo utilizado em diversos estudos, principalmente da próstata (CAMPOS et al., 2011; CASTRO et al., 2021; CORRADI et al., 2017; GUERRA et al., 2019; ROCHEL et al., 2007; TABOGA; VILAMAIOR; GÓES, 2009) e a aplicação de testosterona exógena nos gerbilos adultos causam efeitos proliferativos, sugerindo que possam ser utilizados como modelo para os estudos de hiperplasia prostática induzida (CASTRO et al., 2021; SCARANO; VILAMAIOR; TABOGA, 2006).

ARRUZAZABALA et al. (2007) relataram que o CO reduz a HPB através da redução do peso (ARRUZAZABALA et al., 2007), porém, considerando a ação dos diversos componentes do CO, investigações mais minuciosas são necessárias para esclarecer a ação deste óleo nos mecanismos intimamente ligados à HPB.

3. HIPÓTESE

A hipótese levantada neste trabalho é que o óleo de coco, produto facilmente encontrado e que vem sendo largamente utilizado na alimentação, atue como um fator benéfico, reduzindo as características da hiperplasia prostática benigna induzida pela testosterona em machos adultos de gerbilos.

4. OBJETIVOS

4.1. Objetivo geral

O presente estudo teve como objetivo geral avaliar o efeito do óleo de coco sobre na próstata ventral de gerbilos adultos após indução da HPB pela administração de testosterona exógena.

4.2. Objetivos específicos

- Determinar a frequência dos compartimentos prostáticos, assim como fibras colágenas e vasos em condições normais e hiperplásicas sob o efeito da exposição ao óleo de coco;

- Comparar a morfometria do epitélio e estroma da próstata ventral de gerbilos nos diferentes tratamentos;

- Analisar as alterações morfológicas evidenciando as lesões prostáticas e focos inflamatórios, através da incidência e multiplicidade;

- Determinar a expressão de receptores de esteroides (AR, ERα, ERβ) por imunoistoquímica e western blot nas diferentes condições experimentais;

- Verificar a expressões de diversas enzimas (5aR, MMP-2, MMP-9, COX-2) na próstata ventral,

- Investigar e quantificar os diferentes tipos de células inflamatórias na próstata ventral dos gerbilos através dos marcadores (F4/80, CD68 e CD163);

- Avaliar o balanço entre proliferação e morte celular a partir da imunoistoquímica e TÚNEL;

- Quantificar os níveis séricos de testosterona, estradiol e cortisol para avaliar o efeito da administração de testosterona como também a ação do CO na próstata de gerbilos adultos.

5. RESULTADOS

Os resultados desse estudo foram apresentados em forma de dois manuscritos que se encontram em fase de elaboração e serão submetidos às revistas *Molecular and Cellular Endocrinology* e *The Journal of Nutrition*, respectivamente.

5.1. Coconut oil restores morphology, androgen receptors and apoptosis in an experimental model for prostate hyperplasia

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Abstract

Androgens are essential for the maturation of the male sex organs and also act on prostate homeostasis and growth, and may influence the development of benign prostatic hyperplasia (BPH). Medications for BPH have many side effects and therefore analyses are performed on compounds extracted from plants as alternative treatments. Coconut oil (CO) contains components such as lauric acid that have shown beneficial effects for induced BPH in rodents. In the present study, we aim to investigate the effect of CO on the ventral prostate of adult gerbils supplemented with testosterone for the induction of BPH. We divided the animals into 3 groups (n=7): IC (intact control), HI (3mg/kg bw of testosterone cypionate on alternate days, for four weeks) and HCO (3mg/kg bw of testosterone cypionate on alternate days and for a month and then CO 1ml/kg bw daily for 30 days). Body weights did not vary between groups, but the prostate complex, ventral prostate and adrenals increased in the HI and HCO groups, while testes decreased in these groups. Serum testosterone levels increased in the treated groups and serum cortisol was not different between groups. In the HI group the epithelium and muscle stroma increased in the stereology and muscle morphometry analyses and in the HCO group these compartments decreased

relative frequency for both analyses. The expression of ARs and 5 α reductase and PINs increased in HI group, unlike what we observed in HCO group. Furthermore, although the proliferative cells increased on the HCO group, apoptotic cells also increased, demonstrating a balance in overall cell proliferation. These results suggest that CO may act advantageously on BPH in gerbils.

Keywords: ventral prostate, 5α reductase, androgens receptor, androgens, proliferation

Introduction

Testosterone (T) promotes the development and maintenance of the prostate and other male genital organs and dihydrotestosterone (DHT) promotes the growth of the male external genitalia and secondary sexual characteristics, as well as the growth of the adult prostate (SINISI et al., 2003). DHT is the predominant androgen in the prostate, resulting from the conversion of testosterone by the enzyme 5α-reductase (5αR) (ASADA et al., 2001) and is the most active in the gland due to its high affinity for androgen receptors (AR) (GAO; BOHL; DALTON, 2005). Androgens associate with their receptors and activate the transcription machinery of genes related to cell growth (Marker et al., 2003; Vilamaior et al., 2006). Thus, variations in androgen levels, as well as the expression of these receptors, are directly related to the development of malignant lesions and benign prostatic hyperplasia (BPH) in humans and experimental models (CASTRO et al., 2021; CLARK et al., 2004; SCARANO; VILAMAIOR; TABOGA, 2006; SHIRAI et al., 2000).

The Mongolian gerbil (*Meriones unguiculatus*) is a small rodent that been used for prostate studies (CAMPOS et al., 2008; CORDEIRO et al., 2008; DE JESUS et al., 2015; GUERRA et al., 2019; ROCHEL-MAIA et al., 2013) and has shown significant responses to hormonal variations (BIANCARDI et al., 2012; CASTRO et al., 2021; SANTOS et al., 2006; SCARANO; VILAMAIOR; TABOGA, 2006). The prostate of this animal presents lobes very close to each other, therefore more compact compared to other rodent species (PEGORIN DE CAMPOS et al., 2006; ROCHEL et al., 2007).

Although there is no clear homology between the lobes of the prostate and regions of the human prostate, studies had used the ventral prostate due to its sensitivity to androgens and the incidence of hyperplasia and neoplasia (BANERJEE et al., 1998; CORRADI et al., 2004; OLIVEIRA et al., 2007; PEGORIN DE CAMPOS et al., 2006; SHAPPELL et al., 2004; VILAMAIOR et al., 2000). Analyses show that old

gerbils (12 months old) can naturally develop BPH and other prostate diseases (CAMPOS et al., 2008). In addition, the application of exogenous testosterone to adult gerbils causes proliferative and dysplastic effects in the prostate, suggesting that they could be used as a model to study induced BPH (CASTRO et al., 2021; SCARANO; VILAMAIOR; TABOGA, 2006). BPH is defined as the benign, uncontrolled growth of the prostate (MADERSBACHER; SAMPSON; CULIG, 2019; NOA et al., 2005) and is a common condition in men aged 50 years and older (BHARGAVA; CANDA; CHAPPLE, 2004; THORPE; NEAL, 2003). The enlargement of the organ results from both cell hyperplasia and reduced apoptosis. Therefore, DHT plays an important role in the development of BPH as it has a role in maintaining the balance between cell proliferation and death in the normal prostate (CARSON; RITTMASTER, 2003; ISAACS, 1984).

Anti-androgenic drugs such as 5αR inhibitors (finasteride and dutasteride) are indicated for the treatment of BPH (LANZ et al., 2019; YASSIN et al., 2019) , reducing its impacts and symptoms (BARTSCH; RITTMASTER; KLOCKER, 2002; LAM et al., 2003; SANDHU; REPORTS; 2004,). Although these drugs can inhibit the development of BPH, they have several side effects such as dizziness, myopathy, uncontrolled ejaculation, fatigue, erectile dysfunction, and gynecomastia (PATEL; CHAPPLE, 2006). Thus, plant-derived products have been tested as a treatment for BPH to reduce these effects, such as Saw palmetto (*Serenoa repens)* and (BACHMANN, 2015; CARBAJAL et al., 2005; KWON, 2019), purple rice extracts (*Oryza sativa L. indica*) (KIRIYA et al., 2019), and Bawu decoction (Korean compound of 8 herbs) (EOM et al., 2017a). Recent analyses have investigated the effect of some food oils on induced prostate hyperplasia in rats and found that several of them act advantageously on BPH (OYELOWO et al., 2019). One of these oils is coconut oil (ARRUZAZABALA et al., 2007).

Coconut oil, obtained from the species *Cocos nucifera* from fresh coconut pulp, coconut milk, or coconut milk waste, has been widely used as a food and supplement. Due to this increase in its use, its biological properties have been investigated. There are recent studies on the various health effects of CO (KABARA, 2013; SACKS, 2020; WALLACE, 2019), and its role in cardiovascular diseases is the most studied (EYRES et al., 2016; JAYAWARDENA et al., 2020; TENG et al., 2020) . However, relatively few works evaluating the effect of this oil on the prostate (ARRUZAZABALA et al., 2007). Coconut oil is saturated because it composed of about 90% fatty acids of this nature,

although the exact composition of CO can vary depending on the source. Lauric acid is the most abundant (AKPAN et al., 2006), followed by myristic, palmitic, caprylic, capric, and stearic acids (HUI, 1996). Saturated fats are essential for the physiological and structural functions of the cell. Nonetheless, consuming more than the human body can metabolize can cause harm to health (WALLACE, 2019). Studies have shown that the more abundant fatty acids that make up this oil, especially lauric acid, can inhibit the enzyme 5αR (HABIB et al., 2005; LIANG; LIAO, 1992; NIEDERPRÜM et al., 1994; RAYNAUD et al., 2002).

There is also a small portion of unsaturated compounds (about 9%), including oleic and linoleic acids. These long-chain fatty acids are important constituents of cells and may be involved in cellular function, interfere with membrane fluidity, and regulate cell signaling and gene expression (CALDER, 2008). In addition, gene expression can act to reduce cell proliferation (HAGEN; RHODES; LADOMERY, 2013). The oleic acid can also inhibit the action of the enzyme 5αR (RAYNAUD et al., 2002). Although coconut oil has been shown to reduce prostate weight and testosterone-induced BPH in rats, histological analyses are needed to better understand these effects. As such, considering the action of these components and their possible effects on mechanisms linked to BPH, this study aimed to analyze the effect of coconut oil on the morphophysiology of the hyperplastic prostate of adult gerbils after supplementation by exogenous testosterone.

Materials and Methods

Animals

This experiment utilized 36 male adults (120 days old) Mongolian gerbils (*Meriones unguiculatus*) from the Animal Breeding Center of the Institute of Biosciences, Humanities and Exact Sciences (IBILCE) of the São Paulo State University (UNESP) and maintained in polyethylene cages under controlled conditions of light (12 h dark/ 12 h light) and temperature (24 °C). They received feed and filtered water *ad libitum*. The experiments were carried out following the ethical principles recommended by the National Council for Animal Experimentation Control (CONCEA) and the Ethics Committee on the Use of Animals at IBILCE / UNESP (Proc. No. 175/2017) assessed the procedures involved.

Gas chromatography of coconut oil

Coconut oil sample preparations were conducted into a derivation process using 10% (w/w) methanolic solution of boron trifluoride under N_2 atmosphere, according to Joseph & Ackman (1992). It was weighted around 6-12 mg of fat in a test-tube. Then 1.5 mL of 0.5 mol L^{-1} methanolic solution of sodium hydroxide were added to the sample tube and this mixture were kept at 100 ºC in during 5 min, followed by cooling step at room temperature. To continue the procedure, 2 mL of BF_3 in methanol were added to the sample tube and the solution was warmed at 100 ºC for more 30 min. After cooling at room temperature, 1 mL of isooctane was added to the sample tube and it was vortexed for 30 s. Then 5 mL of saturated aqueous solution of sodium chloride was added and it was vortexed again for 30 s. It was observed the formation of two phases, from which it was collected the superior phase (the organic one). Analysis were performed in triplicate *(n=3)*.

For quantification reasons, it was necessary that derivated samples have had 1 mg mL^{-1} of methyl tricosonoate (C23:0Me), used as internal standard (IS). Proper volume of 5 mg mL^{-1} of stock solution were added to the derivate sample to obtain a final concentration of 1 mg mL^{-1} of IS.

FAME analyses were performed using a gas chromatography system (model GC-2014, Shimadzu) with flame ionization detector (FID). Separations conditions used was as described: Nukol™ capillary column (30 m x 0.25 mm d.i. x 0.25 µm); injector temperature at 220 ºC; injection volume of 1 µL; split ratio of 1:10; N_2 flow; oven isothermal ramp at 200 ºC for 35 min; detector temperature at 220ºC. Sample analyze were performed in triplicate (n=3). A standard solution of FAME (GLC-85, Nu-check, EUA) was injected under the same running conditions.

Chromatograms of sample were manually integrated using software GCSolution (Shimadzu). FAME amount present in fat was calculated using chromatogram's area for each peak (each component). Then fatty acids mass is given by the following relation:

$$M_X = \frac{A_X \times M_{IS} \times T_{CF}}{A_{IS} \times M_S \times F_{MEC}}$$

where M_X is fatty acid mass (mg/g fat); M_{IS} is internal standard mass (mg); M_S is the sample mass (g fat); A_X is the fatty acid peak area; A_{IS} is the internal standard peak area; T_{CF} is the theorical correction factor and F_{MEC} is the methyl ester correction factor

to certain fatty acid. T_{CF} is used to correct the chromatographic detector's answer, which is responsive to combustion of components (C-H bonds), e.g., carbons atoms bonded to hydrogens atoms called active carbons (C*). That is the reason why T_{CF} is obtained from ratio of internal standard C* percentage ($\%cp\ *$) and fatty acid percentage ($\%cA\ *$):

$$T_{CF} = \frac{\%_{cp\ *}}{\%_{cA\ *}}$$

Experimental design and histological processing

The animals were equally and randomly divided into three groups (n=12). Intact control (IC): the animals received no treatment during the experiment; Hyperplasia Induced (HI): the animals received, on alternate days and for 30 days, subcutaneous injections of testosterone cypionate (Deposteron, EMS) diluted in corn oil (3 mg/Kg bw) (ARRUZAZABALA et al., 2007; JEON et al., 2017) and were euthanized 30 days after the end of supplementation ; and Hyperplasia plus Coconut oil (TCO): the animals received, on alternate days and for a 30 days, subcutaneous injections of testosterone cypionate diluted in corn oil (3 mg/Kg bw) and then, via gavage, followed by 1 m/kg bw of Coconut oil *(Copra, Brazil*) (ARRUZAZABALA et al., 2007) daily for 30 days. All animals were anaesthetized with Xylazine (3 mg/kg bw) and Ketamine (10 mg/kg bw), weighed, and euthanized by decapitation. The blood was collected and centrifuged at 3000 rpm for 20 min for retrieval of the serum which was stored at −80 °C for hormone dosages. The prostate complex, ventral prostate, testis, adrenal, liver, and pelvic fat were removed, weighed, and fixed in 4% paraformaldehyde for 24 h, dehydrated in ethanol, clarified in xylene, and then included in Histosec (Merck, Darmstadt, Germany). Ventral prostates were sectioned at 4µm. Considering that the organs have different sizes due to treatment, random slides for each third of the organ (beginning, middle, and end) were used for the study. The remaining organs were destined for other work in the group.

Biometric analysis

We used for the comparative analyses the weights of the animals, prostate complex, ventral prostate, testis, adrenal, liver, and pelvic fat. The relative weight of the prostate complex and ventral prostate as a ratio by dividing the organ weight by

the body weight. For the adrenal and testicular weight analyses, we averaged the weights of the animal's right and left organs.

Morphometric, stereological and, karyometric analysis

We performed morphometric analysis of histological features in sections stained with Hematoxylin-Eosin (HE) by measuring the height (μm) of the secretory epithelial cells and the thickness (μm) of the smooth muscle layer and the nucleus area (μm^2). We evaluated under light microscopy and the images were captured at ×200 magnification using a BX61VS camera (Olympus Corporation, Tokyo, Japan) coupled to an Olympus VS120® Slide Scanning System (VS120-S5) virtual microscopy system using Image Pro-Plus 6.0 software (Media Cybernetics, Inc., MD, USA). For each analysis, 34 measurements were obtained per animal (n=7), totaling 238 measurements per group. In the stereological analyses, we obtained the relative volume of the different prostatic compartments (epithelium, lumen, muscular stroma, non-muscular stroma). We captured at × 400 magnification thirty random fields from each group, performed the measurements according to the M130 multipoint test system proposed by Weibel (WEIBEL, 1963), and applied to the prostate by Huttunen (HUTTUNEN; ROMPPANEN; HELMINEN, 1981). Thus, from the data obtained for each field analyzed, we calculated the relative frequency of the compartments.

Proliferative lesions

We used five slides per animal stained by Hematoxylin-Eosin (HE) and scanned at 400x magnification for the analysis of proliferative disorders (n=7) using the slide scanner system (Olympus VS120-S5). We performed the histopathological classification of prostate changes according to the criteria described and was previously applied to the gerbils (CAMPOS et al., 2008; CASTRO et al., 2021). For this analysis, we considered the following atypias: Papillary epithelial atypia (PEA), corresponding to foci of epithelial cells that cluster, leading to stratification of the epithelium that progresses toward the lumen; flat epithelial atypia (FEA), corresponding to cells that cluster along the acinus forming a region of flat stratification in the epithelium; and prostatic intraepithelial neoplasia (PIN) consisting of a cluster of epithelial cells with irregular stratification and spacing, enlarged nuclei of different sizes (anisionucleosis) and normal chromatin and infrequent nuclei, with occasional presence of luminal junctions, and basal cells and intact basement.

The incidence of prostatic changes was calculated by dividing the number of animals with changes by the number of animals analyzed per group, expressed as %. The multiplicity was obtained from the number of alterations present in each animal divided by the number of slides analyzed per animal.

Immunohistochemistry analysis

We deparaffinized and rehydrated the histological sections for subsequent antigen recovery in 10 mM citrate buffer pH 6.0 at 92°C for 45 minutes (AR), Tris EDTA pH 9.0 for 60 minutes at 98° C (PHH3 and 5α reductase). We then performed blockade of endogenous peroxidases (3% hydrogen peroxide for 30 minutes), followed by blockade of non-specific proteins (5% skimmed milk/TBS+ 0.1% tween). Sections were incubated overnight at 4°C with the following primary antibodies diluted in 1% BSA: enzyme 5 α-reductase 2 (rabbit polyclonal, sc-20659, Santa Cruz Biotechnology CA, USA, dilution 1: 75); androgen receptor (AR) (rabbit polyclonal IgG, N-20, sc-816, Santa Cruz Biotechnology CA, USA, dilution 1: 75) and PHH3 (Rabbit Monoclonal (IgG, D3A7, Ser 10, #9145, Cell Signalling, Danvers, MA, USA, dilution 1:75). In some sections, we replaced the primary antibody with 1% BSA for the negative control of the reaction. After washing, we incubated with a Polymer kit (Novocastra Novolink RE7230-CE, Leica Biosystems, Buffalo Grove, USA) for 1h at room temperature. We then stained with DAB (Sigma, St. Louis, MO, USA) and counterstained with Mayer's Haematoxylin. For all immunohistochemical analyses, we analyzed the sections using the brown DAB precipitate - which indicates the immunoreactivity of the antibodies. We then captured the images at 400x magnification using a slide scanner system (Olympus VS120-S5). We analyzed ten random images per animal (n=7), for a total of 70 images per group. We used Image-Pro-Plus software (Media Cybernetics) to quantify the positively marked cells or regions.

In the analysis of AR-positive cells, we used the frequency of positive cells, calculated by dividing the number of positive nuclei by the total number of nuclei per compartment of each analyzed field.

For the analysis of the 5αR enzyme, we evaluated prostate tissue using a multipoint system with 160 intersections (modified from Weibel et al. 1963). We determined the results by counting the immunoreactivity coinciding with the grid intersection divided by the total number of points and expressed as a relative frequency of positive staining for the enzyme in all experimental groups.

We obtained the cell proliferation analysis from PHH3, by counting the brown nuclei of the ventral prostate. The result is of the total number of positively stained nuclei from both compartments, epithelial and stromal, for all experimental groups. We calculated the proliferation index by dividing the average number of TUNEL positive cells (apoptosis) by the average number of PHH3 positive cells (proliferation) in each group (A/P).

Apoptosis analysis

For the analysis of apoptotic cells, the ApopTag® Plus Peroxidase in Situ Apoptosis Detection Kit (S7101, Merck CA, USA) TUNEL assay was used according to the manufacturer's instructions and counterstained with methyl green. A total of 70 random images per group (10 fields per animal) were captured at 400x magnification and the result was expressed as the total number of nuclei from both compartments stained positive for all experimental groups.

Serum hormonal analysis

The serum levels of testosterone and cortisol were quantified by ELISA using a specific commercial kit of high sensitivity (IBL International, Hamburg, Germany - item number 52151 and 52061, respectively), according to the manufacturer’s instructions. We performed the readings with a microplate reader (TECAN- Infinite F50).

Western blot

We used the ventral prostate of five gerbils per group stored at -80°C for protein extraction. The protein content of the ventral prostate sample was extracted with a lysis buffer according to the protocol described by Li et al (2009). Samples were crushed and left for extraction at 4°C for 1 h under 68 shaking, before being centrifuged for 20 min at 14000 rpm at 4°C. Then homogenized in RIPA extraction buffer (Sigma-Aldrich®, StLouis, MO, USA), protease inhibitor cocktail (Sigma- Aldrich®, StLouis, MO, USA), and Triton X100. We stored the supernatants at -80°C until analysis. We used the Pierce BCA Protein Assay Kit (23227-Thermo Scientific, Rockford, IL, USA) to dose the extracts and the absorbance was read on an absorbance microplate reader (SPECTROstar Omega, BMG Labtech, Ortenberg, Germany).

For western blot, we placed 15µl of protein each well on SDS gel and subjected it to electrophoresis (105V for approximately 120 minutes). The bands were transferred

(360mA for 60 minutes) to a nitrocellulose membrane (Amersham Protram, 10600003, GE Healthcare, Darmstadt, Germany). For immunoblot, we washed the membranes in TBSt, performed a nonspecific binding block (5% skimmed milk in 0.1% tween TBS) for 1h at room temperature. Incubation was performed with anti-AR (rabbit polyclonal IgG, N-20, sc-816, Santa Cruz Biotechnology CA, USA, 1:300) and GAPDH (rabbit monoclonal, IgG, #2118, Cell Signalling, Danvers, MA, USA, 1: 1000) diluted in 1% skimmed milk in TBSt and kept for approximately 12 h under stirring at 4°C. Subsequently, the membranes were washed (TBSt) and incubated with the secondary anti-rabbit antibody (goat, IgG, #7074, Cell Signalling, Danvers, MA, USA) labeled with peroxidase conjugate (1:2000) for 1 h at room temperature under stirring. Then the membranes were washed again, and antibody detection was revealed with ECL Substrate Pierce reagents (32109, Thermo Fischer, Rockford, IL, USA). The imaging system (ChemiDoc MP, BioRad, Hercules, CA, USA) was used to visualize the bands and we used Image J densitometry software (version 1.52a, Wayne Rasband, NIH, USA) to analyze the band densities.

Statistical analysis

We submitted all obtained quantitative data to statistical analysis using GraphPad Prism 6.0 software (GraphPad Software, Inc., CA, USA). We performed the quantitative analyses according to parametric (ANOVA) and nonparametric (Kruskal-Wallis) tests for group differences, followed by Bonferroni and Dunn's multiple comparison tests, respectively. the results in terms of mean ± standard deviation and consider $p \leq 0.05$ values statistically significant.

Results

Gas chromatography of coconut oil

We observed that the fatty acids present in larger quantities are lauric, myristic and palmitic acids. The mass of fatty acids obtained are described in table 1.

Biometric analysis

In Table 2, the results of the biometric analyses. The absolute weight of the prostatic complex, the ventral prostate and adrenal increased in the HI and HCO groups compared to the IC group. We observed the same pattern in the relative weights, although we did not observe this significant change in the bodyweight of the groups studied. The average weight of the testes in the HI and HCO groups decreased compared to the IC group. We found no difference in body weight, liver weight, and pelvic fat among the groups.

Serum hormone analysis

Serum testosterone levels increased in the HI and HCO groups compared to the IC group. There was no significant difference between the groups for serum cortisol levels (Table 2).

Morphometric, stereological, and karyometric analysis

The morphometric analysis (Table 3) showed higher epithelium heigh of the epithelium in the HI group (Figure 1. D) compared to the IC (Figure 1. B) and HCO (Figure 1. F) groups. We verified the same in the muscular stroma thickness analysis that increased in the HI group (Figure 1.D) compared to the other groups. In the stereology analysis (Table 3) the epithelium and muscular stroma increased significantly in the HI group compared to the other groups, while the lumen was smaller in the HI group compared to the other groups (Figure 1.A, C, E). We observed no difference among the groups in the non-muscular stroma. The nuclear area of the HI group was significantly higher than the other groups, while the HCO group showed the smallest nuclear area compared to the others (Table 3).

Immunohistochemistry

We identified AR-positive cells in the epithelium and stroma (Figure 2.A-C) and we detected the 5αR enzyme in the ventral prostate of all groups (Figure 2. E-G). We observed the reduction of AR-positive cells in the HCO group compared to the other experimental groups in both epithelium and stroma (Figure 2.J). The frequency of the tissue expression of enzyme 5 alpha-reductase increased significantly in the HI group compared to the IC and HCO groups, and the HCO group showed fewer markers compared to the other groups (Figure 2 I).

Western Blot

Quantification of AR by western blot showed that the amount of androgen receptors expressed in the HI group is higher than in the other groups evaluated although not significant (Figure 2.K).

Proliferation and death

Immunohistochemistry for PHH3 (Figure 3. A-C) showed that its expression is higher in the ventral prostate in the HCO group compared to the HI group (Figure 3. G). In the TUNEL analysis (Figure 3.A-C) we observed an increase of apoptotic cells in the HCO group when compared to the IC and HI groups (Figure 3.G). This index was higher in the HCO group (Figure 3. H).

Proliferative lesions

From the analysis of the incidence of alterations, we observed that all groups evaluated presented FEA, PEA, and PIN (Figure 4.J), however, these atypias were more prevalent in the HI group concerning to the other groups studied (Figure 4.D-F). The multiplicity analysis showed the increase of the PEA in the HCO group compared to the IC group. There was no difference in the presence of FEA between the groups, and PIN was higher in the HI group in comparison to the IC group (Figure 4.J).

Discussion

We observed in our analyses the increase of wet weight of prostate complex, ventral prostate, and adrenals, as well as higher serum testosterone levels in the HI and HCO groups, while the testes decreased in these groups, indicating the action of exogenous testosterone. In stereology and morphometry analyses, CO was shown to reduce the epithelium and muscle stroma compared to the induced hyperplasia group, as well as reduced the expression of AR, 5 α reductase and PINs. In addition, proliferative cells increased in the HCO group due to the effect of exogenous T that was also observed in the HI group, but despite this expected proliferation, apoptotic cells increased significantly, demonstrating that CO can act by minimizing the proliferative effects of T through the pro-apoptotic effect.

Previous studies have shown that animals with testosterone-induced BPH exhibited significantly greater weight than control animals (ARRUZAZABALA et al., 2007; CASTRO et al., 2021; EOM et al., 2017a; JEON et al., 2017; SCARANO; VILAMAIOR; TABOGA, 2006). Testosterone, in addition to having a direct hormonal function, plays an important role through its metabolites, including dihydrotestosterone (DHT) and estradiol. Therefore, intraprostatic DHT levels are not equivalent to circulating T levels. Furthermore, it should be noted that in BPH, intraprostatic androgen levels do not differ from androgen levels in the normal prostate, thus prostate growth does not depend solely on higher T levels (RASTRELLI et al., 2019). However, we observed no differences in prostate weights between the HI and HCO groups, and although data in the current literature indicates that coconut oil decreases BPH by decreasing weight (ARRUZAZABALA et al., 2007), here we demonstrated that there was no reduction in weight after treatment with CO, but the morphological changes provided surprising data.

Testosterone synthesis is controlled by the hypothalamic-pituitary-gonadal axis and its production originates 95% from the testis and 5% from the adrenal gland (MADERSBACHER; SAMPSON; CULIG, 2019), being one of the main steroids secreted by the gerbil adrenal gland (NICKERSON, 1972). Disturbances at any level of this axis can lead to impaired reproductive function and the clinical syndrome of hypogonadism (CORRADI; CORRADI; GREENE, 2016). Testosterone supplementation in neonatal rats has been observed to cause dysregulation of the hypothalamic-pituitary-gonadal axis and hypogonadism in adult rats (VANDERSTICHELE et al., 1987). Our results showed a reduction in testis weight in the HI and HCO groups compared to the IC group, and the significant increase in serum T levels in the T-supplemented groups, thus the elevation of serum levels of this hormone acts in negative feedback inhibiting GnRH secretion causing testicular atrophy. The testosterone-treated groups had higher adrenal weights than in group IC, but serum cortisol levels were not different among the groups, indicating that testosterone supplementation for 30 days was not sufficient to increase cortisol levels, as has also been shown in other studies, and therefore did not influence the effects observed in this study (MUNIYAPPA et al., 2010; NICKERSON, 1972).

Some studies have shown that the main morphological changes observed in BPH are an increased glandular epithelium and thickened stroma, as well as a decrease in the lumen area (SCARANO; VILAMAIOR; TABOGA, 2006). Thus, the

ventral prostate of the HI group shows these hyperplastic characteristics, with larger epithelium and muscular stroma than the other groups observed in the stereology and morphometry analyses, and consequent reduction of the lumen area. In the HCO group, we observed the opposite, a reduction of the epithelium and muscle stroma, and an increase of the lumen. Indicating that coconut oil influenced in reducing the hyperplasia caused by T supplementation.

The nuclear analysis is another morphological feature used to assess the degree of malignancy of hyperplasia and tumor in humans and other animals (CHOI et al., 1999; DI DONATO; LAUFER-AMORIM; PALMIERI, 2017; VELTRI et al., 2002). We found larger nuclei in the secretory epithelium of the HI group, as was observed by Scarano et al. (2006) in which the nuclear area of the prostatic epithelium of testosterone-treated gerbils was larger than the other groups, whereas in the HCO group the nucleus decreased compared to the other groups (SCARANO; VILAMAIOR; TABOGA, 2006). Choi et al. (1999) checked the nuclear shapes in prostate cancer (PCA) and BPH of men and found that PCA nuclei were more irregular and larger with increasing cell malignancy (CHOI et al., 1999). This demonstrates a possible relationship with increased nuclear activity and proliferation of these cells due to increased androgens in the prostate (GALBRAITH; DUCHESNE, 1997; VELTRI et al., 2007). Therefore, nuclear analysis can be an important parameter to compare some epithelial modifications (DOS SANTOS et al., 2003), but it should be interpreted together with other results (SCARANO et al., 2008).

Androgen receptors increased in both epithelium and stroma of the prostate in the HI group compared to the other groups. Scarano et al. (2006) also observed increased AR in the groups supplemented with T (SCARANO; VILAMAIOR; TABOGA, 2006). We believe that the increase in these prostatic compartments observed in stereology and morphometry, is related to the increase in AR-positive cells in this group since AR activation by androgens regulates prostate growth through the relationship between proliferation and cell death (TAN et al., 2015). Furthermore, signaling of this receptor is known to play a critical role in tumorigenesis and PCa progression (DIALLO et al., 2008), as well as in the development of BPH (VICKMAN et al., 2020). As such, one of the treatments used to alleviate the clinical symptoms of these diseases is the inhibition of AR signaling (ALFARO; FRANCISCO; PROTTER, 2013; MIYAMOTO; MESSING; CHANG, 2004).

In this study, we found that CO was able to reduce AR expressions after treatment with T, indicating an important role in the suppression of these receptors and consequent reduction of hyperplasia. The results observed in immunohistochemistry are consistent with the pattern found in the expression of this receptor by western blot, although in this case, the result was not significant.

In the prostate, the production of specific growth factors such as IGF-I is androgen-dependent (HUYNH et al., 2001) and these factors act by activating or inhibiting genes that control the cell cycle, directly associated with cell proliferation (GALBRAITH; DUCHESNE, 1997). When analyzing cell proliferation and cell death we found that in the testosterone-treated groups the incidence of PHH3-positive cells increased, indicating increased cell proliferation, a feature of BPH (MCPHERSON et al.,2001). However, when analyzing cell death, apoptotic cells increased significantly in the HCO group compared to the others, indicating that these processes remained in balance in this group. In the HI group, apoptosis was not relevant, as in the HCO group, indicating an imbalance between these opposing phenomena, which resulted in abnormal prostate growth. Data from the index (A/P) show that in the HCO group it was higher than in the others, indicating that there was a higher incidence of cell death than proliferative cells. These data agree what has been observed in several studies, that lauric acid, the predominant component in HCO, has pro-apoptotic effects on cancer cells (LAPPANO et al., 2017; SHEELA et al., 2019) and that the pro-apoptotic activity in the HCO group, along with its anti-proliferative activity, were responsible for suppressing prostate cell proliferation and reducing the volume of specific prostate compartments. In vitro studies showed that oleic and linoleic acid, components of coconut oil, reduced cell proliferation and prostate cancer viability in most cell lines analyzed (HAGEN; RHODES; LADOMERY, 2013) suggesting them anticancer activities (CESANO et al., 1998; SONG et al., 2004).

Considering the higher expressions of AR in the HI group compared to HCO, as well as the lower index (A/P), we can explain the morphological changes found in this group, as well as the increase in PINs. According to CAMPOS et al. (2008) and the Bar Harbor Classification System for the mouse prostate, developed by the National Cancer Institute Mouse Models of Human Cancer Consortium Prostate Steering Committee (CAMPOS et al., 2008; SHAPPELL et al., 2004), the PIN is recognized histologically as a proliferation of atypical epithelial cells within pre-existing glands, and this proliferation is most commonly due to stratification of epithelial cells, and these

epithelial cells show nuclear atypia. Other studies have shown that the development of histopathological changes, such as PINs, is enhanced in the presence of testosterone in adult gerbils (CASTRO et al., 2021; PEGORIN DE CAMPOS et al., 2006). Furthermore, gerbils have a high rate of spontaneous histopathological changes during aging (PEGORIN DE CAMPOS et al., 2006), similar to what happens in humans, suggesting the existence of genetic predisposition that can be enhanced after hormone supplementation (SCARANO; VILAMAIOR; TABOGA, 2006).

In the prostate, the 5αR enzyme converts testosterone into a more active form of androgen, DHT, which has a higher affinity for the AR than T (ASADA et al., 2001). The enzyme 5-reductase is described from two isoforms (type 1 and type 2) (RUSSELL; WILSON, 1994). Type 1 is found predominantly in the skin, both in hair follicles and sebaceous glands and in the liver, prostate, and kidney (THIBOUTOT et al., 1995) and type 2 in the male genitalia and prostate (THIGPEN et al., 1992), so we used type 2 in our analyses. We observed that inhibition of the 5αR enzymes was able to cause several changes in the morphology of the ventral prostate of gerbils, including changes in tissue architecture, in addition to altering serum levels of steroid hormones. They also point out that the actions of these enzymes are crucial for the maintenance tissue architecture and extracellular matrix arrangement, but for the function of ARs (CORRADI et al., 2009). In addition, decreasing dihydrotestosterone availability by therapeutic targeting with 5αR inhibitors decreases AR activity and results in reduced prostate size and symptoms in some BPH patients (VICKMAN et al., 2020). Thus, drugs with anti-androgenic activities, mainly 5αR inhibitors are used in the treatment of BPH (ALCÁNTARA MONTERO; MÜLLER-ARTEAGA, 2019; LAM et al., 2003; LANGAN, 2019; SANDHU; REPORTS; 2004 ; SCHULMAN, 2004). Studies show that plant-derived compounds can inhibit 5αR and are effective in reducing BHP symptoms (HABIB et al., 2005; KIM; LARSON; ANDRIOLE, 2015; NIEDERPRÜM; SCHWEIKERT; ZÄNKER, 1994). In this work, the group treated with CO after the induction of hyperplasia had reduced expression of this enzyme compared to the other groups. This suggests that CO acts in inhibiting 5αR and we can relate this effect to some components present in the oil, observed in the analyses presented here, such as oleic acid and lauric acid that have been shown to inhibit the enzyme (HABIB et al., 2005; LIANG; LIAO, 1992; NIEDERPRÜM; SCHWEIKERT; ZÄNKER, 1994; RAYNAUD et al.,1994).

Conclusion

In conclusion, CO treatment significantly inhibited testosterone-induced prostate tissue enlargement as well as nuclear area, demonstrating beneficial effects on hyperplastic prostate in gerbils. With this, the present study provides the first evidence that CO can protect the gerbil prostate from the effects of induced BPH, as demonstrated by its pro-apoptotic action, as well as in inhibition of the 5alpha-reductase enzyme and reduction of androgen receptors. However, as with other studies with plant-extracted products described in the literature, we need further research to understand the action of this oil and its possible potential in the treatment of BPH.

Conflict of interest

The authors declare no conflicts of interest.

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References

AKPAN, E. J. et al. Fatty Acid Profile and Oil Yield in Six Different Varieties of Fresh and Dry Samples of Coconuts (Cocos nucifera). **Pakistan Journal of Nutrition**, v. 5, p. 106–109, 2006.

ALCÁNTARA MONTERO, A.; MÜLLER-ARTEAGA, C. 5-alpha reductase inhibitors: New evidences on benefits and harms beyond benign prostatic hyperplasia. **Actas Urologicas Espanolas**, v. 43, n. 1, p. 52–53, 2019.

ALFARO, E.; FRANCISCO, G.; PROTTER, A. A. Enzalutamide , an Androgen Receptor Signaling Inhibitor , InducesTumor Regression in a Mouse Model of Castration-Resistant Prostate Cancer. v. 1305, n. November 2012, 2013.

ARRUZAZABALA, M. DE L. et al. Effects of coconut oil on testosterone-induced prostatic hyperplasia in Sprague-Dawley rats. **Journal of Pharmacy and Pharmacology**, v. 59, n. 7, p. 995–999, jul. 2007.

ASADA, Y. et al. 5 ␣ -Reductase Type 2 Is Constitutively Expressed in the Dermal Papilla and Connective Tissue Sheath of the Hair Follicle in Vivo But Not during Culture in Vitro *. v. 86, n. 6, p. 1–6, 2001.

BACHMANN, C. Serenoa repens for benign prostatic hyperplasia. **Schweizerische Zeitschrift fur GanzheitsMedizin**, v. 27, n. 4, p. 202–206, 2015.

BANERJEE, P. P. et al. Age-dependent and lobe-specific spontaneous hyperplasia in the Brown Norway rat prostate. **Biology of Reproduction**, v. 59, n. 5, p. 1163–1170, 1998.

BARTSCH, G.; RITTMASTER, R.; KLOCKER, H. **Dihydrotestosterone and the concept of 5alpha-reductase inhibition in human benign prostatic hyperplasiaWorld journal of urology**, 2002.

BHARGAVA, S.; CANDA, A. E.; CHAPPLE, C. R. A rational approach to benign prostatic hyperplasia evaluation : recent advances. p. 1–6, 2004.

BIANCARDI, M. F. et al. Prenatal testosterone exposure as a model for the study of endocrine-disrupting chemicals on the gerbil prostate. **Experimental Biology and Medicine**, v. 237, n. 11, p. 1298–1309, 2012.

CALDER, P. C. **Polyunsaturated fatty acids, inflammatory processes and inflammatory bowel diseasesMolecular Nutrition and Food Research**, ago. 2008.

CAMPOS, S. G. P. et al. Age-related histopathological lesions in the Mongolian gerbil ventral prostate as a good model for studies of spontaneous hormone-related disorders. **International Journal of Experimental Pathology**, v. 89, n. 1, p. 13–24, 2008.

CARBAJAL, D. et al. Therapeutic effect Of D-004, a lipid extract from roystonea regia fruits, on prostate hyperplasia induced in rats. **Drugs under Experimental and Clinical Research**, v. 31, n. 5–6, p. 193–197, 2005.

CARSON, C.; RITTMASTER, R. THE ROLE OF DIHYDROTESTOSTERONE IN BENIGN. p. 2–7, 2003.

CASTRO, N. F. DA C. et al. Therapeutic effects of β-caryophyllene on proliferative disorders and inflammation of the gerbil prostate. **Prostate**, n. May, 2021.

CESANO, A. et al. Opposite effects of linoleic acid and conjugated linoleic acid on human prostatic cancer in SCID mice. **Anticancer research**, v. 18, n. 3A, p. 1429–1434, 1998.

CHOI, N. G. et al. Apoptosis and nuclear shapes in benign prostate hyperplasia and prostate adenocarcinoma: Comparison with and relation to Gleason score. **International Journal of Urology**, v. 6, n. 1, p. 13–18, 1999.

CLARK, R. V. et al. Marked Suppression of Dihydrotestosterone in Men with Benign Prostatic Hyperplasia by Dutasteride, a Dual 5α-Reductase Inhibitor. **Journal of Clinical Endocrinology and Metabolism**, v. 89, n. 5, p. 2179–2184, 2004.
CORDEIRO, R. S. et al. Androgen receptor in the Mongolian gerbil ventral prostate: Evaluation during different phases of postnatal development and following androgen blockage. **Micron**, v. 39, n. 8, p. 1312–1324, 2008.
CORRADI, L. S. et al. Inhibition of 5-α-reductase activity induces stromal remodeling and smooth muscle de-differentiation in adult gerbil ventral prostate. **Differentiation**, v. 72, n. 5, p. 198–208, 2004.
CORRADI, L. S. et al. Increased androgen receptor and remodeling in the prostatic stroma after the inhibition of 5-alpha reductase and aromatase in gerbil ventral prostate. **Microscopy Research and Technique**, v. 72, n. 12, p. 939–950, 2009.
CORRADI, P. F.; CORRADI, R. B.; GREENE, L. W. Physiology of the Hypothalamic Pituitary Gonadal Axis in the Male. **Urologic Clinics of North America**, v. 43, n. 2, p. 151–162, 2016.
DE JESUS, M. M. et al. Histopathological alterations in the prostates of Mongolian gerbils exposed to a high-fat diet and di-n-butyl phthalate individually or in combination. **Reproductive Toxicology**, v. 52, p. 26–39, 2015.
DI DONATO, G.; LAUFER-AMORIM, R.; PALMIERI, C. Nuclear morphometry in histological specimens of canine prostate cancer: Correlation with histological subtypes, Gleason score, methods of collection and survival time. **Research in Veterinary Science**, v. 114, n. April, p. 212–217, 2017.
DIALLO, J. S. et al. Co-assessment of cytoplasmic and nuclear androgen receptor location in prostate specimens: Potential implications for prostate cancer development and prognosis. **BJU International**, v. 101, n. 10, p. 1302–1309, maio 2008.
DOS SANTOS, F. C. A. et al. Structure, histochemistry and ultrastructure of the epithelium and stroma in the gerbil (Meriones unguiculatus) female prostate. **Tissue and Cell**, v. 35, n. 6, p. 447–457, 2003.
EOM, J. H. et al. Bawu decoction () ameliorates benign prostatic hyperplasia in rats. **Chinese Journal of Integrative Medicine**, v. 23, n. 8, p. 611–616, 2017.
EYRES, L. et al. Coconut oil consumption and cardiovascular risk factors in humans. **Nutrition Reviews**, v. 74, n. 4, p. 267–280, 2016.
GALBRAITH, S. M.; DUCHESNE, G. M. Clinical Oncology Update: Prostate Cancer Androgens and Prostate Cancer: Biology, Pathology and Hormonal Therapy INTRODUCTION-PATHWAYS OF ANDROGEN PRODUCTION AND MECHANISMS OF ACTION. **European 3oumal of Cancer**, v. 33, n. 4, p. 545–554, 1997.
GAO, W.; BOHL, C. E.; DALTON, J. T. Chemistry and structural biology of androgen receptor. **Chemical Reviews**, v. 105, n. 9, p. 3352–3370, 2005.
GUERRA, L. H. A. et al. Do mineral and corn oil serve as potential endocrine disruptors in the gerbil prostate? **Reproductive Toxicology**, v. 90, p. 141–149, 1 dez. 2019.
HABIB, F. K. et al. Serenoa repens (Permixon®) inhibits the 5α-reductase activity of human prostate cancer cell lines without interfering with PSA expression. **International Journal of Cancer**, v. 114, n. 2, p. 190–194, 2005.
HAGEN, R. M.; RHODES, A.; LADOMERY, M. R. Conjugated linoleate reduces prostate cancer viability whereas the effects of oleate and stearate are cell line-dependent. **Anticancer research**, v. 33, n. 10, p. 4395–4400, out. 2013.
HUI, Y. H. **Bailey's Industrial Oil and Fat Products, Volume 5, Edible Oil and Fat Products: Processing Technologies, 6th Edition**. 1996.

HUTTUNEN, E.; ROMPPANEN, T.; HELMINEN, H. J. A histoquantitative study on the effects of castration on the rat ventral prostate lobe. **Journal of anatomy**, v. 132, n. Pt 3, p. 357–35770, 1981.
HUYNH, H. et al. Co-administration of finasteride and the pure anti-oestrogen ICI 182,780 act synergistically in modulating the IGF system in rat prostate. **The Journal of endocrinology**, v. 171, n. 1, p. 109–118, out. 2001.
ISAACS, J. T. Antagonistic effect of androgen on prostatic cell death. **The Prostate**, v. 5, n. 5, p. 545–557, 1984.
JAYAWARDENA, R. et al. Effect of coconut oil on cardio-metabolic risk: A systematic review and meta-analysis of interventional studies. **Diabetes and Metabolic Syndrome: Clinical Research and Reviews**, v. 14, n. 6, p. 2007–2020, 2020.
JEON, W. Y. et al. Inhibitory effects of Ponciri Fructus on testosterone-induced benign prostatic hyperplasia in rats. **BMC Complementary and Alternative Medicine**, v. 17, n. 1, p. 1–10, 2017.
KABARA, J. J. Health oils from the tree of life. **Pharmacological effect of Lipids**, p. 642–649, 2013.
KIM, E. H.; LARSON, J. A.; ANDRIOLE, G. L. Management of Benign Prostatic Hyperplasia. **annualreviews.org**, v. 67, p. 137–151, 14 jan. 2015.
KIRIYA, C. et al. Purple rice extract inhibits testosterone-induced rat prostatic hyperplasia and growth of human prostate cancer cell line by reduction of androgen receptor activation. **Journal of Food Biochemistry**, v. 43, n. 9, 1 set. 2019.
KWON, Y. Use of saw palmetto (Serenoa repens) extract for benign prostatic hyperplasia. **Food Science and Biotechnology**, v. 28, n. 6, p. 1599–1606, 2019.
LAM, J. et al. Long-term treatment with finasteride in men with symptomatic benign prostatic hyperplasia: 10-year follow-up. **Elsevier**, 2003.
LANGAN, R. C. **Benign Prostatic HyperplasiaPrimary Care - Clinics in Office Practice**W.B. Saunders, , 1 jun. 2019. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/31030823/>. Acesso em: 24 jun. 2021
LANZ, C. et al. The importance of antiandrogen in prostate cancer treatment. **Annals of Translational Medicine**, v. 7, n. S8, p. S362–S362, 2019.
LAPPANO, R. et al. The lauric acid-activated signaling prompts apoptosis in cancer cells. **Cell Death Discovery**, v. 3, n. 1, 4 dez. 2017.
LIANG, T.; LIAO, S. Inhibition of steroid 5α-reductase by specific aliphatic unsaturated fatty acids. **Biochemical Journal**, v. 285, n. 2, p. 557–562, 1992.
MADERSBACHER, S.; SAMPSON, N.; CULIG, Z. Pathophysiology of Benign Prostatic Hyperplasia and Benign Prostatic Enlargement: A Mini-Review. **Gerontology**, v. 65, n. 5, p. 458–464, 2019.
MARKER, P. C. et al. Hormonal, cellular, and molecular control of prostatic development. **Developmental Biology**, v. 253, n. 2, p. 165–174, 2003.
MCPHERSON, S. et al. Elevated androgens and prolactin in aromatase-deficient mice cause enlargement, but not malignancy, of the prostate gland. **academic.oup.com**, 2001.
MIYAMOTO, H.; MESSING, E. M.; CHANG, C. Androgen deprivation therapy for prostate cancer: Current status and future prospects. **Prostate**, v. 61, n. 4, p. 332–353, 2004.
MUNIYAPPA, R. et al. Effects of testosterone administration on nocturnal cortisol secretion in healthy older men. **Journals of Gerontology - Series A Biological Sciences and Medical Sciences**, v. 65 A, n. 11, p. 1185–1192, nov. 2010.

NICKERSON, P. A. Effect of testosterone, dexamethasone and hypophysectomy on membranous whorls in the adrenal gland of the Mongolian gerbil. **The Anatomical Record**, v. 174, n. 2, p. 191–203, 1972.
NIEDERPRÜM, H. et al. Testosterone 5α-reductase inhibition by free fatty acids from Sabal serrulata fruits. **Elsevier**, 1994.
NIEDERPRÜM, H. J.; SCHWEIKERT, H. U.; ZÄNKER, K. S. Testosterone 5α-reductase inhibition by free fatty acids from Sabal serrulata fruits. **Phytomedicine**, v. 1, n. 2, p. 127–133, 1 set. 1994.
NOA, M. et al. Effect of D-004, a lipid extract from Cuban royal palm fruit, on histological changes of prostate hyperplasia induced with testosterone in rats. **International Journal of Tissue Reactions**, v. 27, n. 4, p. 203–211, 2005.
OLIVEIRA, S. M. et al. Cellular and extracellular behavior in the gerbil (Meriones unguiculatus) ventral prostate following different types of castration and the consequences of testosterone replacement. **Cell Biology International**, v. 31, n. 3, p. 235–245, 2007.
OYELOWO, O. et al. Fatty acids in some cooking oils as agents of hormonal manipulation in a rat model of benign prostate cancer. **Nigerian Journal of Physiological Sciences**, v. 34, n. 1, p. 69–75, 2019.
PATEL, A. K.; CHAPPLE, C. R. Benign prostatic hyperplasia : treatment in primary care. v. 333, n. September, p. 535–539, 2006.
PEGORIN DE CAMPOS, S. G. et al. Biological behavior of the gerbil ventral prostate in three phases of postnatal development. **Anatomical Record - Part A Discoveries in Molecular, Cellular, and Evolutionary Biology**, v. 288, n. 7, p. 723–733, 2006.
RASTRELLI, G. et al. Testosterone and Benign Prostatic Hyperplasia. **Sexual Medicine Reviews**, v. 7, n. 2, p. 259–271, 2019.
RAYNAUD, J. et al. Inhibition of type 1 and type 2 5α-reductase activity by free fatty acids, active ingredients of Permixon®. **Elsevier**, 2002.
ROCHEL-MAIA, S. S. et al. Estrogen receptors alpha and beta in male and female gerbil prostates. **Biology of Reproduction**, v. 88, n. 1, p. 1–7, 2013.
ROCHEL, S. S. et al. Lobe identity in the Mongolian gerbil prostatic complex: A new rodent model for prostate study. **Anatomical Record**, v. 290, n. 10, p. 1233–1247, 2007.
RUSSELL, D. W.; WILSON, J. D. STEROID 5ct-REDUCTASE : TWO GENES / TWO ENZYMES. **Annual Review of Biochemistry**, v. 63, p. 25–61, 1994.
SACKS, F. M. Coconut Oil and Heart Health: Fact or Fiction? Running Title: Coconut Oil and Heart Health: Fact or Fiction? p. 1–6, 2020.
SANDHU, J.; REPORTS, A. T.-C. U.. The role of 5-α-reductase inhibition as monotherapy in view of the MTOPS data. **Springer**, 2004.
SANTOS, F. C. A. et al. Testosterone stimulates growth and secretory activity of the female prostate in the adult gerbil (Meriones unguiculatus). **Biology of Reproduction**, v. 75, n. 3, p. 370–379, 2006.
SCARANO, W. R. et al. Oestrogen supplementation following castration promotes stromal remodelling and histopathological alterations in the Mongolian gerbil ventral prostate. **International Journal of Experimental Pathology**, v. 89, n. 1, p. 25–37, 2008.
SCARANO, W. R.; VILAMAIOR, P. S. L.; TABOGA, S. R. Tissue evidence of the testosterone role on the abnormal growth and aging effects reversion in the gerbil (Meriones unguiculatus) prostate. **Anatomical Record - Part A Discoveries in Molecular, Cellular, and Evolutionary Biology**, v. 288, n. 11, p. 1190–1200, 2006.

SCHULMAN, C. Use of 5-α-reductase inhibitors in the prevention of prostate cancer. **Annales d'urologie**, v. 38, n. SUPPL. 2, p. S35–S42, 2004.
SHAPPELL, S. B. et al. Prostate Pathology of Genetically Engineered Mice: Definitions and Classification. The Consensus Report from the Bar Harbor Meeting of the Mouse Models of Human Cancer Consortium Prostate Pathology Committee. **Cancer Research**, v. 64, n. 6, p. 2270–2305, 2004.
SHEELA, D. L. et al. Lauric acid induce cell death in colon cancer cells mediated by the epidermal growth factor receptor downregulation: An in silico and in vitro study. **Human and Experimental Toxicology**, v. 38, n. 7, p. 753–761, 1 jul. 2019.
SHIRAI, T. et al. Experimental prostate carcinogenesis - Rodent models. **Mutation Research - Reviews in Mutation Research**, v. 462, n. 2–3, p. 219–226, 2000.
SINISI, A. A. et al. Sexual differentiation. **Journal of endocrinological investigation**, v. 26, n. 3 Suppl, p. 23–28, 2003.
SONG, H.-J. et al. Conjugated linoleic acid inhibits proliferation and modulates protein kinase C isoforms in human prostate cancer cells. **Nutrition and cancer**, v. 49, n. 1, p. 100–108, 2004.
TAN, M. E. et al. Androgen receptor: Structure, role in prostate cancer and drug discovery. **Acta Pharmacologica Sinica**, v. 36, n. 1, p. 3–23, 2015.
TENG, M. et al. Impact of coconut oil consumption on cardiovascular health: A systematic review and meta-Analysis. **Nutrition Reviews**, v. 78, n. 3, p. 249–259, 2020.
THIBOUTOT, D. et al. Activity of the type 1 5α-reductase exhibits regional differences in isolated sebaceous glands and whole skin. **Journal of Investigative Dermatology**, v. 105, n. 2, p. 209–214, 1995.
THIGPEN, A. E. et al. Molecular genetics of steroid 5α-reductase 2 deficiency. **Journal of Clinical Investigation**, v. 90, n. 3, p. 799–809, 1992.
THORPE, A.; NEAL, D. Benign prostatic hyperplasia. v. 361, p. 1359–1367, 2003.
VANDERSTICHELE, H. et al. The effects of neonatal androgenization of male rats on testosterone metabolism by the hypothalamus-pituitary-gonadal axis. **Journal of Steroid Biochemistry**, v. 26, n. 4, p. 493–497, 1987.
VELTRI, R. W. et al. Saw palmetto alters nuclear measurements reflecting DNA content in men with symptomatic BPH: Evidence for a possible molecular mechanism. **Urology**, v. 60, n. 4, p. 617–622, 2002.
VELTRI, R. W. et al. Significant variations in nuclear structure occur between and within Gleason grading patterns 3, 4, and 5 determined by digital image analysis. **The Prostate**, v. 67, n. 11, p. 1202–1210, ago. 2007.
VICKMAN, R. E. et al. The role of the androgen receptor in prostate development and benign prostatic hyperplasia: A review. **Asian Journal of Urology**, v. 7, n. 3, p. 191–202, 2020.
VILAMAIOR, P. S. L. et al. Collagen fiber reorganization in the rat ventral prostate following androgen deprivation: A possible role for smooth muscle cells. **Prostate**, v. 45, n. 3, p. 253–258, 2000.
VILAMAIOR, P. S. L.; TABOGA, S. R.; CARVALHO, H. F. Postnatal growth of the ventral prostate in Wistar rats: A stereological and morphometrical study. **Anatomical Record - Part A Discoveries in Molecular, Cellular, and Evolutionary Biology**, v. 288, n. 8, p. 885–892, 2006.
WALLACE, T. C. Health Effects of Coconut Oil—A Narrative Review of Current Evidence. **Journal of the American College of Nutrition**, v. 38, n. 2, p. 97–107, 2019.

WEIBEL, E. R. Principles and methods for the morphometric study of the lung and other organs. **Laboratory investigation; a journal of technical methods and pathology**, v. 12, p. 131–155, fev. 1963.
YASSIN, A. et al. **Testosterone, testosterone therapy and prostate cancerAging Male**Taylor and Francis Ltd, , 2 out. 2019. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/30614347/>. Acesso em: 28 jun. 2021

Table 1. Results of fatty acids methyl esters from coconut oil.

	Relative percentage*		mg/g fat**	
FAME	Value	%RSD	Value	%RSD
C8:0 - Methyl Octanoate	3.61	3.03	30.13	1.81
C10:0 - Methyl Decanoate	5.85	4.70	47.97	3.75
C12:0 - Methyl Laurate	50.33	2.23	402.18	1.13
C14:0 - Methyl Myristate	19.50	2.11	152.73	0.91
C16:0 - Methyl Palmitate	9.83	1.94	75.78	0.70
C16:1 - Methyl Palmitoleate	0.02	15.03	0.13	15.80
C18:0 - Methyl Stereate	3.03	1.89	23.03	0.68
C18:1 - Methyl Oleate	6.56	1.85	49.88	0.65
C18:2 - Methyl Linoleate	1.10	1.59	8.32	0.45
C20:0 - Methyl Arachidate	0.07	3.76	0.54	3.02

*Relative percentage: it is a semi quantitation calculation given by relative percentage of fatty acids in the sample;
** mg/g fat: it is the quantitative method (using internal standard) for fatty acids per fat gram.

Table 2. Biometric and hormonal parameters of Mongolian gerbils in IC (Intact Control), HI (Hyperplasia Induced) and HCO (Hyperplasia Coconut Oil) groups (n=7).

Paremeters	**Groups**		
Biometric data (n=7)	**IC**	**HI**	**HCO**
Body weight (g)	70.29 ± 8.597	79.14 ± 7.198	80.57 ± 8.923
Prostatic complex weight (g)	0.685 ± 0.130^{a}	1.208 ± 0.1404^{b}	1.269 ± 0.095^{b}
Relative weight of prostate complex ($x10^{-3}$)	9.69 ± 0.722^{a}	15.31 ± 1.724^{b}	15.84 ± 1.252^{b}
Ventral prostate weight (g) ($x10^{-3}$)	19.86 ± 6.094^{a}	44.86 ± 6.309^{b}	41 ± 11.83^{b}
Relative weight of ventral prostate ($x10^{-4}$)	2.824 ± 0.864^{a}	5.727 ± 1.042^{b}	5.054 ± 1.192^{b}
Testis weight (g) ($x10^{-2}$)	51.56 ± 4.139^{a}	32.70 ± 2.587^{b}	31.63 ± 7.313^{b}
Adrenal weight (g) ($x10^{-3}$)	16.71 ± 1.799^{a}	22.00 ± 4.000^{b}	23.57 ± 4.614^{b}
Liver weight (g)	2.656 ± 0.432	2.970 ± 0.358	2.983 ± 0.474
Pelvic fat weight (g)	1.013 ± 0.242	1.520 ± 0.4912	1.623 ± 0.5424
Hormonal data (n=7)			
Testosterone (pg/ml)	1.909 ± 0.522^{a}	7.365 ± 3.120^{b}	6.454 ± 0.622^{b}
Cortisol (pg/ml)	79.0 ± 27.12	125.6 ± 79.12	140.0 ± 83.52

Values expressed as mean ± standard deviation. Differences between control and treatments are statistically significant at $p \leq 0.05$. Superscript letters (a,b) represent statistically significant differences between the groups.

Table 3. Values of the Stereological and morphometric parameters of the gerbil ventral prostate (n=7).

Parameters	IC	HI	HCO
Morphometry (µm)			
Epithelium height	13.24 ± 5.098[a]	23.9 ± 9.342[b]	11.21 ± 4.206[c]
Muscular stroma thickness	6.188 ± 2.075[a]	13.65 ± 5.33[b]	6.913 ± 2.668[a]
Stereology (%)			
Epithelium	15.62 ± 5.226[a]	22.66 ± 7.463[b]	13.89 ± 5.694[a]
Lumen	57.54 ± 13.42[a]	43.68 ± 13.82[b]	63.08 ± 13.65[a]
Muscular stroma	7.179 ± 2.942[a]	9.167 ± 4.411[b]	5.516 ± 2.156[a]
Non muscular stroma	13.76 ± 7.358	14.59 ± 7.068	13.34 ± 9.507
Karyometric data (µm²)			
Nuclear area	19.79 ± 5.786[a]	24.85 ± 6.604[b]	13.29 ± 4.064[c]

Values expressed as mean ± standard deviation. Differences between control and treatments are statistically significant at P≤0.05. Superscript letters (a,b,c) represent statistically significant differences between the groups.

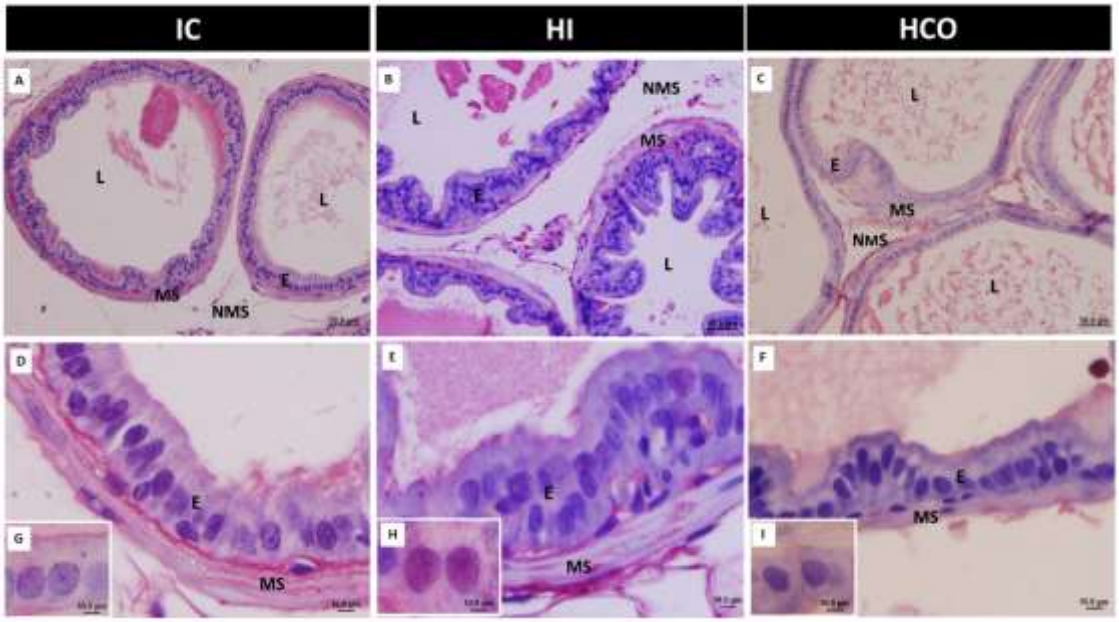


Figure 1. Morphological analysis of the gerbil ventral prostate stained by picrosirius. Group IC (A,D,G), HI (B,E,H) and HCO (C,F,I). L (lumen), MS (muscular stroma), NMS (non muscular stroma), E (epithelium).

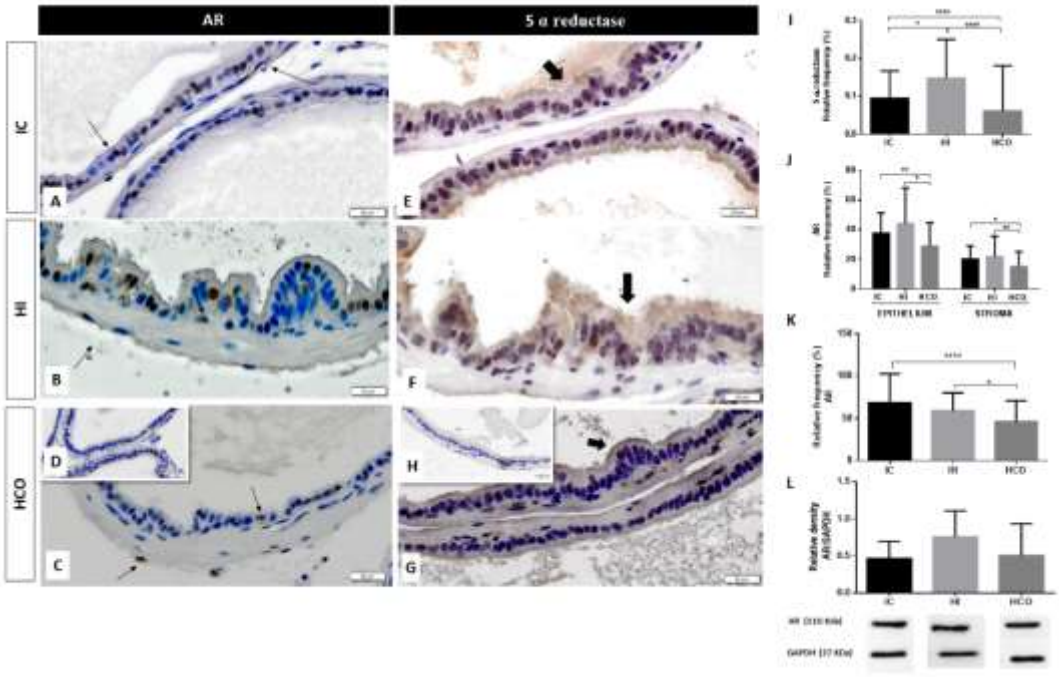
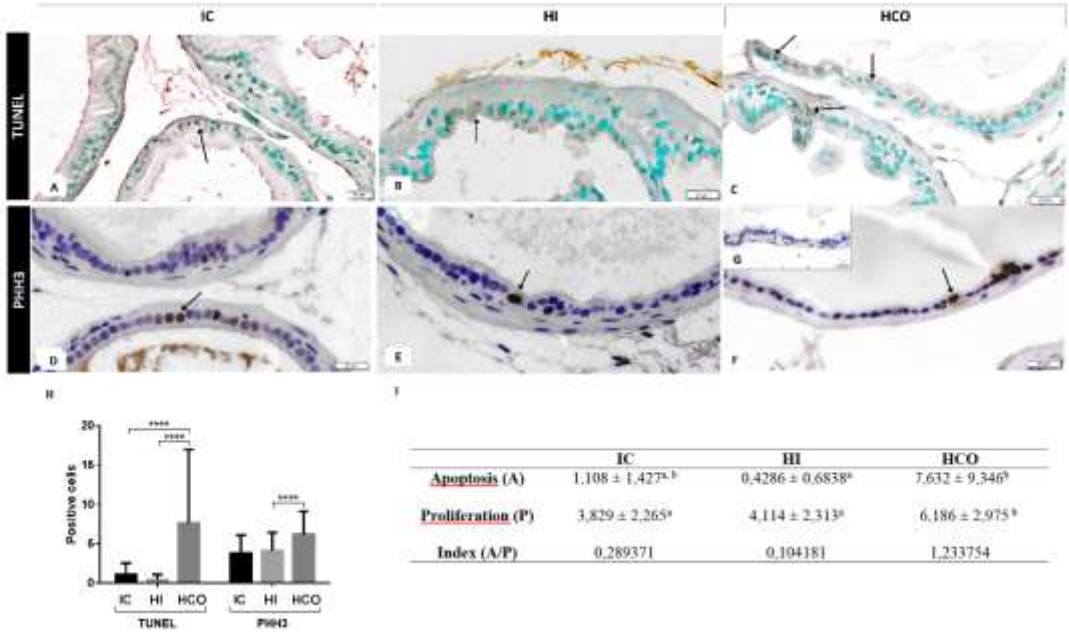
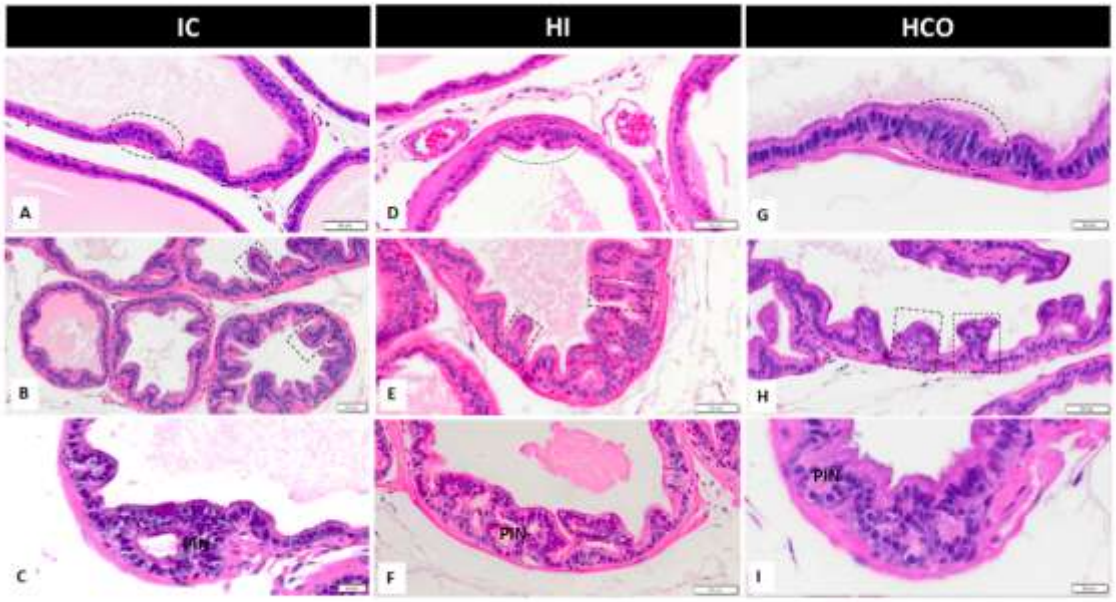


Figure 2. Histological sections of gerbils ventral prostate submitted to immunohistochemistry for Androgen receptor (AR) (A-C) and 5 α-reductase (E-G). Negative control of the reactions (D, H). AR positive markings (thin arrows), 5 α-reductase (wide arrows). Relative frequency (%) of total 5 α-reductase (I) and of AR-positive cells present in epithelium and stroma (J) and sum of compartments (K) of groups IC, HI and HCO. Relative density of AR and normalized by GAPDH (positive control) (L). Statistically significant differences between groups (* p <0.05; ** p <0.01; **** p <0.0001), according to parametric (ANOVA) and non-parametric (Kruskal-Wallis) tests for group differences, followed by Bonferroni and Dunn's multiple comparison tests, respectively.



	IC	HI	HCO
Apoptosis (A)	1,108 ± 1,427[a,b]	0,4286 ± 0,6838[a]	7,632 ± 9,346[b]
Proliferation (P)	3,829 ± 2,265[a]	4,114 ± 2,313[a]	6,186 ± 2,975[b]
Index (A/P)	0,289371	0,104181	1,233754

Figure 3. Histological sections of gerbils ventral prostate submitted to TUNEL reaction (apoptosis) (A-C) and immunostaining PHH3 (proliferating cells) (D-F) and cell death by TUNEL reaction (D-F). IC (A,D), HI (B,E) and HCO (C, F). Negative control of the PHH3 reaction (G). Relative frequency (%) of PHH3-positive cells and apoptosis cells present in ventral prostate of groups IC, HI and HCO (H). Index of proliferation and apoptosis (A/P) cells in gerbil ventral prostate (I). Values expressed as mean ± standard deviation. Differences between control and treatments are statistically significant at P≤0.05. Superscript letters (a,b) represent statistically significant differences between the groups.



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Lesions	IC	HI	HCO
Multiplicity			
Papillary epithelial atypia (PEA)	14-36 (15, 95) [a]	8-61 (23,19) [a,b]	7-42 (22,36) [b]
Flat epithelial atypia (FEA)	7 - 42 (17,86)	4 - 31 (17,95)	10 - 47 (24)
Prostatic intraepithelial neoplasia (PIN)	2 - 9 (4, 54) [a]	0 - 62 (16,66) [b]	4 - 41 (11,63) [a,b]
Incidence (%)			
Papillary epithelial atypia (PEA)	100%	100%	100%
Flat epithelial atypia (FEA)	100%	100%	100%
Prostatic intraepithelial neoplasia (PIN)	100%	100%	100%

Figure 4. Histological sections of the ventral prostate of Mongolian gerbils stained by Hematoxylin-eosin from different experimental groups. Flat epithelial atypia (circles), papillary epithelial atypia (squares) and prostatic intraepithelial neoplasia (PIN). Analyses of multiplicity and incidence (J) of prostatic lesions in ventral lobes of Mongolian gerbils. Differences between control and treatments are statistically significant at $p \leq 0.05$. Superscript letters (a,b) represent statistically significant differences among the groups.

5.2. Coconut oil: friend or foe in benign prostatic hyperplasia?

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Abstract

Benign prostatic hyperplasia (BPH) affects over 40% of men over 40 years of age and causes several symptoms that affect the quality of life of these patients. Even with a high prevalence, its pathophysiology is misunderstood. Some factors that contribute to this condition are hormonal changes, as well as the imbalance of its receptors and chronic inflammation. Coconut oil (CO) is composed of substances that to reduce inflammation, but its effects of this oil on the prostate are still scarce. The present study evaluated the effects of CO on the hyperplastic prostate of gerbils. We used 36 adult male gerbils divided into 3 groups: C (control group), T (3mg/kg of testosterone cypionate on alternate days, for 30 days) and TCO (3mg/kg of testosterone cypionate on alternate days and for 30 days and then CO 1ml/kg daily for 30 days). We observed the reduction in the expression of estrogen receptors type α and β, COX-2, MMP-9 and in inflammatory foci in the TCO group compared to the T group. The frequency of MMP-2 increased in both groups receiving testosterone, and there was a reduction of F4/80-positive macrophages in the stroma and CD68 and CD163-positive macrophages in the epithelium. These results demonstrate that CO may minimize inflammation in this gland, and suggests a beneficial natural product to treatment of BPH.

Keywords: inflammation, macrophages, estrogen receptor, testosterone, gerbils

Introduction

The benign and uncontrolled growth of the prostate, called benign prostate hyperplasia (BPH), results from stromal and epithelial hyperplasia of the gland (NOA et al., 2005). This increase of the prostate can compress the urethra channel, causing its partial or total interruption, interfering with the normal flow of urine and causing difficulty urinating, incontinence, and terminal dripping that affects the quality of life of patients, which are common symptoms of LUTS (lower urinary tract symptoms) (BAUMAN et al., 2014; CARRERO-LÓPEZ; MI, 2016). It is the most common benign neoplasm of aging in men, being present in approximately 40% of men in the 4th decade of life and its prevalence increases almost linearly to about 80% in those over 80 years of age (LANGAN, 2019). BPH has a high prevalence and the pathophysiology of this disease is still not completely understood (LANGAN, 2019; MADERSBACHER; SAMPSON; CULIG, 2019).

Some factors that contribute to this condition have been observed as hormonal changes and the imbalance of its receptors (CHOI et al., [s.d.]), which cause proliferation of the epithelial and stromal cells of the organ (KRUŠLIN et al., 2017) and chronic inflammation (KRUŠLIN et al., 2017; MENDES et al., 2018). Albeit the prostate development and maintenance are directed by androgens, estrogens also influence prostate homeostasis as it expresses both alpha-type (ERα) and beta-type (ERβ) estrogen receptors (GRINDSTAD et al., 2016; HORVATH et al., 2001). These receptors stimulate specific activities in prostate cells, such as the proliferation of epithelial and stromal cells (CUNHA et al., 1987; MCPHERSON et al., 2001). ERβ has action anticancer and pro-apoptotic, thus being predominantly protective, whereas ERα is oncogenic and promotes cell proliferation and survival (ATTIA; EDERVEEN, 2012; HORVATH et al., 2001; MCPHERSON et al., 2001; RISBRIDGER et al., 2001; ZHU et al., 2004). Thus, variation in the expression of ERα and ERβ receptors has been considered one of the hormonal risk factors associated with the development of BPH and prostate cancer (CHOI et al., 1999).

In addition, chronic inflammation has been observed to coexist with histological changes of BPH, suggesting that inflammation plays a role in the development of BPH, through the secretion of cytokines, chemokines, and growth factors involved in the inflammatory response, causing the growth of the prostate (KRUŠLIN et al., 2017; MENDES et al., 2018; SOLER et al., 2013). The origin of inflammation in the prostate is believed to be multifactorial, due to a chronic wound healing process, which activates

proliferation pathways and results in the enlargement of the gland (BOSTANCI et al., 2013; QUINTERO-GARCÍA et al., 2018).

Several medications are used to relieve these symptoms as well as to prevent disease progression and the development of complications. However, these benefits need to be balanced against the potential side effects of these treatments (PATEL; CHAPPLE, 2006; SANDHU; REPORTS; 2004 ; SHUKLA et al., [s.d.]). Studies suggest that natural compounds are effective in treating, improving BPH symptoms such as *Serenoa repens* extract, *Cucurbita pepo* (pumpkin) seed extract, *Urtica dioica (*stinging nettle) root extract, *Opuntia* (cactus) flower extract, *Hypoxis rooperi* (South African star herb) extract, *Pygeum africanum* (African plum tree) extract (CICERO et al., 2019; EOM et al., 2017a; HABIB et al., 2005; PATEL; CHAPPLE, 2006).

The coconut oil is a plant-based product that reduced prostate gland weight and size in rats with induced BPH was coconut oil (ARRUZAZABALA et al., 2007). This oil obtained from the species *Cocos nucifera* from fresh coconut pulp, has been used as food, supplementation and recently its biological properties have been widely investigated (JAMJAI et al., 2019; KABARA, 2013; SHEELA et al., 2019; TENG et al., 2020; WALLACE, 2019; YEAP et al., 2015). Studies show the effectiveness of coconut oil to promote immune system response and aid in anti-inflammatory reactions (RATHEESH et al., 2017; WAN; GRIMBLE, 1987). This effect is related to its composition which has about 90% saturated fatty acids, with lauric acid being the most abundant (AKPAN et al., 2006), followed by myristic, palmitic, caprylic, capric, and stearic acids (HUI, 1996). The lauric and the capric acids are components that have been shown to be important in acting on the anti-inflammatory response (HUANG et al., 2014; PEREIRA; DA SILVA; LANGONE, 2004; WITCHER; NOVICK; SCHLIEVERT, 1996). The fatty acids present in the unsaturated portion of this oil (about 9%), which have two or more double bonds are called polyunsaturated fatty acid (PUFA). These comprise oleic and linoleic acids and these are important constituents of cells and can act as substrates for the synthesis of eicosanoids, which include prostaglandins, thromboxanes, and leukotrienes that are key mediators and regulators of inflammation (BENNETT; GILROY, 2016; CALDER, 2008; FANG et al., 2007; ISHIHARA; YOSHIDA; ARITA, 2019). Therefore, the effect of coconut oil on these inflammatory mediators may influence the anti-inflammatory response in the prostate and reduce the effects of BPH.

Nevertheless, usually, the coconut oil extracts are not submitted to the same analyses as conventional drugs and, therefore, investigations covering more thorough histological analyses are necessary so that treatments with these compounds can be safely used. Thus, this work aimed to evaluate the effect of coconut oil on the ventral prostate of adult gerbils testosterone-induced hyperplasia.

Material and Methods

Animals and Experimental design

Thirty six adult (120 days old) males Mongolian gerbils (*Meriones unguiculatus*) were obtained from the Animal Reproduction Center of the Institute of Bioscience, Humanities and Exact Sciences (IBILCE) of the São Paulo State University (UNESP) and kept in polyethylene cages under controlled conditions of light (12 h dark/ 12 h light) and temperature (24 °C), with food and filtered water ad libitum. The experiments were performed following the ethical principles recommended by the National Council for the Control of Animal Experimentation (CONCEA) and the procedures involved were evaluated by the Ethics Committee for the Use of Animals of IBILCE / UNESP (Proc. No. 175/2017).

The animals were divided into three groups (n=12): Control (C) which received no treatment, Testosterone (T), received subcutaneous injections of Testosterone Cypionate (3 mg/Kg) diluted in corn oil (ARRUZAZABALA et al., 2007; JEON et al., 2017) on alternate days for 30 days and were euthanized 30 days after the end of supplementation, and Testosterone with Coconut Oil (TCO), animals received, on alternate days for 30 days, subcutaneous injections of Testosterone Cypionate (3 mg/Kg) diluted in corn oil and then, via gavage, for 30 consecutive days, 1 ml / kg / day of coconut oil (ARRUZAZABALA et al., 2007).

After the treatment period, all animals were anesthetized with xylazine hydrochloride (3 mg/kg) and ketamine hydrochloride (10 mg/kg), weighed and euthanized by decapitation to collect body blood, which was centrifuged at 3000 rpm for 20 min for serum recovery and stored at -80 °C. The ventral prostate was removed and weighed. The glands from 5 animals/group were stored and frozen at -80°C for protein quantification by blotting and 7 were fixed in 4% paraformaldehyde for 24 h, dehydrated in ethanol, clarified in xylene, and then included in Histosec (Merck, Darmstadt, Germany). Subsequently, the blocks were sectioned at 5µm for morphological analyses stained with Hematoxylin-eosin for the general analyses of

prostate morphological aspects and Picrosirius and Gomori's Reticulin for collagen fiber analysis.

All images obtained were captured using a slide scanner system (Olympus VS120-S5).

Stereology

The histological sections stained with hematoxylin eosin and picrosirius were digitized using a BX61VS camera (Olympus Corporation, Tokyo, Japan) coupled to an Olympus VS120® Slide Scanning System virtual microscopy (VS120-S5) using Image Pro-Plus 6.0 software (Media Cybernetics, Inc., MD, USA). For the stereological analyses, 30 random field images per group were captured at ×200 magnification and quantification was performed according to the M130 multipoint testing system proposed by Weibel (1968) (WEIBEL, 1963) and applied to the prostate by Huttunen et al. (1981) (HUTTUNEN; ROMPPANEN; HELMINEN, 1981). Thus, we obtain the relative frequency of collagen and blood vessels.

Inflammatory foci

We used 5 histological section per animal stained by HE and scanned at 400x magnification using a Slide Scanner System (Olympus VS120-S5) to analyze the inflammatory foci: intraluminal, periductal and subepithelial. The histopathological classification of foci used follows well-established criteria that has been previously applied for gerbils (CAMPOS et al., 2008; CASTRO et al., 2021; DE JESUS et al., 2015). Multiplicity was calculated from the number of alterations present in each animal and divided by the number of histological section analyzed per animal (average). The incidence of inflammatory foci (expressed in %) was calculated by dividing the number of animals with alterations by the number of animals analyzed per group.

Immunohistochemistry analyses

All immunohistochemical analyses performed in this study are described in Table 1. The sections were deparaffinized and rehydrated, then subjected to antigen retrieval in Tris EDTA pH 9.0 for 60 minutes at 98° C (COX-2, F4/80, CD68 and CD 163) and for 20 minutes at 98°C in 10mM citrate buffer pH 6.0 (MMP-2 and MMP-9). Blocking of endogenous peroxidases was performed with 3% H2O2, followed by non-specific protein blocking with 5% skimmed milk in TBS + 0.1% tween (TBSt) for 30

minutes. The sections were then incubated with primary antibody overnight, and in some sections, the primary antibody was replaced with 1% BSA to obtain the negative control of the reaction. Subsequently, the sections were washed with buffer and incubated with a secondary antibody labeled with peroxidase (CD 163 and CD68: Rabbit ABC Staining System SC-2018 -Santa Cruz Biotechnology CA, USA) or Dako Polymer (COX-2, F4/80 and MMP-2 and MMP-9: Novocastra Novolink RE7230-CE kit, Leica Biosystems, Buffalo Grove, USA), and then stained with diaminobenzidine (DAB, Sigma, St. Louis, MO, USA) and counterstained with Mayer's Haematoxylin. Next, the slides were dehydrated in ethanol, clarified, and mounted in Canada balsam, subsequently they were evaluated under conventional light microscopy. Ten random images were obtained per animal (n=7), for a total of 70 images per group. Were evaluated using DAB brown precipitate that indicates antibody immunoreactivity, positively labeled cells or regions were quantified using Image-Pro-Plus software (Media Cybernetics). To evaluate the expression of estrogen receptors type α and β, we used the frequency of positive cells, calculated by dividing the number of positive nuclei by the total number of nuclei per compartment of the analyzed fields. For the quantification of immunohistochemistry reactions, positive and negative cells were quantified from approximately four thousand cells per group. All epithelial and stromal cells from 10 random fields per animal in each group were counted. The frequency of positive cells was calculated by the ratio of the number of positive cells to the total number of cells in each field and multiplied by 100 to obtain the percentage.

For the analyses of MMP-2 and MMP-9 enzymes, we evaluated prostate tissue using a multipoint system with 160 intersections (modified from Weibel 1963) (WEIBEL, 1963). The results were determined by counting the immunoreactivity, coinciding with the grid intersection divided by the total number of points in the epithelial region of the ventral prostate and expressed as a relative frequency.

The absolute frequency of cells and regions positive with cyclooxygenase 2 (COX-2) (for cells in inflammation) and macrophages (F4/80, CD163 and CD68) were calculated by counting the positively stained cells in the epithelium and stroma. The result expressed is the total number of cells for each compartment of all experimental groups.

Protein extraction

We used ventral prostate (5 from each group), stored at -80°C for protein extraction. They were homogenized in RIPA extraction buffer (Sigma-Aldrich®, StLois, MO, USA), and the protease activity blocked by adding (Sigma- Aldrich®, StLois, MO, USA) and then Triton X100. The samples were crushed and homogenized at 4°C for 60 min under stirring, and then centrifuged for 20 min at 14000 rpm at 4°C for membrane precipitation, the supernatant was removed and stored at -80°C. For quantification, BCA protein assay kit (23227 Thermo Fischer) was used, and the absorbance was measured on an absorbance microplate reader (SPECTROstar Omega, BMG Labtech, Ortenberg, Germany).

Western blot

The expression of ERα and ERβ were evaluated. For this, 15ug proteins were placed in each well on SDS gel and subjected to electrophoresis (90 V for 120 minutes), then the bands were transferred (100 V for approximately 2 hours) to a nitrocellulose membrane (Amersham Protram, 10600003, GE Healthcare, Darmstadt, Germany). The blots were blocked using 5% skimmed milk in TBS + 0.1% tween (TBSt) for 60 minutes at room temperature. Then, they were incubated with the primary antibodies: ER-α (polyclonal rabbit IgG, PA5-34577, Invitrogen, Thermo Fisher Scientific, USA, 1: 1000), diluted in 1% milk and kept overnight at 4°C under agitation and stripped and reprobed for ER-β (polyclonal rabbit IgG, PA1-310B, Invitrogen, Thermo Fisher Scientific, USA, 1: 1: 1000) and GAPDH (monoclonal rabbit, IgG, #2118, Cell Signalling, Danvers, MA, USA, 1:1000) to normalize the quantification. The membranes were then washed in TBSt and incubated at room temperature for 1 hour under agitation with secondary anti-rabbit antibody (goat, IgG, #7074, Cell Signalling, Danvers, MA, USA) labeled with conjugated peroxidase (1:2000). After washing the membranes, antibody detection was revealed with ECL Substrate Pierce reagents (32109, ThermoFischer, Rockford, IL, USA) and a Super Signal (34094, ThermoFischer, Rockford, IL, USA). The membranes were developed on an imaging system machine (ChemiDoc MP, BioRad, Hercules, CA, USA) and the band densities were analyzed by densitometry program - Image J (version 1.52a, Wayne Rasband, NIH, USA).

Serum hormonal analysis

Serum estradiol levels were quantified by ELISA using a high sensitivity kit for estradiol (17beta-estradiol, RE52041H, IBL International, Hamburg, Germany). Readings were performed with a microplate reader (TECAN- Infinite F50).

Statistical analysis

Statistical analysis was performed using GraphPad Prism 6.0 software (GraphPad Software, Inc., CA, USA). Quantitative analysis was based on parametric (ANOVA) and non-parametric (Kruskal-Wallis) tests for group differences, followed respectively by Bonferroni and Dunn's multiple comparison tests. Results are presented in terms of mean ± standard deviation and $p \leq 0.05$ values considered statistically significant.

Results

The results of the stereological analyses for collagen and vessels are presented in table 2. These analyses showed increase of collagen in the T group compared to the C and TCO groups (Figure 1. A; C; E). In the vessels analysis, we found no significant difference, but an apparent increase was observed, although not significant in the T group (Figure 1.C). We observed a change in the pattern of organization of the reticular fibers, as seen by the increase in the T group (Figure 1.D).

We noted inflammatory cell foci in the luminal compartment in some sections of all groups (Figure 2), with no significant difference between them (Table 3). In the subepithelial and periductal stromal region we noticed a great concentration of inflammatory cells in the T group (Figure 2.B; E), confirmed in the multiplicity test (Table 2), in which there was an increase in these foci in group T in relation to the other groups and the TCO showed the same proportion as group C (Table 3).

We detected the expression of MMP 2 (Figure 3. A-C) and MMP 9 (Figure 3. E-G) in the ventral prostate of all groups and quantified by immunoreactive regions in the cytoplasm, lumen, and non-muscle stroma for the respective antibodies. We observed increased expression of MMP-2 in the groups T and TCO when compared to C group (Figure 3.D). In the analysis of MMP-9 enzyme, we found that increase in the T group compared to the other groups and decrease in the TCO group when compared to the T and C groups (Figure 3.I).

We observed positive markings for COX-2 enzyme in both stroma and epithelium of all groups analyzed (Figure 3.J-L). Our analyses showed increased COX-2-positive cells in the T group in both compartments when compared to C and TCO, while in the TCO group these markings showed no difference to the C group (Figure 3.N).

The immunohistochemical analysis for F4/80- macrophages, revealed positive markings in the ventral prostate of all groups (Figure 4. A-C). From the quantification of F4/80-positive cells we observed the reduction of this cells in the epithelium of the T group compared to the other groups (Figure 4-E), but in the stroma, we observed a higher incidence of these cells in the T group when compared to TCO group (Figure 4.E).

In the CD163-macrophages analysis (Figure 4. F-H), we observed a reduction of CD163-positive cells in the TCO group compared to the T, and in the stroma no difference was revealed between the groups analyzed (Figure 4. J). The CD68-positive cells increased in the epithelium of the T and TCO groups when compared to C group (Figure 4.K-M). In the stroma, there was no difference between groups (Figure 4-N).

We investigated ERα positive cells in the epithelial and stromal compartments and noted positive markings in all experimental groups (Figure 5). From the relative frequency of these cells, in the epithelium, we did not observed differences among the groups (Figure 5.E). However, the ERα-positive cells decreased in the stromal compartment of the TCO group when compared to C group (Figure 5.E). When summing the frequencies of both compartments, we noticed the increase of Erα-positive cells in the T group compared to the other groups (Figure 5.F), a pattern also observed in the quantification by western blot, although there was no significant difference (Figure 5.G).

The frequency of ERβ positive cells and their distribution in the prostatic compartments were observed in all experimental groups (Figure 6.E). In the epithelium, no differences were observed among the groups analyzed, while in the stroma there was an increase ERβ-positive cells in the T group (Figure 6.E). We found a reduction in the frequency of these cells in the TCO, compared to C and T groups (Figure 6.F) and the same patterns observed in the analysis of these receptors by western blot (Figure 6.G).

Serum estradiol levels were detected only in groups C (17.55 ±12.62) and T (12.41 ± 16.92), in the HCO group they were not detected because they showed levels

below the sensitivity of the kit, whose datasheet does not provide a value. Therefore, lower than those observed in groups C and T (Table 2).

Discussion

We evaluated how coconut oil affect on testosterone-induced BPH in gerbils, analyzing inflammatory and hormonal parameters not investigated so far for this oil. From our analyses, we observed that CO reduced the expression of COX-2, MMP-9 and inflammatory foci, as well as the presence of specific macrophages in the epithelium and general macrophages in the stroma of the hyperplastic ventral prostate. In addition, it decreased the α- and β-type estrogen receptors in the stroma, indicating that it may act to reduce inflammation in the gland with induced hyperplasia, and is a potential treatment for BHP.

BPH is associated with a condition of inflammation (KRUŠLIN et al., 2017; MENDES et al., 2018). The inflammation process promotes modification of vessel width to facilitate blood flow and permeability to allow plasma proteins to exit and lymphocytes to migrate to the focus of injury (GIL, 2002). Although the results are not significant, we observed a tendency for greater vascularization in the T group. In this group we also observed a significant increase in the collagen fibers frequency when compared to the other groups. Inflammation can cause injury to the epithelium and prostatic stroma (KRUŠLIN et al., 2017), promoting tissue remodeling and altering the pattern of organization of collagen and reticular fibers, as we saw in group T. The collagen accumulation in the prostate is associated with inflammation and both are linked to progressive lower urinary tract symptoms (LUTS) in men (RUETTEN et al., 2021). The proportion of larger collagen bundles appears increased in BPH nodules, suggesting that these fibers may play a role in BPH / LUTS (BAUMAN et al., 2014). Muntzing et al. 1980 (MUNTZING, 1980) described an association between collagen content and rat ventral prostate growth, suggesting that collagen plays a crucial role in the mechanism of limiting prostate growth. In the TCO group, on the other hand, the frequency was equivalent to the C group, demonstrating the effect of CO in reducing collagen deposition in the prostate. Similarly, a progressive atrophy of collagen fibers around epithelial structures occurs in the gerbil prostate after castration (Vilamaior et al., 2000).

Bergers et al. (2000) associated angiogenesis in tumors with increased expression of matrix metalloproteinases (MMPs) and Silva et al. (2015) reported collagen deposition, stromal remodeling, increased chronic inflammation and BPH in

obese rats and associated with high expressions of MMPs (BERGERS et al., 2000; SILVA et al., 2015). MMPs comprise a family of enzymes that degrade the extracellular matrix, participate in physiological cellular processes, and are important modulators of tissue homeostasis. Imbalance of these processes can stimulate cell proliferation, migration, angiogenesis, and malignant development in various tissues, including the prostate (BRUNI-CARDOSO et al., 2010; GILL; PARKS, 2008).

MMP-2 and MMP-9 appear at higher levels in serum or prostate cancer tissue samples (BOWDEN, 1993; LIOTTA, 1986; LOKESHWAR, 1999) and in primary cultures of human prostate tumors (LONDON et al., 2003; PAJOUH et al., 1991). In our analyses, it was possible to observe the increase of MMP-2 with the T-supplemented groups, and the expression of MMP-9 increased in the T group compared to the C and TCO groups. This indicates that testosterone supplementation can alter MMP -2 and -9 levels in the prostate and may be directly involved with BPH, angiogenesis and collagen fibers increase while CO treatment in the hyperplastic prostate, was shown to reduce MMP-9 levels, indicating a relationship with reduced collagen frequency in this group.

Our histological analyses allowed us to observe an increase of inflammatory cells in the subepithelial region and in the periductal stromal region of the T group compared to the others, showing that in the ventral prostate of this group the infiltration of macrophages increased, indicating an inflammatory environment. While the group subsequently treated with CO maintained the same proportion of inflammatory cells as group C, indicating that CO had an anti-inflammatory effect in these conditions caused by testosterone supplementation. Coconut oil showed the same anti-inflammatory effect in other studies that evaluated in several situations (FAMUREWA et al., 2017; INTAHPHUAK; KHONSUNG; PANTHONG, 2010; VARMA et al., 2017; ZAKARIA et al., 2011).

During the inflammatory process, several enzymes are expressed, among them cyclooxygenases (COXs), which are a family of enzymes that catalyze the biosynthesis of prostaglandins. Prostaglandins are important for physiological functions such as vasodilation and platelet aggregation (SOBOLEWSKI et al., 2010), as well as having an important role in mediating inflammation (CHANDRASEKHARAN; SIMMONS, 2004). COX-2 is the isoform regulated by growth factors and different cytokines, such as IL1β, IL6, or TNFα, therefore, overexpressed during inflammation (RAMSAY et al., 2003). The expression of this enzyme was higher in both the epithelium and stroma of

the T group compared to the other groups evaluated. Other studies showed the elevation of this enzyme in animals supplemented with testosterone (CASTRO et al., 2021; KIM et al., 2011). Its expression in prostate tissues, may play a key role in the development of BPH, increasing angiogenesis and tumor metastasis, as well as stimulating cell proliferation and inhibiting apoptosis (CHU et al., 2003; EOM et al., 2017b; KIM et al., 2011; LEUNG et al., 2003; SCARANO; VILAMAIOR; TABOGA, 2006; WADDELL et al., 2018) .

The COX-2 can be detected in inflammatory cells (WANG; BERGH; DAMBER, 2004), thus, the reduction of this enzyme in the epithelium and stroma of the TCO group, while maintaining its expression as in group C, shows that CO may influence the anti-inflammatory response in the prostate, as this oil has been shown to be effective in promoting the immune system response and aiding in anti-inflammatory reactions (WAN; GRIMBLE, 1987) . With this, the changes in MMPs as well as in COX-2 observed in our analyses may be caused by testosterone supplementation and lead to the recruitment and infiltration of inflammatory cells such as macrophages.

Macrophages are not a single cell population with a defined phenotype and biological activity, but a diversity of cell types with different roles depending on homeostatic and pathological conditions (DENARDO, 2019). The F4/80 receptor is generally considered a murine pan macrophage marker (AUSTYN; GORDON, 1981) and is expressed on tumor-associated macrophages (TAMs) (TERRY et al., 2015). Our results showed a reduction of F4/80 positive cells in the epithelium of the T group, but in the stroma we observed the opposite, an increase of these cells in this group. This difference indicates that T supplementation influences the localization of these cells to the stroma, evidencing the relationship between increased macrophage stromal infiltration and BPH (WANG et al., 2012). Furthermore, in the TCO group we observed a low frequency of these cells in both compartments, and this indicate a positive effect of CO in the prostate homeostasis after T supplementation.

We also looked at more specific markers such as CD163, used in association with other markers to identify M2 type macrophages (DENARDO et al., 2009; KRYCZEK et al., 2006; SHIAO et al., 2016; UBIL et al., 2018) and CD68 is a type I transmembrane glycoprotein and is a known pan macrophage marker and can be used to recognize M1 tumoricidal as well as M2 anti-inflammatory macrophages (MYUNG-GYU et al., 2020) . It was possible to verify that in the epithelium of the T group there were more CD163 positive cells than in the TCO group, while in the stroma

there was no difference between the groups, although they were found in larger quantities than in the epithelium. The CD163 marker is specific to identify M2 macrophages and is related to tumor progression (MANIECKI et al., 2012; MEDREK et al., 2012). The reduction of these cells was observed in the epithelium of the TCO group compared to the T group, that can be explained by the action of some components of CO. Among them, the lauric acid, present in higher proportions, proved to be an effective precursor of monolaurin (PEREIRA; DA SILVA; LANGONE, 2004), a compound that was able to modulate the proliferation of immune cells (WITCHER; NOVICK; SCHLIEVERT, 1996) and the capric acid, which significantly inhibited the induction of interleukins, reducing inflammation (HUANG et al., 2014).

The CD68 can also be a marker for TAM (KAYAL et al., 2014) and TAMs mostly polarize towards the M2 phenotype (MANTOVANI et al., 2002) and is associated with poor prognosis (KURAHARA et al., 2011; STEIDL et al., 2010). Therefore, the increase of this marker in the T and TCO groups, may be related to testosterone supplementation, and this increased recruitment is associated with the attempt of organ homeostasis in the face of a hyperplastic condition.

Generally, there is a higher incidence of ERα in an environment with proliferative disorders and prone to the onset of prostatic changes and the development of BPH (ELLEM; RISBRIDGER, 2009; JEON et al., 2017). Both prostatic compartments are sensitive to estrogen, but ERα is mainly detected in the stroma, as it is in the stroma that the aromatase gene is expressed and this enzyme converts androgens to estrogen within the local tissue. The activation of ERα in the stroma is required to activate the epithelial response, so the stroma should be considered as a significant component of the control mechanisms of this organ (ELLEM; RISBRIDGER, 2009).

We observed the reduction of ERα in the stroma of the TCO group compared to the C group, indicating a positive effect of the oil. When analyzing these receptors in both compartments of the ventral prostate of gerbils, we observed the increase of this receptor in group T when compared to the other groups. This result demonstrates the relationship between testosterone supplementation in this group with inflammation, in the same way that the reduction of this receptor in the TCO group compared to the T group, demonstrates the beneficial action of CO on the hyperplastic prostate. This same pattern was observed in the immunoblotting protein expression analysis of this receptor, but it was not sensitive enough to detect this variation, possibly because of the low amount of this protein in the tissue when compared to GAPDH.

ERβ has a protective role, acting against uncontrolled cell proliferation (ATTIA; EDERVEEN, 2012; HORVATH et al., 2001; LINDBERG et al., 2003; MCPHERSON et al., 2001; PRAVETTONI et al., 2007; ZHU et al., 2004), thus, it was expected that there would be a reduction of these receptors in the T group and an increase in the TCO group. The increase of this receptor in the stroma of the T group compared to the other groups suggests that other changes found in our previous studies, such as increased muscle layer thickness, as well as high levels of cell proliferation in the CO-treated group after testosterone supplementation, may be related to the activation of the ERβ pathway.

The low expression of both receptors in the TCO group seen in this analysis may be related to the low levels of serum estrogen found in this group, not allowing their detection by ELISA analysis. Different ratios of PUFAs, as found in CO, have been shown to suppress ER expression in breast cancer cells (ZHANG et al., 2015). DAS et al. 2002 indicates that PUFAs and estrogen may interact with each other and the binding of estrogen to its receptor on the cell membrane may be determined by its lipid content, statins and PUFAs (DAS, 2002).

Conclusion

In this study, we brought new insights into the action of coconut oil on the hyperplastic prostate of adult gerbils. The information obtained reveals that this oil promotes anti-inflammatory effects and can reverse the effects of testosterone supplementation. Thus, these results indicate that CO may be the friend of the prostate in the hyperplasic condition and be an ally of natural medicine in the treatment of BPH.

Conflict of interest

The authors declare no conflicts of interest.

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References

AKPAN, E. J. et al. Fatty Acid Profile and Oil Yield in Six Different Varieties of Fresh and Dry Samples of Coconuts (Cocos nucifera). **Pakistan Journal of Nutrition**, v. 5, p. 106–109, 2006.

ARRUZAZABALA, M. DE L. et al. Effects of coconut oil on testosterone-induced prostatic hyperplasia in Sprague-Dawley rats. **Journal of Pharmacy and Pharmacology**, v. 59, n. 7, p. 995–999, jul. 2007.

ATTIA, D. M. A.; EDERVEEN, A. G. H. Opposing roles of ERα and ERβ in the genesis and progression of adenocarcinoma in the rat ventral prostate. **The Prostate**, v. 72, n. 9, p. 1013–1022, 15 jun. 2012.

AUSTYN, J. M.; GORDON, S. F4 / 80 , a monoclonal antibody directed specifically against the mouse macrophage *. p. 805–815, 1981.

BAUMAN, T. M. et al. Characterization of Fibrillar Collagens and Extracellular Matrix of Glandular Benign Prostatic Hyperplasia Nodules. v. 9, n. 10, p. 1–9, 2014.

BENNETT, M.; GILROY, D. W. Lipid Mediators in In fl ammation. p. 1–21, 2016.

BERGERS, G. et al. Matrix metalloproteinase-9 triggers the angiogenic switch during carcinogenesis. **Nature Cell Biology**, v. 2, n. 10, p. 737–744, 2000.

BOSTANCI, Y. et al. Correlation between benign prostatic hyperplasia and inflammation. **Current Opinion in Urology**, v. 23, n. 1, p. 5–10, 2013.

BOWDEN, G. T. Expression of the Metalloproteinase Matrilysin in DU-145 Cells Increases Their Invasive Potential in Severe Combined Immunodeficient Mice 1. v. 1, n. pH 8, 1993.

BRUNI-CARDOSO, A. et al. MMP-2 regulates rat ventral prostate development in vitro. **Developmental Dynamics**, v. 239, n. 3, p. 737–746, mar. 2010.

CALDER, P. C. **Polyunsaturated fatty acids, inflammatory processes and inflammatory bowel diseasesMolecular Nutrition and Food Research**, ago. 2008.

CAMPOS, S. G. P. et al. Age-related histopathological lesions in the Mongolian gerbil ventral prostate as a good model for studies of spontaneous hormone-related disorders. **International Journal of Experimental Pathology**, v. 89, n. 1, p. 13–24, 2008.

CARRERO-LÓPEZ, V. M.; MI, J. M. C. B. Hiperplasia prostática benigna y síntomas del tracto urinario inferior . Revisión de las evidencias actuales. n. xx, 2016.

CASTRO, N. F. DA C. et al. Therapeutic effects of β-caryophyllene on proliferative disorders and inflammation of the gerbil prostate. **Prostate**, n. May, 2021.

CHANDRASEKHARAN, N. V.; SIMMONS, D. L. The cyclooxygenases. **Genome Biology**, v. 5, n. 9, p. 1–7, 2004.

CHOI, H. et al. Cinnamomi Cortex (Cinnamomum verum) Suppresses Testosterone-induced Benign Prostatic Hyperplasia by Regulating 5α-reductase. **nature.com**, 1999.

CHU, J. et al. Potential involvement of the cyclooxygenase-2 pathway in the regulation of tumor-associated angiogenesis and growth in pancreatic cancer. **Molecular Cancer Therapeutics**, v. 2, n. 1, p. 1–7, 2003.

CICERO, A. F. G. et al. Nutraceutical treatment and prevention of benign prostatic hyperplasia and prostate cancer. n. July, p. 139–152, 2019.

CUNHA, G. R. et al. The endocrinology and developmental biology of the prostate. **Endocrine Reviews**, v. 8, n. 3, p. 338–362, ago. 1987.

DAS, U. N. Estrogen, statins, and polyunsaturated fatty acids: Similarities in their actions and benefits - Is there a common link? **Nutrition**, v. 18, n. 2, p. 178–188, 2002.
DE JESUS, M. M. et al. Histopathological alterations in the prostates of Mongolian gerbils exposed to a high-fat diet and di-n-butyl phthalate individually or in combination. **Reproductive Toxicology**, v. 52, p. 26–39, 2015.
DENARDO, D. G. Macrophages as regulators of tumour immunity and immunotherapy. **Nature Reviews Immunology**, 2019.
DENARDO, D. G. et al. NIH Public Access. v. 16, n. 2, p. 91–102, 2009.
ELLEM, S. J.; RISBRIDGER, G. P. The dual, opposing roles of estrogen in the prostate. **Annals of the New York Academy of Sciences**, v. 1155, p. 174–186, 2009.
EOM, J. H. et al. Bawu decoction () ameliorates benign prostatic hyperplasia in rats. **Chinese Journal of Integrative Medicine**, v. 23, n. 8, p. 611–616, 2017a.
EOM, J. H. et al. Bawu decoction () ameliorates benign prostatic hyperplasia in rats. **Chinese Journal of Integrative Medicine**, v. 23, n. 8, p. 611–616, 1 ago. 2017b.
FAMUREWA, A. C. et al. Virgin coconut oil supplementation attenuates acute chemotherapy hepatotoxicity induced by anticancer drug methotrexate via inhibition of oxidative stress in rats. **Biomedicine and Pharmacotherapy**, v. 87, p. 437–442, 1 mar. 2017.
FANG, Q. et al. Etheno-DNA adduct formation in rats gavaged with linoleic acid, oleic acid and coconut oil is organ- and gender specific. **Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis**, v. 624, n. 1–2, p. 71–79, 2007.
GIL, Á. Polyunsaturated fatty acids and inflammatory diseases. v. 56, p. 388–396, 2002.
GILL, S. E.; PARKS, W. C. Metalloproteinases and their inhibitors: Regulators of wound healing. **International Journal of Biochemistry and Cell Biology**, v. 40, n. 6–7, p. 1334–1347, 2008.
GRINDSTAD, T. et al. Estrogen receptors α and β and aromatase as independent predictors for prostate cancer outcome. **Nature Publishing Group**, 2016.
HABIB, F. K. et al. Serenoa repens (Permixon®) inhibits the 5α-reductase activity of human prostate cancer cell lines without interfering with PSA expression. **International Journal of Cancer**, v. 114, n. 2, p. 190–194, 2005.
HORVATH, L. G. et al. **Frequent Loss of Estrogen Receptor-Expression in Prostate Cancer 1CANCER RESEARCH**. [s.l: s.n.]. Disponível em: <https://cancerres.aacrjournals.org/content/61/14/5331.short>. Acesso em: 1 out. 2020.
HUANG, W. et al. Anti-bacterial and anti-inflammatory properties of capric acid against Propionibacterium acnes : A comparative study with lauric acid. **Journal of Dermatological Science**, v. 73, n. 3, p. 232–240, 2014.
HUI, Y. H. **Bailey's Industrial Oil and Fat Products, Volume 5, Edible Oil and Fat Products: Processing Technologies, 6th Edition**. 1996.
HUTTUNEN, E.; ROMPPANEN, T.; HELMINEN, H. J. A histoquantitative study on the effects of castration on the rat ventral prostate lobe. **Journal of anatomy**, v. 132, n. Pt 3, p. 357–35770, 1981.
INTAHPHUAK, S.; KHONSUNG, P.; PANTHONG, A. Anti-inflammatory , analgesic , and antipyretic activities of virgin coconut oil. v. 0209, 2010.
ISHIHARA, T.; YOSHIDA, M.; ARITA, M. Omega-3 fatty acid-derived mediators that control inflammation and tissue homeostasis. n. February, 2019.

JAMJAI, U. et al. Inhibition of Free Radicals and Matrix Metalloproteinase of Virgin Coconut Oil and Its Reduction of Inflammatory Product | Journal of Agricultural Research and Extension. v. 8, n. 1, p. 84–94, 2019.
JEON, W. Y. et al. Inhibitory effects of Ponciri Fructus on testosterone-induced benign prostatic hyperplasia in rats. **BMC Complementary and Alternative Medicine**, v. 17, n. 1, p. 1–10, 2017.
KABARA, J. J. Health oils from the tree of life. **Pharmacological effect of Lipids**, p. 642–649, 2013.
KAYAL, S. et al. CD68 tumor-associated macrophage marker is not prognostic. v. 55, n. May, p. 1031–1037, 2014.
KIM, B. H. et al. Urological Oncology Cyclooxygenase-2 Overexpression in Chronic Inflammation Associated with Benign Prostatic Hyperplasia : Is It Related to Apoptosis and Angiogenesis of Prostate Cancer ? p. 253–259, 2011.
KRUŠLIN, B. et al. Inflammation in prostatic hyperplasia and carcinoma-basic scientific approach. **Frontiers in Oncology**, v. 7, n. APR, p. 1–7, 2017.
KRYCZEK, I. et al. B7-H4 expression identifi es a novel suppressive macrophage population in human ovarian carcinoma. v. 203, n. 4, p. 0–10, 2006.
KURAHARA, H. et al. Significance of M2-polarized tumor-associated macrophage in pancreatic cancer. **Journal of Surgical Research**, v. 167, n. 2, p. e211–e219, 2011.
LANGAN, R. C. **Benign Prostatic HyperplasiaPrimary Care - Clinics in Office Practice**W.B. Saunders, , 1 jun. 2019. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/31030823/>. Acesso em: 24 jun. 2021
LEUNG, W. K. et al. Cyclooxygenase-2 upregulates vascular endothelial growth factor expression and angiogenesis in human gastric carcinoma. **International journal of oncology**, v. 23, n. 5, p. 1317–1322, nov. 2003.
LINDBERG, M. K. et al. Estrogen receptor (ER)-β reduces ERα-regulated gene transcription, supporting a “Ying Yang” relationship between ERα and ERβ in mice. **Molecular Endocrinology**, v. 17, n. 2, p. 203–208, 2003.
LIOTTA, L. A. Tumor Invasion and MÃ©tastasesâ€”Roleof the Extracellular Matrix: Rhoads Memorial Award Lecture1. v. 46, n. January, p. 1–7, 1986.
LOKESHWAR, B. A. L. L. MMP Inhibition in Prostate Cancer. p. 271–289, 1999.
LONDON, C. A. et al. A novel antisense inhibitor of MMP-9 attenuates angiogenesis, human prostate cancer cell invasion and tumorigenicity. **Cancer Gene Therapy**, v. 10, n. 11, p. 823–832, 2003.
MADERSBACHER, S.; SAMPSON, N.; CULIG, Z. Pathophysiology of Benign Prostatic Hyperplasia and Benign Prostatic Enlargement: A Mini-Review. **Gerontology**, v. 65, n. 5, p. 458–464, 2019.
MANIECKI, M. B. et al. macrophage-specific receptor CD163 in malignant cells. v. 000, 2012.
MANTOVANI, A. et al. Macrophage polarization: Tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. **Trends in Immunology**, v. 23, n. 11, p. 549–555, 2002.
MCPHERSON, S. et al. Elevated androgens and prolactin in aromatase-deficient mice cause enlargement, but not malignancy, of the prostate gland. **academic.oup.com**, 2001.
MEDREK, C. et al. The presence of tumor associated macrophages in tumor stroma as a prognostic marker for breast cancer patients. **???**, v. 12, n. 1, p. 1, 2012.

MENDES, L. O. et al. Modulation of inflammatory and hormonal parameters in response to testosterone therapy: effects on the ventral prostate of adult rats † Running Title: Testosterone modulates inflammatory response Leonardo O. Mendes. n. February, p. 1–27, 2018.
MUNTZING, J. Androgen and Collagen as Growth Regulators of the Rat Ventral Prostate. v. 78, p. 71–78, 1980.
MYUNG-GYU, K. et al. M2 macrophages predict worse long-term outcomes in human acute tubular necrosis. p. 1–7, 2020.
NOA, M. et al. Effect of D-004, a lipid extract from Cuban royal palm fruit, on histological changes of prostate hyperplasia induced with testosterone in rats. **International Journal of Tissue Reactions**, v. 27, n. 4, p. 203–211, 2005.
PAJOUH, M. S. et al. Expression of metalloproteinase genes in human prostate cancer. **Journal of Cancer Research and Clinical Oncology**, v. 117, n. 2, p. 144–150, 1991.
PATEL, A. K.; CHAPPLE, C. R. Benign prostatic hyperplasia : treatment in primary care. v. 333, n. September, p. 535–539, 2006.
PEREIRA, C. C. B.; DA SILVA, M. A. P.; LANGONE, M. A. P. Enzymatic Synthesis of Monolaurin. In: **Proceedings of the Twenty-Fifth Symposium on Biotechnology for Fuels and Chemicals Held May 4–7, 2003, in Breckenridge, CO**. [s.l.] Humana Press, 2004. p. 433–445.
PRAVETTONI, A. et al. Estrogen receptor beta (ERbeta) and inhibition of prostate cancer cell proliferation: Studies on the possible mechanism of action in DU145 cells. **Molecular and Cellular Endocrinology**, v. 263, n. 1–2, p. 46–54, 2007.
QUINTERO-GARCÍA, M. et al. Iodine prevents the increase of testosterone-induced oxidative stress in a model of rat prostatic hyperplasia. **Free Radical Biology and Medicine**, v. 115, n. April 2017, p. 298–308, 2018.
RAMSAY, R. G. et al. Transcriptional regulation of cyclo-oxygenase expression: three pillars of control. **International journal of immunopathology and pharmacology**, v. 16, n. 2 Suppl, p. 59–67, 2003.
RATHEESH, M. et al. Anti-inflammatory and antioxidant effect of Kerabala: a value-added ayurvedic formulation from virgin coconut oil inhibits pathogenesis in adjuvant-induced arthritis. **Inflammopharmacology**, v. 25, n. 1, p. 41–53, 1 fev. 2017.
RISBRIDGER, G. et al. The metaplastic effects of estrogen on mouse prostate epithelium: proliferation of cells with basal cell phenotype. **academic.oup.com**, 2001.
RUETTEN, H. et al. A uropathogenic E. coli UTI89 model of prostatic inflammation and collagen accumulation for use in studying aberrant collagen production in the prostate. **American journal of physiology. Renal physiology**, v. 320, n. 1, p. F31–F46, jan. 2021.
SANDHU, J.; REPORTS, A. T.-C. U.; . The role of 5-α-reductase inhibition as monotherapy in view of the MTOPS data. **Springer**, 2004.
SCARANO, W. R.; VILAMAIOR, P. S. L.; TABOGA, S. R. Tissue evidence of the testosterone role on the abnormal growth and aging effects reversion in the gerbil (Meriones unguiculatus) prostate. **Anatomical Record - Part A Discoveries in Molecular, Cellular, and Evolutionary Biology**, v. 288, n. 11, p. 1190–1200, 2006.
SHEELA, D. L. et al. Lauric acid induce cell death in colon cancer cells mediated by the epidermal growth factor receptor downregulation: An in silico and in vitro study. **Human and Experimental Toxicology**, v. 38, n. 7, p. 753–761, 1 jul. 2019.
SHIAO, S. L. et al. radiation therapy. v. 3, n. 5, p. 518–525, 2016.

SHUKLA, S. et al. Studies on anti-inflammatory, antipyretic and analgesic properties of Caesalpinia bonducella F. seed oil in experimental animal models. **Elsevier**, 2010.
SILVA, S. A. et al. Prostate hyperplasia caused by long-term obesity is characterized by high deposition of extracellular matrix and increased content of MMP-9 and VEGF. **International Journal of Experimental Pathology**, v. 96, n. 1, p. 21–30, 2015.
SOBOLEWSKI, C. et al. The Role of Cyclooxygenase-2 in Cell Proliferation and Cell Death in Human Malignancies. v. 2010, 2010.
SOLER, R. et al. Future direction in pharmacotherapy for non-neurogenic male lower urinary tract symptoms. **European Urology**, v. 64, n. 4, p. 610–621, 2013.
STEIDL, C. et al. Tumor-Associated Macrophages and Survival in Classic Hodgkin's Lymphoma. **New England Journal of Medicine**, v. 362, n. 10, p. 875–885, 2010.
TENG, M. et al. Impact of coconut oil consumption on cardiovascular health: A systematic review and meta-Analysis. **Nutrition Reviews**, v. 78, n. 3, p. 249–259, 2020.
TERRY, S. Y. A. et al. In-anti-F4 / 80-A3-1 antibody : a novel tracer to image macrophages. p. 1430–1438, 2015.
UBIL, E. et al. Tumor-secreted Pros1 inhibits macrophage M1 polarization to reduce antitumor immune response. v. 128, n. 6, 2018.
VARMA, S. R. et al. Journal of Traditional and Complementary Medicine In vitro anti-in fl ammatory and skin protective properties of Virgin coconut oil. p. 4–13, 2017.
VILAMAIOR, P. S. L. et al. Collagen fiber reorganization in the rat ventral prostate following androgen deprivation: A possible role for smooth muscle cells. **Prostate**, v. 45, n. 3, p. 253–258, 2000.
WADDELL, L. A. et al. ADGRE1 (EMR1, F4/80) is a rapidly-evolving gene expressed in mammalian monocyte-macrophages. **Frontiers in Immunology**, v. 9, n. OCT, p. 1–14, 2018.
WALLACE, T. C. Health Effects of Coconut Oil—A Narrative Review of Current Evidence. **Journal of the American College of Nutrition**, v. 38, n. 2, p. 97–107, 2019.
WAN, J.; GRIMBLE, R. Effect of dietary linoleate content on the metabolic response of rats to Escherichia coli endotoxin. **portlandpress.com**, 1987.
WANG, W.; BERGH, A.; DAMBER, J. E. Chronic inflammation in benign prostate hyperplasia is associated with focal upregulation of cyclooxygenase-2, Bcl-2, and cell proliferation in the glandular epithelium. **Prostate**, v. 61, n. 1, p. 60–72, 2004.
WANG, X. et al. Increased infiltrated macrophages in benign prostatic hyperplasia (BPH): Role of stromal androgen receptor in macrophage-induced prostate stromal cell proliferation. **Journal of Biological Chemistry**, v. 287, n. 22, p. 18376–18385, 2012.
WEIBEL, E. R. Principles and methods for the morphometric study of the lung and other organs. **Laboratory investigation; a journal of technical methods and pathology**, v. 12, p. 131–155, fev. 1963.
WITCHER, K. J.; NOVICK, R. P.; SCHLIEVERT, P. M. **Modulation of Immune Cell Proliferation by Glycerol MonolaurateCLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY**. [s.l: s.n.]. Disponível em: <http://cvi.asm.org/>. Acesso em: 1 out. 2020.
YEAP, S. et al. Antistress and antioxidant effects of virgin coconut oil in vivo. **spandidos-publications.com**,2015.

ZAKARIA, Z. A. . et al. In vivo Antinociceptive and Anti-inflammatory Activities of Dried and Fermented Processed Virgin Coconut Oil. p. 231–236, 2011.
ZHANG, F. et al. Effect of n-3 and n-6 polyunsaturated fatty acids on lipid metabolic genes and estrogen receptor expression in MCF-7 breast cancer cells. **Clinical laboratory**, v. 61, n. 3–4, p. 397–403, 2015.
ZHU, X. et al. Dynamic regulation of estrogen receptor-β expression by DNA methylation during prostate cancer development and metastasis. **American Journal of Pathology**, v. 164, n. 6, p. 2003–2012, 1 jun. 2004.

Table 1. Antibodies utilized in the immunohistochemistry.

Antibodies	Description	Diluition
ERα	Polyclonal rabbit (IgG, MC-20, Santa Cruz Biotechnology, CA, USA)	1:50
ERβ	Polyclonal rabbit (IgG, H-150, Santa Cruz Biotechnology, CA, EUA)	1:50
COX-2	Monoclonal rabbit (IgG, D5H5, ♯12282, Cell Signaling, Danvers,MA, USA)	1:100
MMP-2	Polyclonal rabbit (IgG, SC-20659, Santa Cruz Biotechnology CA, USA)	1:75
MMP-9	Monoclonal mouse (IgG, SC-32351, Santa Cruz Biotechnology CA, USA)	1:100
F4/80	Monoclonal rabbit (IgG, D2S9R, ♯70076, Cell Signaling, Danvers,MA, USA)	1:100
CD68	Polyclonal goat (IgG M-20, SC-7084, Santa Cruz Biotechnology CA, USA)	1:50
CD163	Polyclonal rabbit (IgG, SC-33560, Santa Cruz Biotechnology CA, USA)	1:50

Table 2. Stereology and hormonal parameters of Mongolian gerbils in Control (C), Testosterone suplementation (T) and Testosterone supplementation with coconut oil (TCO) groups (n=7).

Parameters (n=7)	**Groups**		
Stereology (%)	**C**	**T**	**TCO**
Collagen	0.3146 ± 0.2069[a]	0.4989 ± 0.2652[b]	0.2039 ± 0.2039[a]
Vessels	0.2198 ± 0.3989	0.3632 ± 0.5031	0.2978 ± 0.4732
Hormonal data (pg/ml)			
Estradiol	17.55 ±12.62	12.41 ± 16.92	Non detected

Values expressed as mean ± standard deviation. Differences between control and treatments are statistically significant at P≤0.05. Superscript letters (a,b,) represent statistically significant differences between the groups.

Table 3. Analysis of multiplicity and incidence of inflammatory foci of the ventral prostate of the Mongolian gerbil. Control (C), Testosterone suplementation (T) and Testosterone supplementation with coconut oil (TCO). Incidence data indicate the percentage of animals affected.

Inflammatory foci (n=7)	**C**	**T**	**TCO**
Multiplicity			
Intraluminal inflammation	0 - 2 (0.09)	0 -10 (0.57)	0 - 1 (0.09)
Periductal inflammation	0 - 4 (0.90)[a]	0 - 19 (6.38)[b]	0 - 9 (2.04)[a]
Subepithelial inflammation	0 - 1 (0.13)[a]	0 - 15 (4.33)[b]	0 - 1 (0.09)[a]
***Incidence* (%)**			
Intraluminal inflammation	14.28 %	14.28 %	28.57 %
Periductal inflammation	57.14 %	85.71 %	85.71 %
Subepithelial inflammation	42.85 %	85.71 %	28.57 %

Values expressed as mean ± standard deviation. Differences between control and treatments are statistically significant at p≤0.05. Superscript letters (a,b) represent statistically significant differences between the groups.

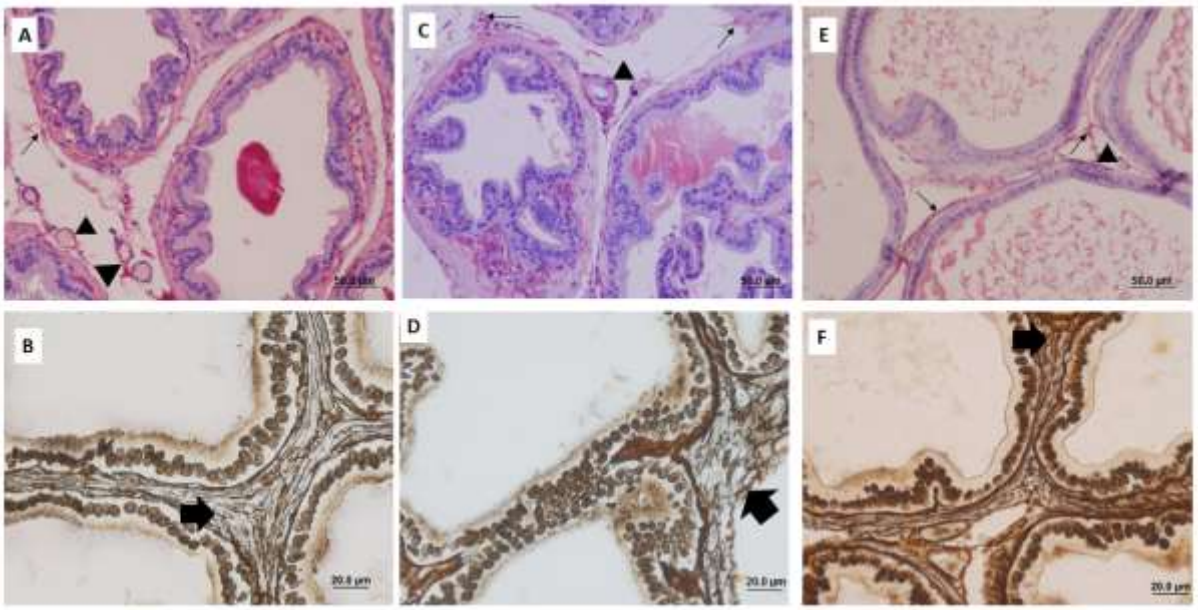

Figure 1. Histological sections of the ventral prostate of Mongolian gerbils stained with Picrosirius (A,C,E) and Gomori's Reticulin (B,D,F). Groups C (A,B), T (C,D) and TCO (E,F). Collagen fibers (thin arrow), reticular fibers (wide arrows) and vessels (arrowhead).

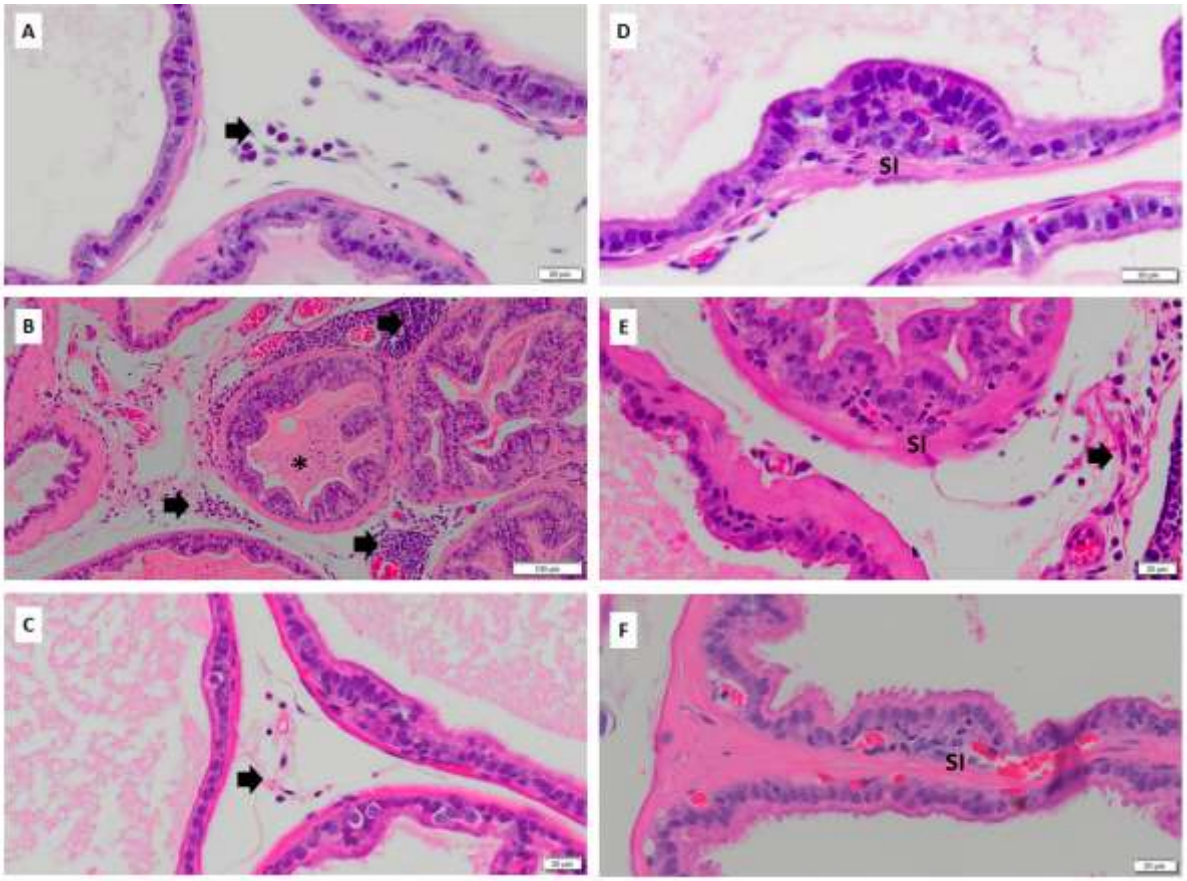

Figure 2. Histological sections of the ventral prostate of Mongolian gerbils stained with Hematoxilin Eosin from different experimental groups. Group C (A and D), T (B and E) and TCO (C and F). Intraluminal inflammatory foci (*), periductal foci (wide arrow), subepithelial inflammatory foci (SI).

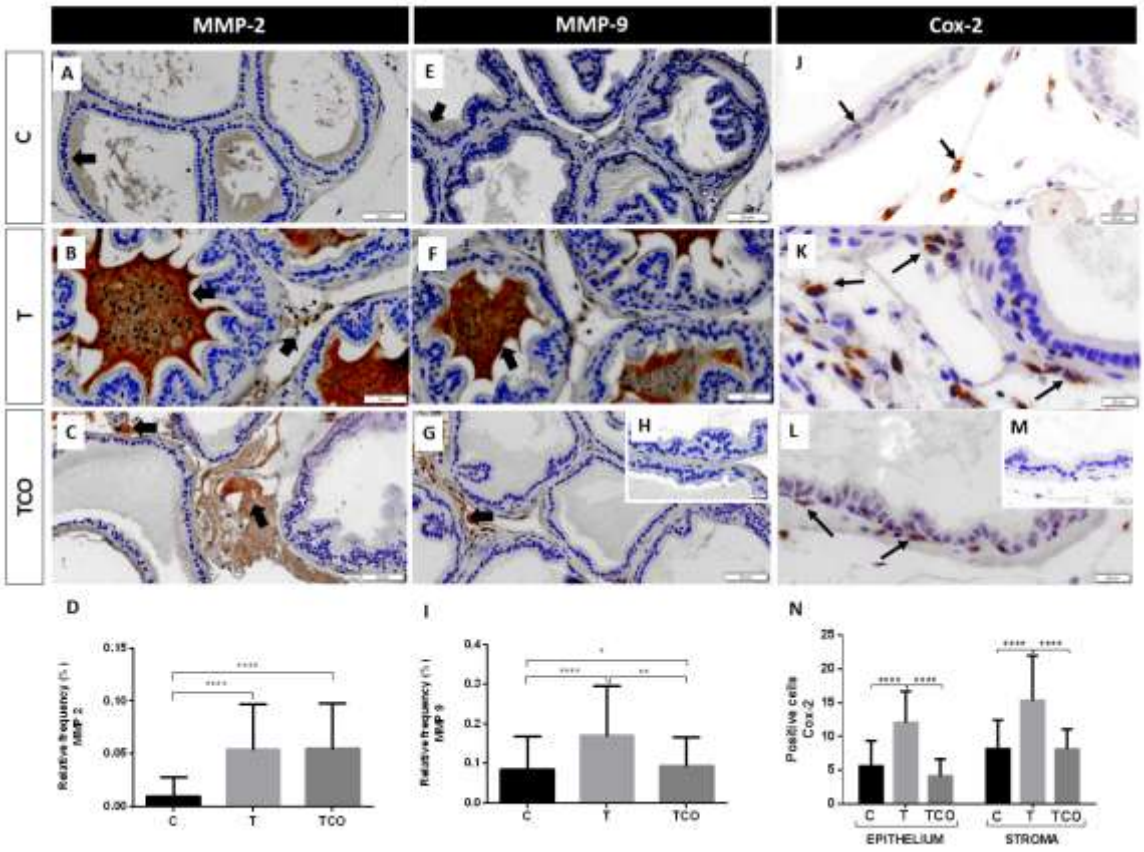

Figure 3. Histological sections of gerbils ventral prostate submitted to immunohistochemistry for MMP-2 (A-C), MMP 9 (E-G), Cox-2 (J-L). Negative control of the reactions (H and M). MMP-2 and MMP-9 (wide arrows), Cox-2 positive markings in epithelium and stroma (thin arrows). Relative frequency (%) of total MMP-2 (D) and MMP-9 (I) positive cells in ventral prostate. Relative frequency (%) of Cox-2 positive cells in epithelium and stroma (N)..Statistical analyses were based on parametric (ANOVA) and nonparametric (Kruskal-Wallis) tests for group differences, followed respectively by Bonferroni and Dunn's multiple comparison tests (* p <0.05; ** p <0.01; **** p <0.0001).

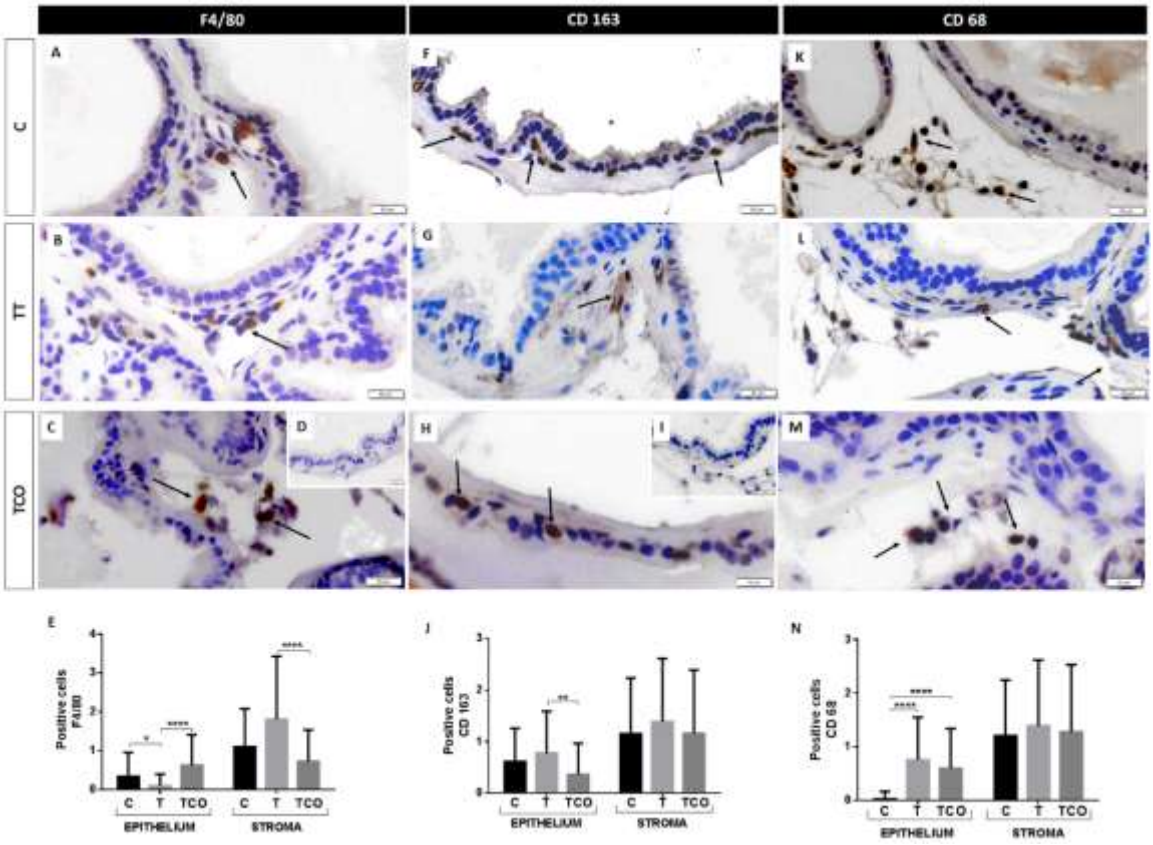

Figure 4. Histological sections of gerbils ventral prostate subjected to immunohistochemistry for F4/80 (A-C), CD163 (F-H), CD68 (K-M). Negative control of reactions (D and I). Positive markings (thin arrows). Relative frequency (%) of F4/80 (E), CD163 (J) and CD68 (N) positive cells in epithelium and stroma. Statistical analyses were based on parametric (ANOVA) and nonparametric (Kruskal-Wallis) tests for group differences, followed respectively by Bonferroni and Dunn's multiple comparison tests (* $p < 0.05$; ** $p < 0.01$; **** $p < 0.0001$).

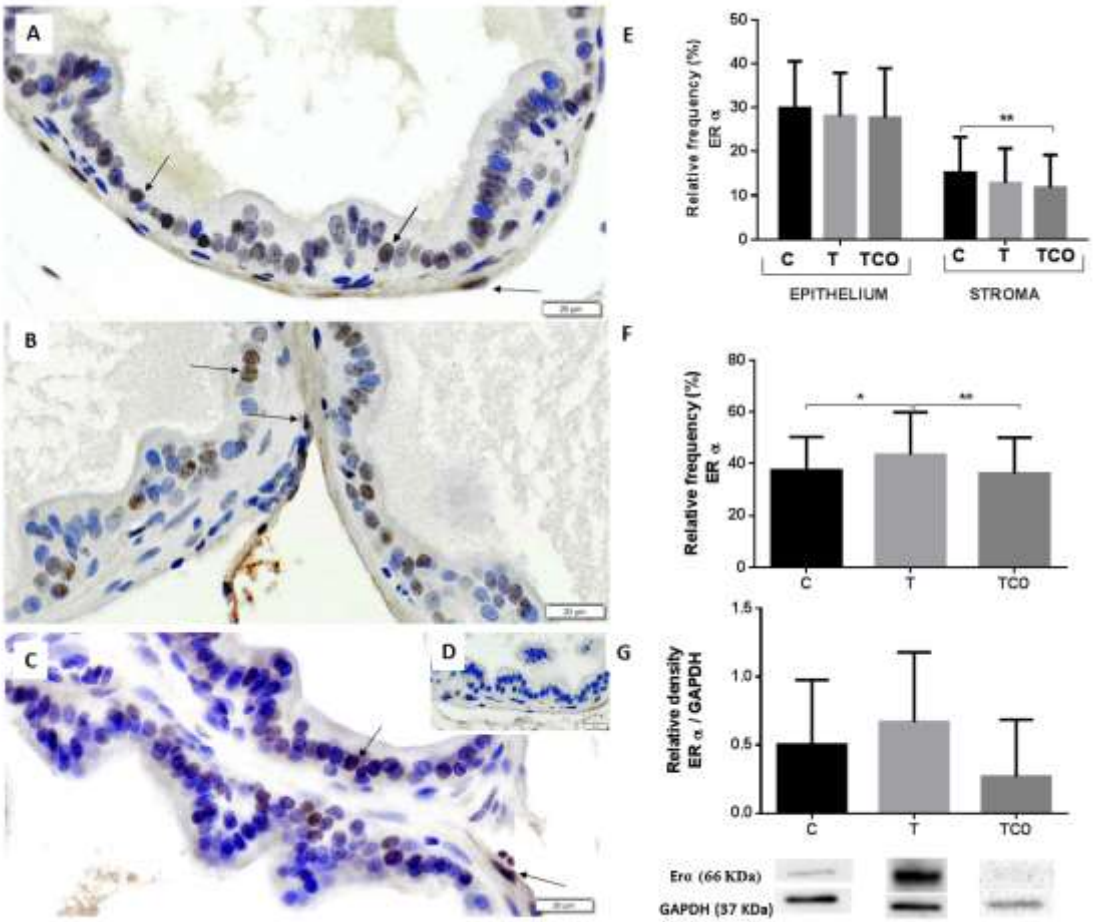

Figure 5. Histological sections of gerbils ventral prostate submitted to immunohistochemistry for identification of alpha type estrogen receptor (ER α). Groups C (A), T(B) and TCO (C), negative reaction control (D). Positive markings for ERα in the nuclei of epithelial and stromal cells (arrows). Relative frequency (%) of ER α-positive cells present in epithelium and stroma (E) and sum of compartments (F) of groups C, T and TCO. Relative density of ER-α, and normalized by GAPDH (positive control) (G). Statistically significant differences between groups (* p <0.05; ** p <0.01), according to parametric (ANOVA) and non-parametric (Kruskal-Wallis) tests for group differences, followed by Bonferroni and Dunn's multiple comparison tests, respectively.

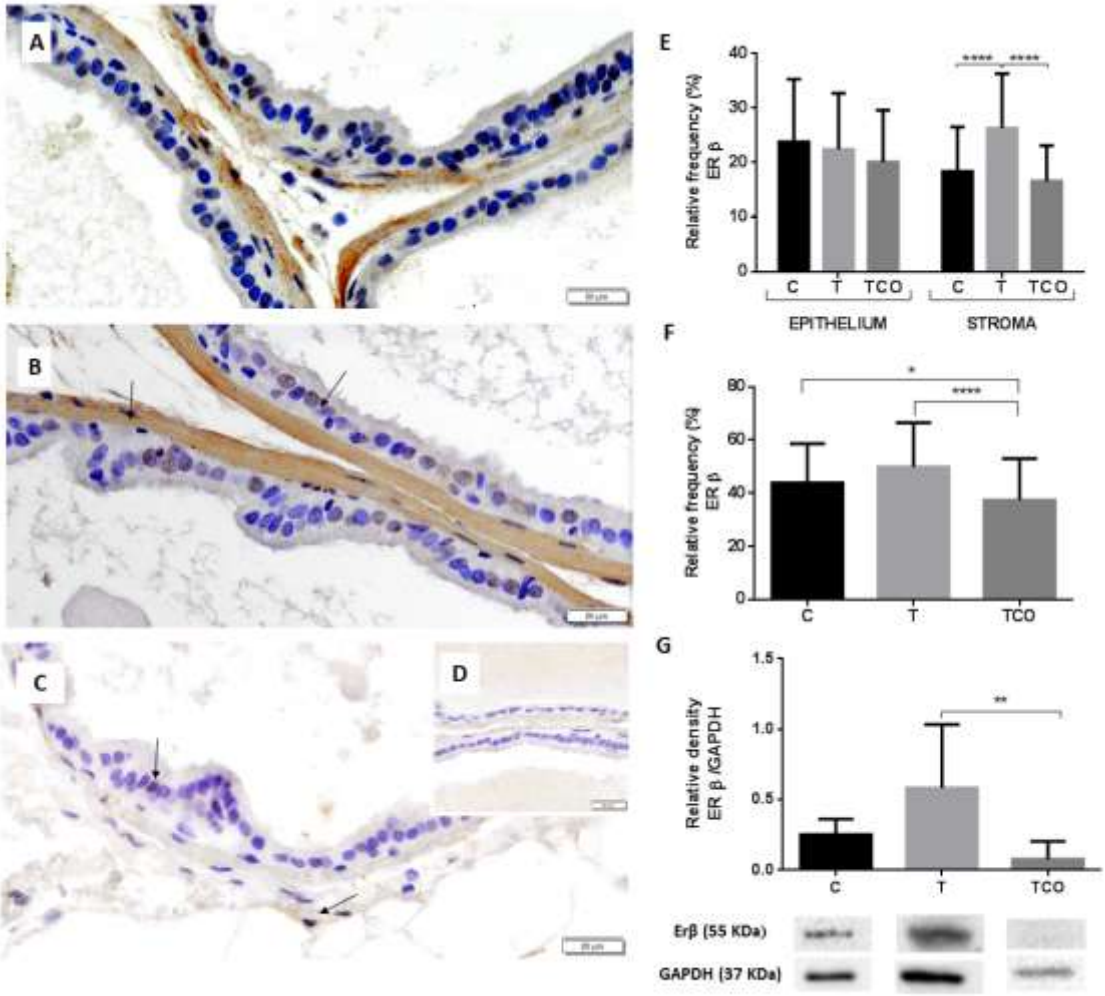

Figure 6. Histological sections of gerbils ventral prostate submitted to immunohistochemistry for identification of estrogen receptor type alpha (ER β). Group C (A), T (B) and TCO (C), negative reaction control (D). Positive markings for ER β in the nuclei of epithelial and stromal cells (arrows). Relative frequency (%) of ER β-positive cells present in epithelium and stroma (E) and sum of compartments (F) of groups C, T and TCO. Relative density of ER-α, and normalized by GAPDH (positive control) (G). Statistically significant differences between groups (* p <0.05; ** p <0.01; **** p <0.0001), according to parametric (ANOVA) and non-parametric (Kruskal-Wallis) tests for group differences, followed by Bonferroni and Dunn's multiple comparison tests, respectively.

6. CONCLUSÕES FINAIS

Neste trabalho, trouxemos novos conhecimentos sobre o efeito do óleo de coco sobre a próstata hiperplásica de gerbilos adultos e concluímos que este óleo foi capaz de inibir o aumento dos tecidos da próstata induzido pela testosterona exógena, bem como a área nuclear, além de reduzir a expressão da enzima 5α redutase e dos receptores de andrógenos e estrógenos. Além disso, observamos seus efeitos pró-apoptóticos e anti-inflamatórios, demonstrando ser benéfico para a próstata em condições hiperplásicas. Estes resultados indicam que o CO pode ser um aliado da medicina natural no tratamento da HPB, porém, tal como em outros estudos com produtos extraídos de plantas descritos na literatura, precisamos de maior aprofundamento nas investigações para compreender a ação deste óleo e o seu possível papel no tratamento da HPB.

REFERÊNCIAS

AARON, L. T.; FRANCO, O. E.; HAYWARD, S. W. Review of Prostate Anatomy and Embryology and the Etiology of Benign Prostatic Hyperplasia. **Urologic Clinics of North America**, v. 43, n. 3, p. 279–288, 2016.

ABDEL-AZIZ, A. M. et al. Amelioration of testosterone-induced benign prostatic hyperplasia using febuxostat in rats: The role of VEGF/TGFβ and iNOS/COX-2. **European Journal of Pharmacology**, v. 889, n. October, p. 173631, 2020.

AKPAN, E. J. et al. Fatty Acid Profile and Oil Yield in Six Different Varieties of Fresh and Dry Samples of Coconuts (Cocos nucifera). **Pakistan Journal of Nutrition**, v. 5, p. 106–109, 2006.

ALCÁNTARA MONTERO, A.; MÜLLER-ARTEAGA, C. 5-alpha reductase inhibitors: New evidences on benefits and harms beyond benign prostatic hyperplasia. **Actas Urologicas Espanolas**, v. 43, n. 1, p. 52–53, 2019.

ALSHAHRANI, S.; MCGILL, J.; AGARWAL, A. Prostatitis and male infertility. **Journal of Reproductive Immunology**, v. 100, n. 1, p. 30–36, 2013.

ARRUZAZABALA, M. DE L. et al. Effects of coconut oil on testosterone-induced prostatic hyperplasia in Sprague-Dawley rats. **Journal of Pharmacy and Pharmacology**, v. 59, n. 7, p. 995–999, jul. 2007.

ASADA, Y. et al. 5 ⎵ -Reductase Type 2 Is Constitutively Expressed in the Dermal Papilla and Connective Tissue Sheath of the Hair Follicle in Vivo But Not during Culture in Vitro *. v. 86, n. 6, p. 1–6, 2001.

ASTORG, P. Dietary n - 6 and n - 3 polyunsaturated fatty acids and prostate cancer risk: A review of epidemiological and experimental evidence. **Cancer Causes and Control**, v. 15, n. 4, p. 367–386, 2004.

ATTIA, D. M. A.; EDERVEEN, A. G. H. Opposing roles of ERα and ERβ in the genesis and progression of adenocarcinoma in the rat ventral prostate. **The Prostate**, v. 72, n. 9, p. 1013–1022, 15 jun. 2012.

BANERJEE, P. P. et al. Age-dependent and lobe-specific spontaneous hyperplasia in the Brown Norway rat prostate. **Biology of Reproduction**, v. 59, n. 5, p. 1163–1170, 1998.

BATCHELDER, M. et al. **Gerbils**. [s.l.] Elsevier Inc., 2012.

BELLO, D. et al. Androgen responsive adult human prostatic epithelial cell lines immortalized by human papillomavirus 18. **Carcinogenesis**, v. 18, n. 6, p. 1215–1223, 1997.

BENNETT, M.; GILROY, D. W. Lipid Mediators in In fl ammation. p. 1–21, 2016.

BHARGAVA, S.; CANDA, A. E.; CHAPPLE, C. R. A rational approach to benign prostatic hyperplasia evaluation : recent advances. p. 1–6, 2004.

CAI, X. et al. High fat diet increases the weight of rat ventral prostate. **Prostate**, v. 49, n. 1, p. 1–8, 2001.

CALDER, P. C. **Polyunsaturated fatty acids, inflammatory processes and inflammatory bowel diseasesMolecular Nutrition and Food Research**, ago. 2008.

CAMPOS, S. G. P. et al. Age-related histopathological lesions in the Mongolian gerbil ventral prostate as a good model for studies of spontaneous hormone-related disorders. **International Journal of Experimental Pathology**, v. 89, n. 1, p. 13–24, 2008.

CAMPOS, S. G. P. et al. Prostatic stromal cells of old gerbils respond to steroidal blockades creating a microenvironment similar to reactive stroma. **Biomedicine and Aging Pathology**, v. 1, n. 2, p. 97–106, 2011.

CARRERO-LÓPEZ, V. M.; MI, J. M. C. B. Hiperplasia prostática benigna y síntomas del tracto urinario inferior . Revisión de las evidencias actuales. n. xx, 2016.

CARSON, C.; RITTMASTER, R. THE ROLE OF DIHYDROTESTOSTERONE IN BENIGN. p. 2–7, 2003.

CASTRO, N. F. DA C. et al. Therapeutic effects of β-caryophyllene on proliferative disorders and inflammation of the gerbil prostate. **Prostate**, n. May, 2021.

CHATTERJEE, B. The role of the androgen receptor in the development of prostatic hyperplasia and prostate cancer. **Molecular and Cellular Biochemistry**, v. 253, n. 1–2, p. 89–101, 2003.

CHOI, H. et al. Cinnamomi Cortex (Cinnamomum verum) Suppresses Testosterone-induced Benign Prostatic Hyperplasia by Regulating 5α-reductase. **nature.com**, 1999.

CICERO, A. F. G. et al. Nutraceutical treatment and prevention of benign prostatic hyperplasia and prostate cancer. n. July, p. 139–152, 2019.

CORDEIRO, R. S. et al. Androgen receptor in the Mongolian gerbil ventral prostate: Evaluation during different phases of postnatal development and following androgen blockage. **Micron**, v. 39, n. 8, p. 1312–1324, 2008.

CORRADI, L. S. et al. Inhibition of 5-α-reductase activity induces stromal remodeling and smooth muscle de-differentiation in adult gerbil ventral prostate. **Differentiation**, v. 72, n. 5, p. 198–208, 2004.

CORRADI, L. S. et al. Structural and ultrastructural evidence for telocytes in prostate stroma. **Journal of Cellular and Molecular Medicine**, v. 17, n. 3, p. 398–406, 2013.

CORRADI, L. S. et al. Long-term inhibition of 5-alpha reductase and aromatase changes the cellular and extracellular compartments in gerbil ventral prostate at different postnatal ages. **International Journal of Experimental Pathology**, v. 90, n. 1, p. 79–94, 2017.

CUNHA, G. R. et al. The endocrinology and developmental biology of the prostate. **Endocrine Reviews**, v. 8, n. 3, p. 338–362, ago. 1987.

CUNHA, G. R. et al. Estrogenic effects on prostatic differentiation and carcinogenesis. **Reproduction, fertility, and development**, v. 13, n. 4, p. 285–296, 2001.

CURIE, M. S. Estou entre aqueles que acham que a ciência tem uma grande beleza.

DE JESUS, M. M. et al. Histopathological alterations in the prostates of Mongolian gerbils exposed to a high-fat diet and di-n-butyl phthalate individually or in combination. **Reproductive Toxicology**, v. 52, p. 26–39, 2015.

DE NUNZIO, C.; PRESICCE, F.; TUBARO, A. Inflammatory mediators in the development and progression of benign prostatic hyperplasia. **Nature Reviews Urology**, v. 13, n. 10, p. 613–626, 2016.

DEBMANDAL, M.; MANDAL, S. Coconut (Cocos nucifera L.: Arecaceae): In health promotion and disease prevention. **Asian Pacific Journal of Tropical Medicine**, v. 4, n. 3, p. 241–247, 2011.

DOS SANTOS, F. C. A. et al. Structure, histochemistry and ultrastructure of the epithelium and stroma in the gerbil (Meriones unguiculatus) female prostate. **Tissue and Cell**, v. 35, n. 6, p. 447–457, 2003.

ELLEM, S. J.; RISBRIDGER, G. P. The dual, opposing roles of estrogen in the prostate. **Annals of the New York Academy of Sciences**, v. 1155, p. 174–186, 2009.

EOM, J. H. et al. Bawu decoction () ameliorates benign prostatic hyperplasia in rats. **Chinese Journal of Integrative Medicine**, v. 23, n. 8, p. 611–616, 2017.

ESCOBAR, E. L. O.; GOMES-MARCONDES, M. C. C.; CARVALHO, H. F. Dietary fatty acid quality affects AR and PPARγ levels and prostate growth. **Prostate**, v. 69, n. 5, p. 548–558, 2009.

EYRES, L. et al. Coconut oil consumption and cardiovascular risk factors in humans. **Nutrition Reviews**, v. 74, n. 4, p. 267–280, 2016.

FANG, Q. et al. Etheno-DNA adduct formation in rats gavaged with linoleic acid, oleic acid and coconut oil is organ- and gender specific. **Mutation Research - Fundamental and Molecular Mechanisms of Mutagenesis**, v. 624, n. 1–2, p. 71–79, 2007.

GRINDSTAD, T. et al. Estrogen receptors α and β and aromatase as independent predictors for prostate cancer outcome. **Nature Publishing Group**, 2016.

GUERRA, L. H. A. et al. Do mineral and corn oil serve as potential endocrine disruptors in the gerbil prostate? **Reproductive Toxicology**, v. 90, p. 141–149, 1 dez. 2019.

HABIB, F. K. et al. Serenoa repens (Permixon®) inhibits the 5α-reductase activity of human prostate cancer cell lines without interfering with PSA expression. **International Journal of Cancer**, v. 114, n. 2, p. 190–194, 2005.

HAGEN, R. M.; RHODES, A.; LADOMERY, M. R. Conjugated linoleate reduces prostate cancer viability whereas the effects of oleate and stearate are cell line-dependent. **Anticancer research**, v. 33, n. 10, p. 4395–4400, out. 2013.

HO, C. K. M.; HABIB, F. K. Estrogen and androgen signaling in the pathogenesis of BPH. **Nature Reviews Urology**, v. 8, n. 1, p. 29–41, 2011.

HORVATH, L. G. et al. **Frequent Loss of Estrogen Receptor-Expression in Prostate Cancer 1CANCER RESEARCH**. [s.l: s.n.]. Disponível em: <https://cancerres.aacrjournals.org/content/61/14/5331.short>. Acesso em: 1 out. 2020.

HUANG, W. et al. Anti-bacterial and anti-inflammatory properties of capric acid against Propionibacterium acnes : A comparative study with lauric acid. **Journal of Dermatological Science**, v. 73, n. 3, p. 232–240, 2014.

HUERTA-YÉPEZ, S.; TIRADO-RODRIGUEZ, A. B.; HANKINSON, O. Papel de las dietas ricas en omega-3 y omega-6 en el desarrollo del cáncer. **Boletin Medico del Hospital Infantil de Mexico**, v. 73, n. 6, p. 446–456, 2016.

HUI, Y. H. **Bailey's Industrial Oil and Fat Products, Volume 5, Edible Oil and Fat Products: Processing Technologies, 6th Edition**. 1996.

HUTCH, J. A.; RAMBO, O. S. A study of the anatomy of the prostate, prostatic urethra and the urinary sphincter system. **The Journal of urology**, v. 104, n. 3, p. 443–452, 1970.

INTAHPHUAK, S.; KHONSUNG, P.; PANTHONG, A. Anti-inflammatory , analgesic , and antipyretic activities of virgin coconut oil. v. 0209, 2010.

ISHIHARA, T.; YOSHIDA, M.; ARITA, M. Omega-3 fatty acid-derived mediators that control inflammation and tissue homeostasis. n. February, 2019.

JAMJAI, U. et al. Inhibition of Free Radicals and Matrix Metalloproteinase of Virgin Coconut Oil and Its Reduction of Inflammatory Product | Journal of Agricultural Research and Extension. v. 8, n. 1, p. 84–94, 2019.

JAYAWARDENA, R. et al. Effect of coconut oil on cardio-metabolic risk: A systematic review and meta-analysis of interventional studies. **Diabetes and Metabolic Syndrome: Clinical Research and Reviews**, v. 14, n. 6, p. 2007–2020, 2020.

JESIK, C. J.; HOLLAND, J. M.; LEE, C. An anatomic and histologic study of the rat prostate. **The Prostate**, v. 3, n. 1, p. 81–97, 1982.

KABARA, J. J. Health oils from the tree of life. **Pharmacological effect of Lipids**, p. 642–649, 2013.

KIRIYA, C. et al. Purple rice extract inhibits testosterone-induced rat prostatic hyperplasia and growth of human prostate cancer cell line by reduction of androgen receptor activation. **Journal of Food Biochemistry**, v. 43, n. 9, 1 set. 2019.

KRUŠLIN, B. et al. Inflammation in prostatic hyperplasia and carcinoma-basic scientific approach. **Frontiers in Oncology**, v. 7, n. APR, p. 1–7, 2017.

LAM, J. et al. Long-term treatment with finasteride in men with symptomatic benign prostatic hyperplasia: 10-year follow-up. **Elsevier**, 2003.

LANGAN, R. C. **Benign Prostatic HyperplasiaPrimary Care - Clinics in Office Practice**W.B. Saunders, , 1 jun. 2019. Disponível em: <https://pubmed.ncbi.nlm.nih.gov/31030823/>. Acesso em: 24 jun. 2021

LIANG, T.; LIAO, S. Inhibition of steroid 5α-reductase by specific aliphatic unsaturated fatty acids. **Biochemical Journal**, v. 285, n. 2, p. 557–562, 1992.

MADERSBACHER, S.; SAMPSON, N.; CULIG, Z. Pathophysiology of Benign Prostatic Hyperplasia and Benign Prostatic Enlargement: A Mini-Review. **Gerontology**, v. 65, n. 5, p. 458–464, 2019.

MARION-LETELLIER, R.; SAVOYE, G.; GHOSH, S. Polyunsaturated fatty acids and inflammation. **IUBMB Life**, v. 67, n. 9, p. 659–667, 2015.

MARKER, P. C. et al. Hormonal, cellular, and molecular control of prostatic development. **Developmental Biology**, v. 253, n. 2, p. 165–174, 2003.

MCNEAL, J. E. Anatomy of the prostate and morphogenesis of BPH. **Progress in clinical and biological research**, v. 145, p. 27–53, 1984.

MCPHERSON, S. et al. Elevated androgens and prolactin in aromatase-deficient mice cause enlargement, but not malignancy, of the prostate gland. **academic.oup.com**, 2001.

NIEDERPRÜM, H. et al. Testosterone 5α-reductase inhibition by free fatty acids from Sabal serrulata fruits. **Elsevier**, 1994.

NOA, M. et al. Effect of D-004, a lipid extract from Cuban royal palm fruit, on histological changes of prostate hyperplasia induced with testosterone in rats. **International Journal of Tissue Reactions**, v. 27, n. 4, p. 203–211, 2005.

NOLAN, C. C.; BROWN, A. W.; CAVANAGH, J. B. Regional variations in nerve cell responses to trimethyltin intoxication in Mongolian gerbils and rats; further evidence for involvement of the Golgi apparatus. **Acta Neuropathologica**, v. 81, n. 2, p. 204–212, 1990.

OLIVEIRA, S. M. et al. Cellular and extracellular behavior in the gerbil (Meriones unguiculatus) ventral prostate following different types of castration and the consequences of testosterone replacement. **Cell Biology International**, v. 31, n. 3, p. 235–245, 2007.

PARSONS, J. K. et al. Obesity and Benign Prostatic Hyperplasia: Clinical Connections, Emerging Etiological Paradigms and Future Directions. **Journal of Urology**, v. 182, n. 6 SUPPL., p. S27–S31, 2009.

PATEL, A. K.; CHAPPLE, C. R. Benign prostatic hyperplasia : treatment in primary care. v. 333, n. September, p. 535–539, 2006.

PEGORIN DE CAMPOS, S. G. et al. Biological behavior of the gerbil ventral prostate in three phases of postnatal development. **Anatomical Record - Part A Discoveries in Molecular, Cellular, and Evolutionary Biology**, v. 288, n. 7, p. 723–733, 2006.

PEREIRA, C. C. B.; DA SILVA, M. A. P.; LANGONE, M. A. P. Enzymatic Synthesis of Monolaurin. In: **Proceedings of the Twenty-Fifth Symposium on Biotechnology for Fuels and Chemicals Held May 4–7, 2003, in Breckenridge, CO**. [s.l.] Humana Press,. p. 433–445. 2004.

PINHEIRO, P. F. F. et al. Structure of the pelvic and penile urethra - Relationship with the ducts of the sex accessory glands of the Mongolian gerbil (Meriones unguiculatus). **Journal of Anatomy**, v. 202, n. 5, p. 431–444, 2003.

PRICE, D. COMPARATIVE ASPECTS OF DEVELOPMENT AND STRUCTURE IN THE PROSTATE. **National Cancer Institute monograph**, v. 12, p. 1–27, out. 1963.

RAHMAN, N. U. et al. An animal model to study lower urinary tract symptoms and erectile dysfunction: The hyperlipidaemic rat. **BJU International**, v. 100, n. 3, p. 658–663, 2007.

RATHEESH, M. et al. Anti-inflammatory and antioxidant effect of Kerabala: a value-added ayurvedic formulation from virgin coconut oil inhibits pathogenesis in adjuvant-induced arthritis. **Inflammopharmacology**, v. 25, n. 1, p. 41–53, 1 fev. 2017.

RAYNAUD, J. et al. Inhibition of type 1 and type 2 5α-reductase activity by free fatty acids, active ingredients of Permixon®. **Elsevier**, 2001.

RICH, S. T. The Mongolian gerbil (Meriones unguiculatus) in research. **Laboratory animal care**, v. 18, n. 2, p. Suppl:235-43, abr. 1968.

RISBRIDGER, G. et al. The metaplastic effects of estrogen on mouse prostate epithelium: proliferation of cells with basal cell phenotype. **academic.oup.com**, 2001.

RISBRIDGER, G. P.; TAYLOR, R. A. **CHAPTER 23 - Physiology of the Male Accessory Sex Structures: The Prostate Gland, Seminal Vesicles, and Bulbourethral Glands A2 - Neill, Jimmy D. BT - Knobil and Neill's Physiology of Reproduction (Third Edition)**. Third Edit ed. [s.l.] Elsevier Inc., 2006.

ROCHEL, S. S. et al. Lobe identity in the Mongolian gerbil prostatic complex: A new rodent model for prostate study. **Anatomical Record**, v. 290, n. 10, p. 1233–1247, 2007.

ROEHRBORN, C. G. Pathology of benign prostatic hyperplasia. **International Journal of Impotence Research**, v. 20, n. SUPPL. 3, 2008.

RUMPOLD, H. et al. The development of benign prostatic hyperplasia by trans-differentiation of prostatic stromal cells. **Experimental Gerontology**, v. 37, n. 8–9, p. 1001–1004, 2002.

SACKS, F. M. Coconut Oil and Heart Health: Fact or Fiction? Running Title: Coconut Oil and Heart Health: Fact or Fiction? p. 1–6, 2020.

SANCHES, B. D. A. et al. Budding process during the organogenesis of the ventral prostatic lobe in mongolian gerbil. **Microscopy Research and Technique**, v. 77, n. 6, p. 458–466, 2014.

SANDHU, J.; REPORTS, A. T.-C. U.; 2004, UNDEFINED. The role of 5-α-reductase inhibition as monotherapy in view of the MTOPS data. **Springer**, 2004.

SANKARARAMAN, S.; SFERRA, T. J. Ficamos loucos óleo de coc. p. 6–8, 2018.

SANTOS, S. A. A. DOS et al. Hyperglycemic condition during puberty increases collagen fibers deposition in the prostatic stroma and reduces MMP-2 activity. **Biochemical and Biophysical Research Communications**, v. 493, n. 4, p. 1581–1586, 2017.

SCARANO, W. R. et al. Tissue remodeling in Guinea pig lateral prostate at different ages after estradiol treatment. **Cell Biology International**, v. 29, n. 9, p. 778–784, 2005.

SCARANO, W. R. et al. Oestrogen supplementation following castration promotes stromal remodelling and histopathological alterations in the Mongolian gerbil ventral prostate. **International Journal of Experimental Pathology**, v. 89, n. 1, p. 25–37, 2008.

SCARANO, W. R.; VILAMAIOR, P. S. L.; TABOGA, S. R. Tissue evidence of the testosterone role on the abnormal growth and aging effects reversion in the gerbil (Meriones unguiculatus) prostate. **Anatomical Record - Part A Discoveries in Molecular, Cellular, and Evolutionary Biology**, v. 288, n. 11, p. 1190–1200, 2006.

SFANOS, K. S. et al. The inflammatory microenvironment and microbiome in prostate cancer development. **Nature Reviews Urology**, v. 15, n. 1, p. 11–24, 2018.

SHAPPELL, S. B. et al. Prostate Pathology of Genetically Engineered Mice: Definitions and Classification. The Consensus Report from the Bar Harbor Meeting of the Mouse Models of Human Cancer Consortium Prostate Pathology Committee. **Cancer Research**, v. 64, n. 6, p. 2270–2305, 2004.

SHEELA, D. L. et al. Lauric acid induce cell death in colon cancer cells mediated by the epidermal growth factor receptor downregulation: An in silico and in vitro study. **Human and Experimental Toxicology**, v. 38, n. 7, p. 753–761, 1 jul. 2019.

SHIRAI, T. et al. Experimental prostate carcinogenesis - Rodent models. **Mutation Research - Reviews in Mutation Research**, v. 462, n. 2–3, p. 219–226, 2000.

SHUKLA, S. et al. Studies on anti-inflammatory, antipyretic and analgesic properties of Caesalpinia bonducella F. seed oil in experimental animal models. **Elsevier**, 2010.

SOLER, R. et al. Future direction in pharmacotherapy for non-neurogenic male lower urinary tract symptoms. **European Urology**, v. 64, n. 4, p. 610–621, 2013.

SUGIMURA, Y.; CUNHA, G. R.; DONJACOUR, A. A. Morphogenesis of ductal networks in the mouse prostate. **Biology of reproduction**, v. 34, n. 5, p. 961–971, 1986.

TABOGA, S. R.; VILAMAIOR, P. S. L.; GÓES, R. M. Modulação androgênica e estrogênica na próstata: uma abordagem em modelos experimentais de roedores com enfoque na biologia estrutural. **Arquivos Brasileiros de Endocrinologia & Metabologia**, v. 53, n. 8, p. 946–955, 2009.

TENG, M. et al. Impact of coconut oil consumption on cardiovascular health: A systematic review and meta-Analysis. **Nutrition Reviews**, v. 78, n. 3, p. 249–259, 2020.

THOMSON, A. A.; CUNHA, G. R.; MARKER, P. C. Prostate development and pathogenesis. **Differentiation**, v. 76, n. 6, p. 559–564, 2008.

THORPE, A.; NEAL, D. Benign prostatic hyperplasia. v. 361, p. 1359–1367, 2003.

TIMMS, B. G.; MOHS, T. J.; DIDIO, L. J. A. Ductal budding and branching patterns in the developing prostate. **Journal of Urology**, v. 151, n. 5, p. 1427–1432, 1994.

TISSELL, L. E.; SALANDER, H. Anatomy of the human prostate and its three paired lobes. **Progress in clinical and biological research**, v. 145, p. 55–65, 1984.

UNTERGASSER, G. et al. Hiperplasia benigna da próstata: remodelação tecidual relacionada à idade. **Elsevier**, 2001.

VANELLA, L. et al. Correlation between lipid profile and heme oxygenase system in patients with benign prostatic hyperplasia. **Urology**, v. 83, n. 6, p. 1444.e7-1444.e13, 2014.

VENÂNCIO, D. P. et al. Sleep deprivation alters rat ventral prostate morphology, leading to glandular atrophy: A microscopic study contrasted with the hormonal assays. **Journal of Biomedicine and Biotechnology**, v. 2012, 2012.

VERZE, P.; CAI, T.; LORENZETTI, S. The role of the prostate in male fertility, health and disease. **Nature Reviews Urology**, v. 13, n. 7, p. 379–386, 2016.

VICKMAN, R. E. et al. The role of the androgen receptor in prostate development and benign prostatic hyperplasia: A review. **Asian Journal of Urology**, v. 7, n. 3, p. 191–202, 2020.

VILAMAIOR, P. S. L. et al. Collagen fiber reorganization in the rat ventral prostate following androgen deprivation: A possible role for smooth muscle cells. **Prostate**, v. 45, n. 3, p. 253–258, 2000.

VILAMAIOR, P. S. L.; TABOGA, S. R.; CARVALHO, H. F. Postnatal growth of the ventral prostate in Wistar rats: A stereological and morphometrical study. **Anatomical Record - Part A Discoveries in Molecular, Cellular, and Evolutionary Biology**, v. 288, n. 8, p. 885–892, 2006.

WALLACE, T. C. Health Effects of Coconut Oil—A Narrative Review of Current Evidence. **Journal of the American College of Nutrition**, v. 38, n. 2, p. 97–107, 2019.

WAN, J.; GRIMBLE, R. Effect of dietary linoleate content on the metabolic response of rats to Escherichia coli endotoxin. **portlandpress.com**, 1987.

WANG, X. et al. Increased infiltrated macrophages in benign prostatic hyperplasia (BPH): Role of stromal androgen receptor in macrophage-induced prostate stromal cell proliferation. **Journal of Biological Chemistry**, v. 287, n. 22, p. 18376–18385, 2012.

WANG, Y. et al. Cell differentiation lineage in the prostate. **Differentiation**, v. 68, n. 4–5, p. 270–279, 2001.

WILSON, J. D. The Critical Role of Androgens in Prostate Development. **Endocrinology and Metabolism Clinics of North America**, v. 40, n. 3, p. 577–590, 2011.

WITCHER, K. J.; NOVICK, R. P.; SCHLIEVERT, P. M. **Modulation of Immune Cell Proliferation by Glycerol MonolaurateCLINICAL AND DIAGNOSTIC LABORATORY IMMUNOLOGY**. [s.l: s.n.]. Disponível em: <http://cvi.asm.org/>. Acesso em: 1 out. 2020.

YANG, Y. J. et al. Comparison of fatty acid profiles in the serum of patients with prostate cancer and benign prostatic hyperplasia. **Clinical biochemistry**, v. 32, n. 6, p. 405–409, ago. 1999.

YEAP, S. et al. Antistress and antioxidant effects of virgin coconut oil in vivo. **spandidos-publications.com**, 2015.

ZHU, X. et al. Dynamic regulation of estrogen receptor-β expression by DNA methylation during prostate cancer development and metastasis. **American Journal of Pathology**, v. 164, n. 6, p. 2003–2012, 1 jun. 2004.

ANEXO A- Certificado de Experimentação Animal

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Câmpus de São José do Rio Preto

COMISSÃO DE ÉTICA NO USO DE ANIMAIS – IBILCE/UNESP-CSJRP

CERTIFICADO

Certificamos que a proposta intitulada "Avaliação morfofisiológica da próstata de gerbilos adultos tratados com óleo de coco após a suplementação pela testosterona", registrada com o nº. 175/2017 - CEUA, sob a responsabilidade da Professora Doutora Patrícia Simone Leite Vilamaior, que envolve a produção, manutenção ou utilização de animais pertencentes ao filo Chordata, subfilo Vertebrata (exceto humanos), para fins de pesquisa científica (ou de ensino), encontra-se de acordo com os Preceitos da Lei nº 11.794, de 08 de outubro de 2008, do Decreto nº 6.899, de 15 de julho de 2009, e com as normas editadas pelo Conselho Nacional de Controle da Experimentação Animal (CONCEA), e foi aprovada pela COMISSÃO DE ÉTICA NO USO DE ANIMAIS (CEUA), do IBILCE/UNESP, em reunião de 17 de outubro de 2017.

Finalidade	( ) Ensino (X) Pesquisa Científica
Vigência da autorização	01/10/2017 a 28/02/2021
Espécie/linhagem/Raça	*Meriones unguiculatus* (Gerbilo da Mongólia)
Nº de animais	60 (sessenta)
Peso/Idade	70g / 90 dias
Sexo	Machos
Origem	Biotério de criação e manutenção de roedores do Laboratório de Microscopia e Microanálise do Instituto de Biociências, Letras e Ciências Exatas de São José do Rio Preto.

São José do Rio Preto, 16 de outubro de 2017.

Prof. Dr. Luiz Henrique Florindo
Presidente da CEUA

Instituto de Biociências, Letras e Ciências Exatas – Comissão de Ética no Uso de Animais
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São José do Rio Preto, 30 / 10 / 2021

Assinatura do autor