Original Article

Protective Role of 4-(4-Hydroxy-3-methoxyphenyl)-2-Butanone on Prostatic Cells Hyperplasia of Rats and Human, 5α-reductase Inhibition Pathway

Ahmed S. Ahmed, Mohamed Gaber Soliman[1]

Department of Anatomy and Embryology, College of Medicine, Tanta University, [1]Department of Urology, Tanta University Hospitals, Tanta, Egypt

Abstract

Background: Prostate gland is an exocrine gland that could be affected by various pathological conditions. Benign prostatic hyperplasia (BPH) is an age-dependent medical condition caused by increased activity of 5α-reductase enzyme (5α-R). Medical treatment by finasteride is considered during treatment, but it has unavoidable side effects. Hence, there is an increasing need to use natural ingredients for BPH treatment**.** Gingerol oil (ginger extract) is transferred by heating into zingerone. Recent studies reported the effect of zingerone on prostate cancer cells. **Aims and Objectives:** The aim of the present research is to investigate the protective effect of zingerone against BPH. **Materials and Methods:** Sixty male Albino Wistar rats were divided into three groups: control group, prostatic hyperplasia group treated with saline, and prostatic hyperplasia group treated with zingerone (PH-Z-G). At day 28, all rats were sacrificed, epididymis and prostate samples were collected for histopathological examination and Western blotting for androgen receptors (ARs) proteins and steroid 5 alpha-reductase 1 (SRD5A1). Human RWPE-1 prostatic cell line was assessed for viability and cycle after treated with zingerone 500 μg/day for 10 days. **Results:** PH-S group showed significant ($P < 0.05$) thickening of connective tissue septa associated with narrowing of acinar lumen. PH-Z group showed regain of the normal histological feature. SRD5A1 and AR expression was significantly ($P < 0.05$) reduced in PH-Z group in comparison with PH-S group. Cell line proliferation was significantly reduced after application of zingerone with G2/M cell cycle arrest. **Conclusion:** Our results showed that natural herbal zingerone decreased the prostatic tissue levels of (5α reductase and AR) in rat BPH model, which could be a promising herbal medicine for BPH treatment. Further human clinical trials are required.

Keywords: 4-(4-Hydroxy-3-methoxyphenyl)-2-butanone, 5α-reductase, prostatic hyperplasia, zingerone

Introduction

Prostate gland is one of the internal components of male reproductive system, formed of exocrine acini (lined with simple columnar epithelium) embedded in rich connective tissue stroma. It secretes an alkaline fluid, forming about 40% of semen volume, to protect sperms from vaginal acidity. Many pathological conditions can affect the prostate, such as inflammation, neoplasms, and benign prostatic hyperplasia (BPH); the latter is a hyperplasia of prostatic epithelium that may interfere with normal evacuation of the urinary bladder if left untreated.[1-3]

As prostatic growth rate is 3% per year, BPH is an age-dependent condition. BPH prevalence reaches its peak by the age of ninety. It has genetic, lifestyle, and diet predisposing factors. It is age related and caused by increased activity of 5α-reductase enzyme (5α-R) in the prostatic acinar epithelium, which facilitates the irreversible conversion of testosterone into dihydrotestosterone (DHT), which has a more potent agonist effect on androgen receptors (ARs) causing hyperplasia of prostatic epithelial tissue. DHT helps in the differentiation of external genitalia of males during embryonic life (5α-R deficiency during fetal life results in

Received: 17-05-2020 **Revised:** 17-05-2020
Accepted: 03-09-2020 **Published:** 24-05-2021

Access this article online

Quick Response Code:

Website: http://www.jmau.org/

DOI: 10.4103/JMAU.JMAU_37_20

Address for correspondence: Dr. Ahmed S. Ahmed, Department of Anatomy and Embryology, College of Medicine, Tanta University, Tanta, Egypt.
E-mail: ahmedsahmed.tanta@gmail.com

How to cite this article: Ahmed AS, Soliman MG. Protective role of 4-(4-Hydroxy-3-methoxyphenyl)-2-butanone on prostatic cells hyperplasia of rats and human, 5α-reductase inhibition pathway. J Microsc Ultrastruct 2021;9:164-9.

underdeveloped external genitalia) and has very weak anabolic effect if administered exogenously.[4-6]

Medical treatment by α-blockers, 5α-R inhibitors, and phytotherapy is considered the first line of treatment. Surgery is indicated for patients with persistent symptoms even though medical treatment is initiated. 5α-R inhibiters such as finasteride are capable of lowering the level of circulating DHT by 70% helping in the reduction of prostate gland size in cases of BPH and reducing the incidence of prostatic cancer if considered for long-term usage. It has some side effects such as erectile dysfunction with loss of libido anxiety, depression, and gynecomastia. Hence, there is an increasing need to use natural remedy for BPH treatment.[7-9]

Ginger is a plant with flowers; its root (rhizome) is widely used as spices and in folk medication. It originates in Asia and then transported all over the world. Gingerol oil constitutes about 2% of the weight of ginger roots, transferred by heating (cooking) into zingerone, which is responsible for the sweet flavor of cooked ginger. Zingerone (vanillylacetone), 4-(4-Hydroxy-3-methoxyphenyl)-2-butanone, C11 H14 O3, is recognized as a free radicle scavenger and has potent antioxidant capacity. Recent studies revealed its effect on prostate cancer cells. The aim of the present research is to investigate the effect of zingerone against BPH and explore underlying pathway.[10-12]

Materials and Methods

Chemicals

Testosterone propionate, 50 mg per pack, NMID710, was purchased from Sigma-Aldrich company, 3050 Spruce Street, Saint Loui, Missouri, 63103, USA. Zingerone, 50 mg per pack, was purchased from TAKARA Bio. 1290 Terra Bella Ave., Mountain View, California, 94043, USA.

Animals

Sixty male rats (Albino Wistar), aged 6 months, weighted 200 g, were obtained from animal house, Tanta university. Rats were housed individually under normal condition with temperature 25°C, humidity 55%, and 12-hour light/dark cycle. Free access to water and chow was ensured (in accordance with the national and institutional guidelines). This research study was approved by the Research and Ethics Committee, Quality Assurance Unit, Faculty of Medicine, Tanta University, Egypt (2019/GKJDWLS-03-002).

Experimental designs

Rats were divided into three groups (n = 20). In control group (C-G), 3 ml of saline was injected intraperitoneally for twenty-eight days and gavage was orally inserted once daily. In prostatic hyperplasia treated with saline, BPH model was performed to rats and gavage was orally inserted once daily for twenty-eight days. In prostatic hyperplasia treated with zingerone (PH-Z-G), BPH model was performed and zingerone 100 mg/kg b.w.[13] was administered orally once daily for 28 days by gavage. BPH model was induced by intraperitoneal injection of 5 mg/kg/day and testosterone propionate for 28 days. At day 28 under general anesthesia, all rats were sacrificed, and epididymis and prostate samples were collected [Figure 1].

Body weight, prostatic weight, and prostatic index

Rats' total body weight was recorded once weekly using a digital scale-Tor Rey LEQ 5/10 (Tor Rey, EQ-4HP, Torrey, Mexico City, Mexico). After rat scarification, prostate was extracted and weighted individually. Prostate weight was divided by total body weight to calculate the prostatic index.[14]

Sperm count

Each epididymis sample was cut into four sections, inserted into Petri dish, mixed with four milliliters of saline to allow sperm to disperse into saline for one minute. Fluid was collected with Eppendorf tube (Eppendorf Middle East and Africa FZ-LLC, Dubai Science Park Nucleotide Complex, Office G08A, Ground Floor, Al Barsha South 2, Um Suqeim Road) and cytometer is used for sperm counting.[15,16]

Histopathological examination

Fresh prostate samples were fixed in 10% paraformaldehyde, dehydrated, embedded in paraffin, and sectioned at 4 μm. Then, it was stained with hematoxylin and eosin for histopathological examination at ×1000 magnification. Three fields per section were analyzed by ImageJ 1.24 v. software to detect septal thickness and acinar width.[17,18]

In vitro cell line and viability

RWPE-1 prostatic cell line was purchased from ATCC® (LGC Standards S. L. U., Spain). Phosphate-buffered saline (PBS) was used to rinse the cells. We added 1 mL of trypsin inhibitor (TI), in PBS, and then cells were aspirated and seeded in the 60 mm plates and placed in incubators at 37°C. Subculture was done when cell concentration reached 100 cells/cm^2. Keratinocyte serum-free medium was renewed every 48 h. Temperature was kept at 37°C, and CO_2 concentration was kept 5%. 500 μg/day of zingerone (dissolved in dimethyl sulfoxide as a vehicle) was added to prostatic cell line for two days, and cell proliferation was detected

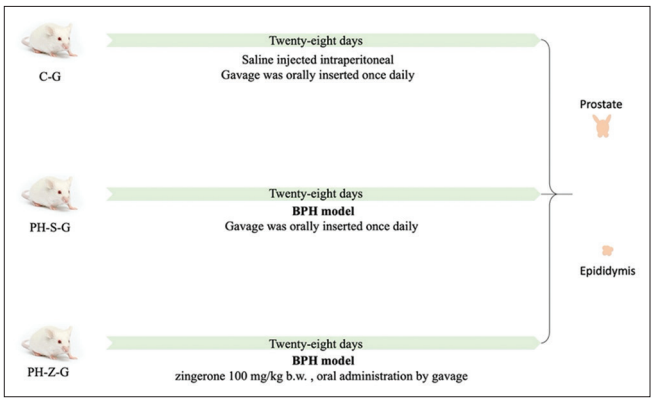

Figure 1: Schematic representative of experimental study. Rats are divided into 3 groups: control group, prostatic hyperplasia-treated with saline, prostatic hyperplasia-treated with zingerone (PH-Z-G). At the end of the study, epididymis and prostate samples were collected

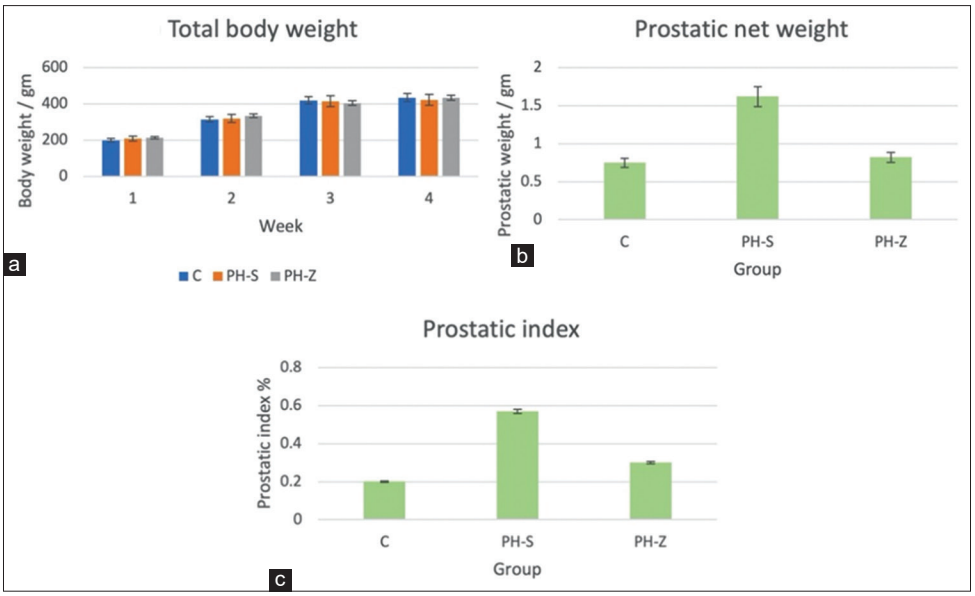

Figure 2: (a) Rats' total body weight, (b) prostatic weight (net weight without urethra or capsule), (c) prostatic index. There is no significant difference in body weight between C, PH-S, and PH-Z groups along the whole length of study. Prostatic weight and prostatic index of PH-S were significantly increased when compared to control group. Parameters were reduced in PH-Z group in relation to PH-S group

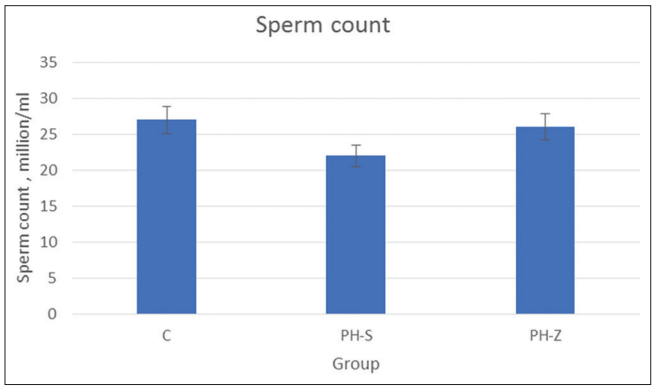

Figure 3: Rat sperm count in three groups. Control group showed normal sperm count. There was a slight reduction in number in PH-S group. The sperm number returned to normal value in PH-Z group

every 24 h. Dimethylthiazol–diphenyltetrazolium bromide assay was performed to detect cellular viability by checking for the presence of nicotinamide adenine dinucleotide phosphate-dependent oxi-reductase by adding tetrazolium and the resultant purple color was measured by microplate reader, Hidex Sense Beta Plus (HVD Life Science Vestries).[19-21]

Cell cycle analysis

RWPE-1 cells were extracted from medium plates, washed, fixed with 80% ethanol,[22] and finally stained with propidium iodide. The cell cycle analyses were performed using NxT Flow Cytometer (Becton Dickinson, Canada). Cell cycle phases were analyzed by FlowJo software (FlowJo LLC, USA).[23,24]

Western blot

It was used to estimate the prostatic tissue content of AR and SRD5A1 as indicators for AR and 5α-R expression. Methodology started by extraction of proteins from prostatic tissues by the help of radioimmunoprecipitation assay buffer followed by protein separation by gel electrophoresis, transfer to polyvinylidene fluoride membrane, blocking the rest of surface of high protein affinity membrane, incubation with primary antibody (N-20/sc-816, 26001-1-AP, Sigma Aldrich) overnight, then incubation with secondary antibody for one hour finally analysis of the data expressed by Image J 1.24 v. software (IBM, USA).[25,26]

Statistical analysis

SPSS software, 22 V. (IBM, Saudi Arabia office (IBM, USA) was used for data analysis; the data were expressed in mean ± standard deviation; and the probability value was considered significant if <0.05.

Results

Body weight, prostatic weight, and prostatic index

There was no significant difference in body weight between C, PH-S, and PH-Z groups ($P > 0.05$). Prostatic weight and prostatic index of PH-S were significantly increased in PH-S when compared to C-G ($P < 0.05$). Parameters were significantly reduced in PH-Z group in relation to PH-S group ($P < 0.05$) [Figure 2].

Sperm count

C-G showed normal sperm count. There was a slight insignificant reduction in number in PH-S group. The sperm number returned to normal value in PH-Z group [Figure 3].

Histopathological examination

Histopathological examination of prostate sections of C-G showed normal histological architecture. The prostate tissue is formed of connective tissue stroma with glands embedded. PH-S group showed significant ($P < 0.05$) apparent hyperplasia of the glandular lining epithelium with thickening of connective

tissue septa in between associated with narrowing of acinar lumen when compared to C-G. PH-Z group showed regain of the normal histological feature with no significant thickening of C. T septa or reduction of acinar lumen [Figure 4].

Cell line and viability

Cell proliferation was detected to be significantly reduced after application of zingerone 500 µg/day ($P < 0.05$). Cell viability was depressed by 37% after 72 h and by 77% after 192 h (8 days) [Figure 5].

Cell cycle

Zingerone was found to cause G2/M cell cycle arrest at a dose of 500 µg as shown in Figure 6; G2/M was 34.78% after 48 h.

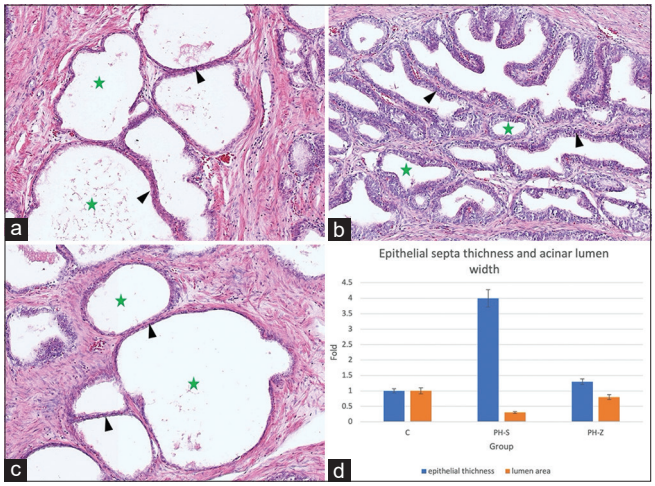

Figure 4: Photomicrograph of rats' prostatic sections stained with hematoxylin and eosin (×1000). (a) Control group showed normal histological architecture. The prostate tissue was formed of connective tissue stroma with numerous exocrine glands embedded in it. (b) PH-S group showed thickening of connective tissue septa, hyperplasia of the glandular lining epithelium with reduction of acinar lumen. (c) PH-Z group showed regain of normal histological features with no significant thickening of C. T septa or reduction of acinar lumen. Note C. T septa, black arrow, acini green star. (d) Quantification of septal thickness and lumen width

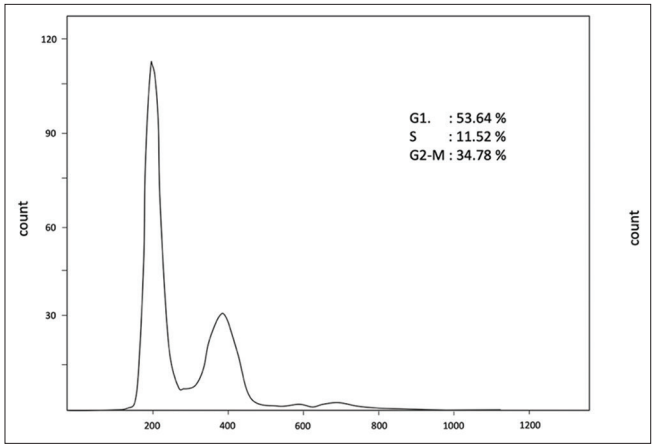

Figure 6: Flow cytometry analysis of epithelial cells at 500 µg concentration of zingerone; G2/M was 34.78% after 48 h

Western blot

SRD5A1 and AR expression in prostatic tissue cells were detected by the help of Western blot; markers' expression was significantly ($P < 0.05$) reduced in PH-Z group in comparison with PH-S group as shown in Figure 7.

Discussion

BPH is an age-related pathological condition which affects the elderly men. Medical treatment is the first line of treatment, but many side effects were reported. A need to explore natural alternative is rising in the horizon. In our study, we explored the potential protective effect of one of the natural ginger derivatives, zingerone, against the prostatic hyperplasia induced in rats together with its effect on *in vitro* human noncancerous prostatic cells.[27-29]

In our four-week research, we noticed that there was no significant difference in body weight between C, PH-S, and PH-Z groups. However, other researchers reported that ginger derivatives can cause hypoglycemia with reduction of body weight in a ten-week length study.[30] The same fact was emphasized by Han *et al*.[31] Previous study[32] has reported the stable body weight and absence abdominal fat deposition after testosterone administration. On the other hand, Steckler *et al*. reported body weight increase if testosterone administered prenatally.[33]

Our study demonstrated that prostatic weight and prostatic index of PH-S were significantly increased; this comes in

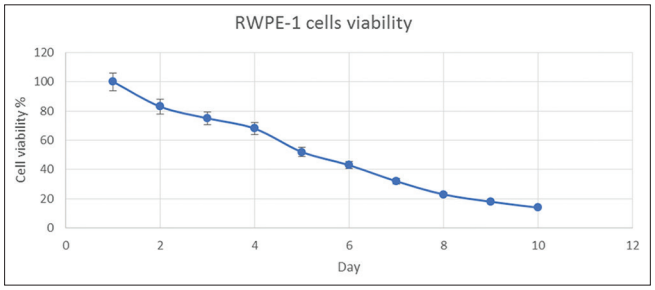

Figure 5: Quantitative expression of cell viability after zingerone application, Cell viability was depressed by 37% after 72 h and by 77% after 192 h (dimethylthiazol–diphenyltetrazolium bromide assay)

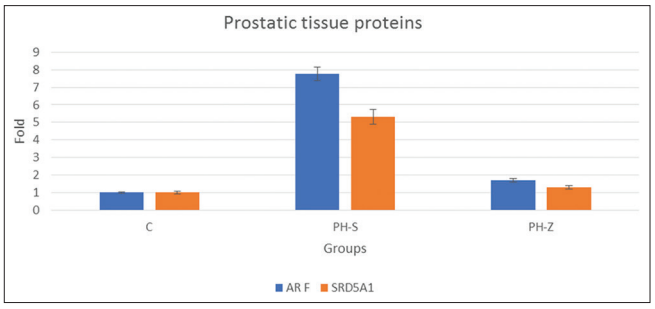

Figure 7: Western blot for SRD5A1 and AR expression in prostatic tissue cells; markers' expression was reduced in PH-Z group in comparison to PH-S group

agreement with Ishola *et al*.,[34] who reported prostatic weight increase by 20% and a prostatic index amplification by 66% after testosterone administration. In a six-week length study performed by Choi *et al*.,[35] they reported the same effect on prostate after exogenous testosterone administration. Our study revealed that these parameters were reduced in PH-Z group as a response to zingerone administration, which comes in consistence with Karna *et al*.[36] This could be related to the beneficial effect of zingerone on the prostatic tissue that is noticed in our study.

Our research reported a slight reduction in sperm number in PH-S group, which comes in agreement with Froman and Thurston[37] who added that testosterone caused reduction in semen volume as well, while Scully *et al*.[38] reported a negative effect of prenatal testosterone administration on scrotal size and semen concentration. The increase in sperm number after zingerone treatment which was revealed in our study comes in consistence with Khaki *et al*.[39] as they reported a positive effect of ginger and cinnamon on sperm vitality, number, and motility, which was also reported by Bordbar *et al*.[40]

We noticed that testosterone administration caused hyperplasia of the glandular lining epithelium, thickening of connective tissue septa in addition to narrowing of acinar lumen in PH-S group as mentioned by others[41-43] who examined the effect of testosterone on the histopathological changes in prostate tissue related to exogenous testosterone administration. PH-Z group showed regain of the normal histological feature, which indicates the protective effects of zingerone on prostatic tissue histological feature.[30,36,44]

Eight days after application of 500 µg/day zingerone to non-neoplastic prostatic cell line, we noticed reduction in cellular proliferation and viability. On the other hand, Akimoto *et al*.[45] stated that ginger extract has negative impact on cancerous cells[46,47] via inhibition of mammalian target of rapamycin. Chen *et al*.[48] reported the anti-cancerous activity of ginger extract, which comes in the same context with Vijaya *et al*.[49] In our research, we noticed that zingerone caused human prostatic cell cycle arrest as confirmed by Choi *et al*.[50] who reported inhibition of neuroblastoma cells after application of zingerone. Previously, the same effect was reported on pancreatic cancer cells.[51]

We noticed that SRD5A1 and AR expression was reduced in PH-Z group, which comes in consistence with Li *et al*.[52] who mentioned that Paraquat reduced SRD5A1 expression in testicular tissue. However, to the best of our knowledge, it is the first time to link between exogenous zingerone administration and the lowering of 5α-R activity, which may explain the positive beneficial effect of zingerone on prostatic tissue.

Conclusion

Our results showed that 100 mg/kg b.w. of natural herbal zingerone decreased the prostatic (weight and index) and the prostatic tissue levels of (5α reductase and AR) in rat BPH model. Minimal effect on sperm counts was noticed. These data indicate that zingerone could be a promising herbal medicine for BPH treatment. Human clinical trials are required.

Financial support and sponsorship

Nil.

Conflicts of interest

There are no conflicts of interest.

References

1. Langan RC. Benign prostatic hyperplasia. Prim Care 2019;46:223-32.
2. Kim EH, Larson JA, Andriole GL. Management of benign prostatic hyperplasia. Annu Rev Med 2016;67:137-51.
3. Robert G, De La Taille A, Descazeaud A. Données épidémiologiques en rapport avec la prise en charge de l'HBP Epidemiology of benign prostatic hyperplasia. Prog Urol 2018;28:803-12.
4. Swerdloff RS, Dudley RE, Page ST, Wang C, Salameh WA. Dihydrotestosterone: Biochemistry, physiology, and clinical implications of elevated blood levels. Endocr Rev 2017;38:220-54.
5. Kannenberg F, Fobker M, Schulte E, Pierściński G, Kelsch R, Zitzmann M, *et al*. The simultaneous measurement of serum testosterone and 5α-dihydrotestosterone by gas chromatography-mass spectrometry (GC-MS). Clin Chim Acta 2018;476:15-24.
6. Bormann CL, Smith GD, Padmanabhan V, Lee TM. Prenatal testosterone and dihydrotestosterone exposure disrupts ovine testicular development. Reproduction 2011;142:167-73.
7. Carreño-Orellana N, Moll-Manzur C, Carrasco-Zuber JE, Álvarez-Véliz S, Berroeta-Maurizianо D, Porras-Kusmanic N. Efectos adversos de finasteride: Mitos y realidades. Una revisión actualizada [Finasteride adverse effects: An update]. Rev Med Chil 2016;144:1584-90.
8. Fertig RM, Gamret AC, Darwin E, Gaudi S. Sexual side effects of 5-α-reductase inhibitors finasteride and dutasteride: A comprehensive review. Dermatol Online J 2017;23:13030/qt24k8q743.
9. Chaudhary UB, Turner JS. Finasteride. Expert Opin Drug Metab Toxicol 2010;6:873-81.
10. Banihani SA. Ginger and testosterone. Biomolecules 2018;8:119.
11. Semwal RB, Semwal DK, Combrinck S, Viljoen AM. Gingerols and shogaols: Important nutraceutical principles from ginger. Phytochemistry 2015;117:554-68.
12. Daily JW, Zhang X, Kim DS, Park S. Efficacy of Ginger for Alleviating the Symptoms of Primary Dysmenorrhea: A Systematic Review and Meta-analysis of Randomized Clinical Trials. Pain Med 2015;16:2243-55.
13. Sistani Karampour N, Arzi A, Rezaie A, Pashmforoosh M, Kordi F. Gastroprotective Effect of Zingerone on Ethanol-Induced Gastric Ulcers in Rats. Medicina (Kaunas). 2019 11;55:64.
14. Deng L, Zong W, Tao X, Liu S, Feng Z, Lin Y, *et al*. Evaluation of the therapeutic effect against benign prostatic hyperplasia and the active constituents from Epilobium angustifolium L. J Ethnopharmacol 2019;232:1-0.
15. Lamas CA, Cuquetto-Leite L, do Nascimento da Silva E, Thomazini BF, Cordeiro GDS, Predes FS, *et al*. Grape juice concentrate alleviates epididymis and sperm damage in cadmium-intoxicated rats. Int J Exp Pathol 2017;98:86-99.
16. Corrêa LBNS, da Costa CAS, Ribas JAS, Boaventura GT, Chagas MA. Antioxidant action of alpha lipoic acid on the testis and epididymis of diabetic rats: Morphological, sperm and immunohistochemical evaluation. Int Braz J Urol 2019;45:815-24.
17. Wick MR. The hematoxylin and eosin stain in anatomic pathology-An often-neglected focus of quality assurance in the laboratory. Semin Diagn Pathol 2019;36:303-11.
18. Sy J, Ang LC. Microtomy: Cutting Formalin-Fixed, Paraffin-Embedded Sections. Methods Mol Biol 2019;1897:269-78.
19. Dent MP, Madnick SJ, Hall S, Vantangoli Policelli M, Bars C, Li H, *et al*. A human-derived prostate co-culture microtissue model using epithelial (RWPE-1) and stromal (WPMY-1) cell

lines. Toxicol *In vitro* 2019;60:203-11.
20. Moroz A, Delella FK, Lacorte LM, Deffune E, Felisbino SL. Fibronectin induces MMP2 expression in human prostate cancer cells. Biochem Biophys Res Commun 2013;430:1319-21.
21. Clubbs EA, Bomser JA. Basal cell induced differentiation of noncancerous prostate epithelial cells (RWPE-1) by glycitein. Nutr Cancer 2009;61:390-6.
22. Ren G, Tang L, Zhu M, Yang L, Huang Y. Inhibition of genistein on the proliferation of RWPE-1 cells induced by testosterone under the control of insulin-like growth factor-1. Wei Sheng Yan Jiu 2010;39:447-50.
23. Kolluru V, Tyagi A, Chandrasekaran B, Damodaran C. Profiling of differentially expressed genes in cadmium-induced prostate carcinogenesis. Toxicol Appl Pharmacol 2019;375:57-63.
24. Lin CY, Wang BJ, Chen BC, Tseng JC, Jiang SS, Tsai KK, et al. Histone Demethylase KDM4C Stimulates the Proliferation of Prostate Cancer Cells via Activation of AKT and c-Myc. Cancers (Basel). 2019 13;11:1785.
25. Kim B. Western blot techniques. Methods Mol Biol 2017;1606:133-9.
26. Hnasko TS, Hnasko RM. The Western blot. Methods Mol Biol 2015;1318:87-96.
27. Schoeb, D S; Wullich, B; Dürschmied, D; Heimbach, B; Heupel-Reuter, M; Gross, A J. *et al*. Therapie des benignen Prostatasyndroms beim geriatrischen Patienten – Anwendung und Limitation medizinischer Leitlinien [Treatment of benign prostatic hyperplasia in geriatric patients-use and limitations of existing guidelines]. Urologe A 2019;58:1029-38.
28. Bechis SK, Otsetov AG, Ge R, Olumi AF. Personalized medicine for the management of benign prostatic hyperplasia. J Urol 2014;192:16-23.
29. Paolone DR. Benign prostatic hyperplasia. Clin Geriatr Med 2010;26:223-39.
30. Eid BG, Mosli H, Hasan A, El-Bassossy HM. Ginger ingredients alleviate diabetic prostatic complications: Effect on oxidative stress and fibrosis. Evid Based Complement Alternat Med 2017;2017:107-11.
31. Han LK, Morimoto C, Zheng YN, Li W, Asami E, Okuda H. *et al.* Effects of zingerone on fat storage in ovariectomized rats. Yakugaku Zasshi 2008;128:1195-201.
32. Deyhim F, Moreng RE, Kienholz EW. The effect of testosterone propionate on growth of broiler chickens. Poult Sci 1992;71:1921-6.
33. Steckler TL, Herkimer C, Dumesic DA, Padmanabhan V. Developmental programming: Excess weight gain amplifies the effects of prenatal testosterone excess on reproductive cyclicity-implication for polycystic ovary syndrome. Endocrinology 2009;150:1456-65.
34. Ishola IO, Yemitan KO, Afolayan OO, Anunobi CC, Durojaiye TE. Potential of Moringa oleifera in the Treatment of Benign Prostate Hyperplasia: Role of Antioxidant Defence Systems. Med Princ Pract 2018;27:15-22.
35. Choi HM, Jung Y, Park J, Kim HL, Youn DH, Kang J, *et al*. Cinnamomi Cortex (Cinnamomum verum) Suppresses Testosterone-induced Benign Prostatic Hyperplasia by Regulating 5α-reductase. Sci Rep 2016;6:31906.
36. Karna P, Chagani S, Gundala SR, Rida PC, Asif G, Sharma V, *et al*. Benefits of whole ginger extract in prostate cancer. Br J Nutr 2012;107:473-84.
37. Froman DP, Thurston RJ. The influence of testosterone propionate on semen quality and testes morphology in the domestic turkey, Meleagris gallopavo. Poult Sci 1983;62:169-74.
38. Scully CM, Estill CT, Amodei R, McKune A, Gribbin KP, Meaker M, *et al*. Early prenatal androgen exposure reduces testes size and sperm concentration in sheep without altering neuroendocrine differentiation and masculine sexual behavior. Domest Anim Endocrinol 2018;62:1-9.
39. Khaki A, Khaki AA, Hajhosseini L, Golzar FS, Ainehchi N. The anti-oxidant effects of ginger and cinnamon on spermatogenesis dys-function of diabetes rats. Afr J Tradit Complement Altern Med 2014;11:1-8.
40. Bordbar H, Esmaeilpour T, Dehghani F, Panjehshahin MR. Stereological study of the effect of ginger's alcoholic extract on the testis in busulfan-induced infertility in rats. Iran J Reprod Med 2013;11:467-72.
41. Park HS, Seo CS, Wijerathne CU, Jeong HY, Moon OS, Seo YW, *et al*. Effect of Veratrum maackii on Testosterone Propionate-Induced Benign Prostatic Hyperplasia in Rats. Biol Pharm Bull 2019;42:1-9.
42. Ko JW, Park SW, Shin NR, Kim WI, Kim JC, Shin IS, *et al*. Inhibitory effects of Pycnogenol®, a pine bark extract, in a rat model of testosterone propionate-induced benign prostatic hyperplasia. Lab Anim Res 2018;34:111-7.
43. Jung Y, Park J, Kim H.L, Youn D.H, Kang J, Lim S. *et al*. Vanillic acid attenuates testosterone-induced benign prostatic hyperplasia in rats and inhibits proliferation of prostatic epithelial cells. Oncotarget 2017;8:87194-208.
44. Lechner JF, Stoner GD. Gingers and Their Purified Components as Cancer Chemopreventative Agents. Molecules. 2019;24:2859.
45. Akimoto M, Iizuka M, Kanematsu R, Yoshida M, Takenaga K. Anticancer Effect of Ginger Extract against Pancreatic Cancer Cells Mainly through Reactive Oxygen Species-Mediated Autotic Cell Death. PLoS One 2015;10:e0126605.
46. Al-Daghri NM, Alokail MS, Alkharfy KM, Mohammed AK, Abd-Alrahman SH, Yakout SM, *et al*. Fenugreek extract as an inducer of cellular death via autophagy in human T lymphoma Jurkat cells. BMC Complement Altern Med 2012;12:202.
47. Bae WY, Choi JS, Kim JE, Park C, Jeong JW. Zingerone suppresses angiogenesis via inhibition of matrix metalloproteinases during tumor development. Oncotarget 2016;7:47232-41.
48. Chen SY, Lee YR, Hsieh MC, Omar HA, Teng YN, Lin CY, *et al*. Enhancing the anticancer activity of antrodia cinnamomea in hepatocellular carcinoma cells via cocultivation with ginger: The impact on cancer cell survival pathways. Front Pharmacol 2018;9:780.
49. Vijaya Padma V, Arul Diana Christie S, Ramkuma KM. Induction of apoptosis by ginger in HEp-2 cell line is mediated by reactive oxygen species. Basic Clin Pharmacol Toxicol 2007;100:302-7.
50. Choi JS, Ryu J, Bae WY, Park A, Nam S, Kim JE, *et al*. Zingerone Suppresses Tumor Development through Decreasing Cyclin D1 Expression and Inducing Mitotic Arrest. Int J Mol Sci. 2018 19:2832.
51. Park YJ, Wen J, Bang S, Park SW, Song SY. [6]-Gingerol induces cell cycle arrest and cell death of mutant p53-expressing pancreatic cancer cells. Yonsei Med J 2006;47:688-97.
52. Li H, Zhu Q, Wang S, Huang T, Li X, Ni C, *et al*. Paraquat exposure delays stem/progenitor Leydig cell regeneration in the adult rat testis. Chemosphere 2019;231:60-71.