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...
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 inhibitors 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.\(^{[13-19]}\)

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, C\textsubscript{11} H\textsubscript{14} O\textsubscript{3}, is recognized as a free radiol 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/GKJDWL3-03-002).

**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 Suqieim 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 \(\times1000\) 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® (LG 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.
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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 followed 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.

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.[33] 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 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 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 6: Flow cytometry analysis of epithelial cells at 500 µg concentration of zingerone; G2/M was 34.78% after 48 h

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 Pararquat 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-reductase 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.

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Nil.

**Conflicts of interest**

There are no conflicts of interest.

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