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Antiproliferative Potentials of *Zingiber officinale* in Testosterone Induced Prostate Hyperplastic Albino Wister Rats

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Authors' contributions

This work was carried out in collaboration among all authors. Authors EON and NB designed and supervised this work. Author UAO conducted the experimental aspect of the study and author NN as one of the supervisors, chaired the team that supervised the work. All authors read and approved the final manuscript.

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ABSTRACT

Aims: The present study evaluated the anti-proliferative potential of *Zingiber officinale - Zo* (Ginger) rhizome and Dutasteride (Avodart) singly and in combination on testosterone propionate (TP) induced benign prostatic hyperplastic (BPH) male albino wistar rats.
Study Design: This study is an interventional study.
Place and Duration of Study: This study was conducted at the Department of Pharmacology, University of Port Harcourt, between April and September, 2019.
Methodology: A total of 70 adult male albino wistar rats that weighed between 170-200 g were used for this study. They were fed with commercial rat diet and clean drinking water. Aqueous and ethanolic extracts of Ginger rhizome were prepared using the maceration method. BPH was induced in rats after they were subjected to bilateral orchiectomy by daily injections of TP (4 mg/kg b.wt.sc.). Rats were treated with 500 or 1500 mg/kg b.wt. of aqueous or ethanol extracts of *Zo* rhizome, dutasteride or in combination. Administration of extracts was done by gavage. Plasma prostate

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specific antigen (PSA) was analysed using sandwich ELISA Kits by Shanghai Korain Biotech Co., Ltd, China, prostatic weight (PW) was determined using a weighing balance while rat prostatic volume (PV) was calculated from measured prostatic length, breath and height. Prostatic indices (PI) and percentage prostatic growth inhibition (Percent. I) by the extracts were calculated. Statistical analysis was done using SPSS version 22.0 of Windows Stat Pac and p values <0.05 were considered statistically significant.

Results: The results showed that 500 and 1500 mg/kg b.wt. of *Zo* rhizome administered orally after exogenous injection of TP and BPH had been established for 15 days, significantly decreased (p=0.000) mean PV, PW, PI and PSA levels in treated rat groups compared with BPH induced rat groups. Both doses of the *Zo* extracts individually and in combination with dutasteride also markedly decreased (p=0.000) mean PV, PW, PI and PSA, indicating that there could be synergistic interaction between the *Zo* and the drug. Individual extract and in combination with dutasteride also produced high percentage inhibition of the prostate. Simultaneous administration of aqueous and ethanolic extracts of *Zo* rhizome with TP injection for 30 days also showed anti-proliferative qualities, although the effects were statistically not better than values for treatments done when BPH was established before treatment. Ethanolic extracts of both *Zo* rhizome produced better effects compared to the aqueous extracts.

Conclusion: From the findings, we conclude that ginger rhizome could reduce and inhibit testosterone-induced hyperplasia of the prostate in albino wistar rats and is suggested for further studies, especially in humans.

Keywords: Antiproliferative; Zingiber officinale; testosterone; prostate hyperplastic; albino rats.

1. INTRODUCTION

Benign prostatic hyperplasia (BPH) is characterized by non-malignant enlargement of the prostate. It is one of the most common urological diseases in elderly, Oesterling, [1]; Berry et al. [2]. BPH involves increases in the number of both stromal and epithelial cells in the transitional zone of the prostate and can cause lower urinary tract symptoms, which includes urgency, frequency, dysuria, incontinence and suprapubic pain, O'Leary, [3]; Wasserman [4].

Although aging and androgens are two established factors that contribute to the development of BPH, novel findings highlight the importance of inflammation and production of reactive oxygen species, Izumi et al. [5]. Auto-reactive T cells recognize prostate secretion products, also, animal models of experimental prostatitis have demonstrated an autoimmune component of chronic inflammation, Kramer et al. [6]. Therefore, reducing inflammation may serve a crucial role in the treatment of BPH and may lead to improved clinical outcomes.

In recent times, traditional medicine has continued to receive increasing acceptance in Nigeria among many other African nations. The World Health Organization (WHO) estimated that about 80% of African populations use traditional medicine to meet their primary health care needs. For many people in these countries especially those living in rural areas, traditional medicines are the only available, easily accessible and affordable source of health care.

Various types of pharmaceutical therapies are currently in use for the treatment of BPH, including alternative herbal-based therapies. However, there is currently no drug that can completely cure BPH without accompanying adverse effects. The 5α-reductase inhibitors, Dutasteride and Finasteride are currently in use but come with severe adverse effects, including sexual dysfuction, Shoskes [7].

Ginger (*Zingiber officinale*), a member of the Zingiberaceae family, is a popular spice used globally especially in most of the Asian countries, Demin and Yingying, [8]. Chemical analysis of ginger shows that it contains over 400 different compounds. Evidences from *in vitro*, animal, and epidemiological studies suggest that ginger and its active constituents suppress the growth and induce apoptosis of variety of cancer types including skin, ovarian, colon, breast, cervical, oral, renal, prostate, gastric, pancreatic, liver, and brain cancer. These properties of ginger and its constituents could be associated with antioxidant, anti-inflammatory and antimutagenic properties as well as other biological activities, Srinivasan [9].

2. MATERIALS AND METHODS

2.1 Experimental Design

This study is an interventional study.

2.2 Experimental Animals

A total of seventy (70) male albino wistar rats that weighed between 170-200 g were used for this study. The rats were purchased from the Department of Pharmacology, University of Port Harcourt, Rivers State. The rats were kept in a spacious and well-ventilated cage at room temperature; under natural circadian rhythm and were allowed to acclimatize for fourteen (14) days. They were housed in standard cages and allowed access to feed (Top Feed Finisher Mash, Sapele, Nigeria) and water *ad libitum*. All the animals received humane treatment according to the criteria outlined in the Guide for the Care and Use of Laboratory Animals prepared by the National Institute of Health NIH [10].

2.3 Plant Material

2.3.1 Ginger *(Zingiber officinale)*

Ginger root (rhizome) was bought at the Fruit Garden Market, Port Harcourt.

2.4 Drugs

2.4.1 Avodart (Dutasteride)

Avodart manufactured by GlasoSmithKline, UK was purchased from a Pharmacy in Port Harcourt.

2.4.2 Testosterone (Testosterone propionate)

Benign prostatic hyperplasia was induced by subcutaneous injection of 4mg/kg body weight (b.wt.) of testosterone propionate Obisike et al. [11]. Testosterone propionate (brand name Testost – manufactured by Laborate Pharmaceuticals India Limited) was purchased from a Pharmacy in Port Harcourt.

2.4.3 Ketamine injection (Ketamine hydrochloride)

Ketamine is a medication mainly used for starting and maintaining anaesthesia (brand name Ketalar **–** manufactured by Sular Pharmaceuticals, India) was also purchased from a Pharmacy in Port Harcourt and was used to anaethesize the rats prior to bilateral orchiectomy.

2.5 Castration of Rats (Bilateral Orchiectomy)

The rats were castrated using an anaesthetic agent (ketamine, 25 mg/kg b.wt i.p.) in order to eliminate the influence of endogenous testosterone during the study. Castration involved the removal of both testes and the epididymal fat through the scrota sac by the method of Van Coppenolle et al. [12]. The blood vessels and the spermatic cord were tied up with suture materials (3.0 mm) and resected. The animals were then allowed one (1) week to recuperate before the commencement of the pilot and main study.

2.6 Extraction of Powdered *Zingiber officinale* Rhizome with Absolute Ethanol and Distilled Water

After preparation of powered *Zingiber officinale* through maceration method, finely powdered ginger Rhizome was poured into a beaker and absolute ethanol/ distilled water was measured and poured into the beaker. It was intermittently shaken on a shaker and macerated for 48 hours. After 48 hours' storage, it was filtered and the filtrate was separated through a Whatman's Number One filter paper into a clean beaker. The filtered extracts were concentrated (at low pressure) using the rotary evaporator equipment (Manual Lift Rotary Evaporator Model EV311H by LabTech, U.S.A) after which they were dried on an evaporating dish at a temperature of 50^{o}C to 60^{o}C to a semi-solid form. A sticky semi-solid dark brownish substance was obtained. The extracts were stored in a well corked universal bottle and was kept in the refrigerator prior to use.

2.7 Dose Calculation

2.7.1 Avodart

Calculation of the administered dosages was based on guidelines from U.S. Department of Health and Human Services Food and Drug Administration Center for Drug Evaluation and Research CDER, [13]. Human daily dose is 1 capsule (0.5mg) per day.

The FDA guideline for dose conversion between human and animals in pre-clinical studies was used. To convert human dose in mg/kg to animal equivalent dose (AED) in mg/kg multiply human dose by 6.2. Therefore, if a 60 kg man would

take 0.5 mg Dutasteride, then a 1 kg man would take;

0.5mg/60kg =0.00833 mg

That is 0.00833 mg/kg. Then multiplying by the FDA factor, the AED would be

0.0083 mg/kg X 6.2 = 0.051 mg/kg.

This dose was administered mg per kg body weight of the rats dissolved in appropriate volume of normal saline (FDA guideline). CDER [13].

2.8 Grouping of Animals

The rats were weighed and randomized into twelve (12) of five (5) rats each (apart from normal control and BPH control groups that contained 10 rats each that were further divided into 5 rats for each group, as shown below):

2.8.1 Group 1 (Normal control group – NC and NC_2)

This group contained ten (10) male albino wistar rats. The rats in this group were further divided into two groups; five rats were used as control for the groups that were treated after BPH had been established for 15 days (NC), while the remaining five were used as control for the groups that were simultaneously induced and treated (NC_2). They were not BPH induced but were subjected to sham bilateral orchiectomy and were allowed rat feed for 30 days.

2.8.2 Group 2 (BPH control – BPHC and $BPHC_2$)

Ten (10) male albino wistar rats in this group were subjected to bilateral orchiectomy and divided into two groups of five rats each. BPHC group rats were BPH induced by subcutaneous (s.c.) injection of 4 mg/kg body weight (b.wt.) (for the first 15 days) of testosterone propionate and were not given further treatment for 30 days, while the $BPHC_2$ groups were treatment with 4mg/kg body b.wt. s.c for 30 days. They were allowed normal rat feed from the 16th day for 30 days.

2.8.3 Group 3 (Positive control - PC)

Five (5) male albino wistar rats in this group were subjected to bilateral orchiectomy and BPH induced by subcutaneous injection of 4 mg/kg b.wt. (for the first 15 days) of testosterone propionate and were given oral (gavage) administration of 0.051 mg/kg/day of Avodart (Dutasteride) daily from the 16th day for 30 days.

2.8.4 Group 4 (500Eth.Zin.)

Five (5) male albino wistar rats in this group were subjected to bilateral orchiectomy and BPH induced by subcutaneous injection of 4 mg/kg b.wt. (for the first 15 days) of testosterone propionate and were given oral (gavage) administration of 500 mg/kg b.wt./day ethanol extract of Ginger rhizome from the 16th day for 30 days.

2.8.5 Group 5 (1500EthZin.)

Five (5) male albino wistar rats in this group were subjected to bilateral orchiectomy and BPH induced by subcutaneous injection of 4 mg/kg b.wt. (for the first 15 days) of testosterone propionate and were given oral (gavage) administration of 1500 mg/kg b.wt./day ethanol extract of Ginger rhizome from the 16th day for 30 days.

2.8.6 Group 6 (500AquZin.)

Five (5) male albino wistar rats in this group were subjected to bilateral orchiectomy and BPH induced by subcutaneous injection of 4mg/kg b.wt. (for the first 15 days) of testosterone propionate and were given oral (gavage) administration of 500mg/kg b.wt./day aqueous extract of Ginger rhizome from the 16th day for 30 days.

2.8.7 Group 7 (1500Aqu.Zin.)

Five (5) male albino wistar rats in this group were subjected to bilateral orchiectomy and BPH induced by subcutaneous injection of 4 mg/kg b.wt. (for the first 15 days) of testosterone propionate and were given oral (gavage) administration of 1500 mg/kg b.wt./day aqueous extract of Ginger rhizome from the 16th day for 30 days.

2.8.8 Group 8 (1500EthZin.Dut)

Five (5) male albino wistar rats in this group were subjected to bilateral orchiectomy and BPH induced by subcutaneous injection of 4 mg/kg b.wt. (for the first 15 days) of testosterone propionate and were given oral (gavage) administration of 1500 mg/kg b.wt./day ethanol extract of Ginger root mixed with 0.051 mg/kg b.wt./day of Avodart (Dutasteride) from the 16th day for 30 days.

2.8.9 Group 9 (1500AquZin.Dut)

Five (5) male albino wistar rats in this group were subjected to bilateral orchiectomy and BPH induced by subcutaneous injection of 4 mg/kg b.wt. (for the first 15 days) of testosterone propionate and were given oral (gavage) administration of 1500 mg/kg b.wt./day aqueous extract of Ginger root mixed with 0.051 mg/kg b.wt./day of Avodart (Dutasteride) from the 16th day for 30 days.

2.8.10 Group 10 (Positive control 2 - PC_2)

Five male rats in this group were subjected to bilateral orchiectomy and were simultaneously induced for BPH by the injection of 4 mg/kg b.wt. s.c. daily and administered 0.051 mg/kg of Dutasteride daily for 30 days.

2.8.11 Group 11 (SimAdm1500AquZin)

Five (5) male albino wistar rats in this group were subjected to bilateral orchiectomy and BPH induced by subcutaneous injection of 4 mg/kg b.wt./day of testosterone propionate for 30 days and were simultaneously given oral (gavage) administration of 1500 mg/kg b.wt./day aqueous extract of Ginger rhizome from day 1 (first day of administration of testosterone propionate) for 30 days.

2.8.12 Group 12 (SimAdm1500EthZin)

Five (5) male albino wistar rats in this group were subjected to bilateral orchiectomy and BPH induced by subcutaneous injection of 4 mg/kg b.wt./day of testosterone propionate for 30 days and were simultaneously given oral (gavage) administration of 1500 mg/kg b.wt./day ethanolic extract of Ginger rhizome from day 1 (first day of administration of testosterone propionate) for 30 days.

2.9 Sample Collection and Storage

At the end of the treatments, the rats were anaesthetized with chloroform and blood samples collected through cardiac puncture after 8 hours fast. Immediately after euthanasia, the prostate gland was resected, washed weighed and measured. The length, height and breadth were measured. The prostate volume was calculated as described below. Five (5) ml of blood was put in lithium heparin container for the determination of plasma PSA, The samples in the lithium heparin container were allowed to stand and plasma separated within thirty minutes of sample collection using a centrifuge. The plasma samples were then stored frozen at -20°C, until the time of determination of other parameters.

2.10 Measurement of Prostate Size

Prostate size was measured with a centimeter rule. Immediately after euthanasia, the prostate gland was resected, washed weighed and measured. The length, height and breadth were measured. The prostate volume was calculated using the formula below:

PV (cm^3) = ½ (l x b x h), where

l = length of prostate gland
b = breadth of prostate gland
h = height of prostate gland, all measured in cm.

2.10.1 Calculation of prostatic indices, prostate percentage inhibition and oxidative stress indices

Prostate Index (Hongcai et al., 2018) was calculated using the formula below:

Prostate Index (PI) = Prostate weight (mg)/Body weight (g)

Arbitrary unit = mg/g.

Calculation of Prostate Percentage Inhibition (Percent. I) = 100 – [(T-C)/(B-C)] x 100% (Veeresh et al., 2010).

Where:

T - mean PI for treatment group
C – mean PI for control group
B – mean PI for BPH group
Unit = %

2.11 Laboratory Methods

2.11.1 Estimation of rat plasma prostate specific antigen

McCarthy et al., [14] as modified by Shanghai Korain Biotech Co., Ltd, China. The method used was sandwich enzyme-linked immunosorbent assay (ELISA).

2.12 Statistical Analysis

SPSS version 22.0 of windows statistical package was used to analyze the data

generated. The mean ± standard deviation was determined. One way analysis of variance (ANOVA) with Turkey's Post Hoc test, bar charts and line graph were also done using the same statistical package. From the values obtained statistical decision and inferential evaluation were made. A probability (p) value of less than 0.05 was considered statistically significant.

3. RESULTS AND DISCUSSION

In this study, the mean prostate weight (PW), prostate volume (PV) and prostate specific antigen (PSA) levels of rats in the testosterone induced group were significantly increased (p=0.000) when compared with those of rats in the normal control groups following BPH induction. These values were observed to have decreased markedly after thirty days of daily oral administration of 500mg/kg b.wt. and 1500mg/kg b.wt. of aqueous and ethanolic extracts of *Zingiber officinale* rhizome (Table 1).

This study also showed that aqueous and ethanolic extracts of *Zo* rhizome (500 and 1500 mg/kg/day for 30 days) treatment could significantly inhibit the development of TP-induced BPH, which was confirmed by significant decrease (p=0.000) in elevated PW, PV, PI and high percentage inhibition of the testosterone propionate enlarged prostate. Ginger is one of the most commonly consumed dietary condiments in the world. The oily resin from the rhizomes of ginger contains many bioactive components, such as [6]-gingerol (1-[4′-hydroxy-3′- methoxyphenyl]-5-hydroxy-3-decanone; which is the primary pungent ingredient that is believed to exert a variety of remarkable pharmacological and physiological activities, Surh [15].

These findings are supported by previous investigations demonstrating that dry ginger powder or solvent extracts of ginger roots induced cell cycle arrest and apoptosis in skin, breast, prostate, colon, and ovarian cancer cells, Shukla et al. [16]. The findings that aqueous and ethanolic extracts of *Zo* inhibit prostatic growth agree with a study conducted in 2012 by Prasanthi and colleagues. They reported a novel finding that oral consumption of the extract of whole ginger, a commonly consumed vegetable worldwide, significantly inhibited prostate tumour progression in both in vitro and in vivo rat models. They further reported that the anticancer effect of ginger was coupled with its significant anti-proliferative, cell-cycle inhibitory and pro-apoptotic activity in prostate tumour xenograft models, Prasanthi et al. [17]. Another reason why Ginger extracts ameliorated BPH could possibly be due to the presence of the phytochemical terpenoids (moderately present in ethanolic extract of *Zo* rhizome. Tepenoids are known to have antiproliferative effect on prostate tissues through the induction of cellular apoptosis, Roslin and Anupam, [18].

High amounts of polyphenols in Ginger may also have contributed to the significant decreases in PV, PW, PI and increases in percentage inhibition. Some studies have also linked the ability of ginger to ameliorate BPH with its high content of certain phytochemicals. The majority of these studies have suggested phenolic compounds, particularly 6-gingerol, shogaol and others, were responsible for the anti-proliferative effects of ginger, Krell and Stebbing, [19]. However, careful analysis of the data suggests that these polyphenolic compounds inhibit growth and induce apoptosis of cancer cells at relatively high concentrations of >10 mM, Rhode et al. [20]. In many studies, the phenolic compounds from ginger were shown to inhibit cancer cell proliferation at IC_{50} concentrations of 50–100 mM. The high IC_{50} concentrations of the phenols have likely curtailed additional investigations on the development of ginger-based compounds for the prevention and treatment of ovarian and other solid tumors.

Polyphenols have also been reported to directly inhibit 5-alpha reductase which is the enzyme that converts prostatic testosterone to the more potent dihydrotestosterone (DHT), Eleazu et al. [21]. DHT acts in an autocrine fashion on the stromal cells or in paracrine fashion by diffusing into nearby epithelial cells. In both of these cell types, DHT binds to nuclear androgen receptors and signals the transcription of growth factors that are mitogenic to the epithelial and stromal cells leading to prostatic hyperplasia. DHT is ten times more potent than testosterone because it dissociates from the androgen receptor more slowly, Alberto et al. [22].

Another phytochemical that may have caused ameliorative effect of ginger on BPH by the reduction of the rat prostate indices in the treated groups are alkaloids. According to a study by Liu and colleagues in 2011, they worked on the effect of the phytochemical alkaloids (though extracted from a different plant: *Leonuri herba*), on prostate index and histomorphology of mice. They reported that there were significant reduction in prostate index of mice treated with

alkaloids when compared with model group. They inferred that alkaloids could cause atrophy of hypertrophic prostatic cells, Liu et al. [23].

Higher doses of the two methods of extractions of Ginger did not show any better anti-proliferative capabilities (non-significant differences in PW, PV and PI) (Table 1). Reasons may be that the difference in dose may not be enough to make any significant therapeutic impact.

There are nearly 1500 documented interactions between drugs, herbal medicines, and dietary supplements and these interactions could enhance the therapeutic efficacy of the mixture or may antagonize the effect of each other. In most of the cases, the mixture results to a wide variety of harmful effects, (Tsai et al., 2012).

In different regions and cultures, herbal products are used as single herb, combination of herbs, or combination of herb(s) and drug(s). Chen et al., [24]. When herbs are used in combination, the effects can be complicated as various interactions can occur among the individual components, Colalto, [25]. The most desirable interactions are those which can result in additional therapeutic benefit. This is often the intended or expected outcome when using combination therapy. However, due to the presence of multiple components in the herbal products, the effects arising from herb-herb or herb-drug interactions are often unpredictable and complicated. Various types of pharmacokinetic and pharmacogenomic interactions from herb-drug combinations have been well described and documented in recent literatures.

In the current study, it was observed that treatment with a combination of ginger rhizome and the anti-BPH drug dutasteride showed marked decreases in PV, PW, PI and PSA (Table 2).

A decrease in PSA and other parameters is associated with reduced prostate hyperplasia as a direct consequence of 5α-reductase inhibition or anti-inflammatory actions by the anti-BPH drug dutasteride or in combination with the extracts Sing et al., [26]. BPH is caused by dihydrotestosterone, a metabolite obtained from the conversion of testosterone by 5α –reductase, McConnell et al., [27]. Consequently, inhibitors of 5α-reductase which block production of DHT ultimately slow down the development of BPH.

Ozbay and colleague in 2011 reported that *Zo* oil presents growth inhibitory activity in breast cancer cells of different molecular subtypes, but elevate potential drug antagonism when used in combination with existing targeted therapies in HER2-overexpressing breast cancer, Ozbay and Nahta, [28]. Similarly, ethanol extracts of *Zo* rhizome also showed a better BPH reducing effect than the aqueous extract (Table 2). Increase in prostatic parameters induced by TP were also significantly decreased in treatments that contained a combination of ethanol extracts of ginger, dutasteride when compared with their aqueous counterpacts, (Table 2). Reasons may be, as earlier said, due to the difference in phytochemical contents of both methods of extractions. Previous studies have reported that ethanolic extracts of ginger contained more alkaloids, flavonoids, terpenoids and saponins compared to the aqueous extracts. Terpenoids have also shown to demonstrate high antiproliferative effects through the induction of apoptosis [18].

Taking an herb or supplement could change the way a prescription medicine works in the body by enhancing the effect of the medicine, or it could react in a negative manner, causing symptoms like an overdose, or, it might cause the medicine not to work at all. Although many studies regarding herb–drug interactions emphasize the potential harmful effects of such interactions, the possibility of herbal components beneficially enhancing or facilitating the action of anproliferative pharmaceutical agents (or vice versa) may also exist. Positive interactions between herbs and drugs may lead to enhanced effectiveness of the anti-proliferative agents through additive or synergistic actions. The importance of DHT in causing nodular hyperplasia is supported by clinical observations in which dutasteride, an inhibitor of 5a-reductase is given to men with BPH. Therapeutic use of the 5a-reductase inhibitors markedly reduces the DHT content of the prostate and, in turn, reduces prostate volume and BPH symptoms [22].

This study also showed that the hyperplasia induced by testosterone was less in rats simultaneously given aqueous ginger rhizome along with testosterone, attaining even better protection when the ethanolic extract was administered.

Simultaneous administration of higher doses of aqueous and ethanolic extract *Zo* rhizome with TP for 30 days inhibited prostate enlargement in

Table 1. Prostatic parameters of TP induced BPH male rats treated with a combination of lower and higher doses of ethanolic and aqueous extracts of *zo* rhizome compared with controls

	PW(mg)	LBW(g)	PI (mg/g)	Per.I(%)	PV(cm^3)	PSA (pg/ml)
Grp1(NC (n=5)	216.2 ± 10.5	175 ± 7.31	1.23 ± 0.07	-	0.23 ± 0.05	76.34 ± 3.55
Grp2(BPHC) (n=5)	635.8 ± 78.8^{a}	181 ± 12.70	3.52 ± 0.46^{a}	-	0.83 ± 0.11^{a}	716.96 ± 56.18^{a}
Grp3(PC_1)(n=5)	307.0 ± 11.8^{ba}	178 ± 12.17	1.73 ± 0.17^{b}	78.19	0.33 ± 0.06^{b}	98.44 ± 15.26
Grp4(500EthZin)n=5	301.2 ± 8.24^{ba}	141 ± 12.97^{a}	2.13 ± 0.12^{b}	60.7	0.39 ± 0.08^{b}	170.02 ± 9.19^{a}
Grp5(1500EthZin)n=5	299.2 ± 16.73^{ba}	162 ± 19.66^{a}	1.86 ± 0.31^{b}	72.5	0.38 ± 0.07^{b}	124.26 ± 8.78^{bc}
Grp6(500AquZin)n=5	447.0 ± 97.20^{ba}	129 ± 31.70^{abc}	3.59 ± 0.96^{ac}	86.5	0.54 ± 0.09^{abc}	179.50 ± 58.74^{b}
Grp7(1500AquZin)n=5	596.8±126.50^{ac}	140 ± 8.10^{ab}	4.27 ± 1.02^{a}	88.1	0.71 ± 0.14^{ac}	127.38 ± 27.71^{bc}
F value	28.47	8.01	20.85		26.408	225.80
P value	0.000	0.000	0.000		0.000	0.000
Remark	**S**	**S**	**S**		**S**	**S**

Post hoc test, significant p values: a, b & c – compared with Grps. 1, 2 and 3 respectively

Table 2. Prostatic parameters of TP induced BPH male rats treated with a combination of higher dose of aqueous and ethanolic extracts of mixture of *Zingiber officinale* and dutasteride compared with controls

	PW(mg)	LBW(g)	PI (mg/g)	Per.I(%)	PV (cm^3)	PSA (pg/ml)
Grp1(NC (n=5)	216.2 ± 10.5	175 ± 7.31	1.23 ± 0.07	-	0.23 ± 0.05	76.34 ± 3.55
Grp2(BPHC) (n=5)	635.8 ± 78.8^{a}	181 ± 12.70	3.52 ± 0.46^{a}	-	0.83 ± 0.11^{a}	716.96 ± 56.18^{a}
Grp3(PC_1)(n=5)	307.0 ± 11.8^{a}	178 ± 12.17	1.73 ± 0.17^{ab}	78.19	0.33 ± 0.06^{b}	98.44 ± 15.26^{b}
Grp8(1500EthZinDut)n=4	316.0 ± 15.97^{ab}	152 ± 15.50	2.09 ± 0.29^{ab}	62.45	0.25 ± 0.07^{b}	102.25 ± 8.85^{b}
Grp9(1500AquZinDut) n=4	363.75 ± 32.73^{ab}	154 ± 27.26	2.41 ± 0.50^{ab}	48.48	0.26 ± 0.06^{b}	164.90 ± 16.77^{abc}
F value	75.85	3.34	32.65		51.071	450.41
P value	0.000	0.000	0.000		0.000	0.000
Remark	**S**	**S**	**S**		**S**	**S**

Post hoc test, significant p values: a, b and c – compared with Groups 1, 2 and 3 respectively. Per. I -percentage inhibition

Table 3. Prostatic parameters of male rats simultaneously induced for bph and treated with higher dose of both extracts of mixture of *Zo* rhizome compared with controls

	PW(mg)	LBW(g)	PI (mg/g)	Per.I(%)	PV (cm^3)	PSA (pg/ml)
Grp1(NC_2 (n=5)	219.8 ± 12.9	175 ± 5.34	1.26 ± 0.09	-	0.37 ± 0.07	75.58 ± 5.60
Grp2($BPHC_2$) (n=5)	631.6 ± 69.1[a]	175 ± 4.09	3.60 ± 0.34[a]	-	0.85 ± 0.07[a]	716.72 ± 58.76[a]
Grp10(PC_2)(n=5)	222.8 ± 12.33[b]	134 ± 9.65[ab]	1.66 ± 0.19[b]	81.22	0.21 ± 0.04[b]	72.58 ± 5.85[b]
Grp11(SimAdm1500AquZin)n=5	330.4 ± 15.53[abc]	144 ± 7.71[ab]	2.29 ± 0.09[ab]	53.71	0.22 ± 0.04[b]	462.96 ± 57.85[abc]
Grp12(SimAdm1500EthZin) n=4	312.75 ± 9.21[abc]	151 ± 13.88[ab]	2.08 ± 0.17[ab]	62.88	0.31 ± 0.06[b]	328.75 ± 59.50[abc]
F value	102.51	18.63	60.80		71.478	192.22
P value	0.000	0.000	0.000		0.000	0.000
Remark	**S**	**S**	**S**		**S**	**S**

Post hoc test, significant p values: a, b and c – compared with Groups 1, 2 and 10 respectively

the treatment groups. Mean PV, PW and PSA were significantly decreased (p=0.000). The PC_2 groups being rats treated with daily administration of 4 mg/kg b.wt. sc. TP and 0.051 mg/kg dutasteride showed marked decreases in mean PV, PW and PSA, (Table 3). Again, ethanolic extracts of *Zo* showed better results when compared to the aqueous extract. Dutasteride, an inhibitor of 5α-reductase enzyme, reduced the testosterone-induced prostatic hyperplasia in rats. Exogenous testosterone accelerated the growth of the prostate, castration of the rats paused prostatic growth and testosterone administration to castrated adult male rats caused the gland to grow beyond normal.

Castration of adult male rats causes extensive atrophy of prostate with induction of apoptosis, majorly of the ventral prostate epithelial cells, (Colombel and Buttyan, 1995). Further administration of testosterone exogenously to castrated rats caused suppression of apoptosis and prevented epithelial cell atrophy. The percentage inhibition of the ethanolic extract of *Zo* was also higher. However, the inhibitory effects of dutasteride (PC_2) administered daily for 30 days had better inhibitory effects than both the aqueous and ethanolic extracts of *Zo*.

The extracts apparently tried to inhibit hyperplasia and hypertrophy induced by TP, but their effectiveness were not as strong as that of dutasteride.

4. CONCLUSION

From the findings, we conclude that ginger rhizome can reduce and inhibit testosterone-induced hyperplasia of the prostate in albino wistar rats and is suggested for further studies, especially in humans.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Animal Ethic committee approval has been taken to carry out this study.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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