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Assessment of Antioxidant Effects of Aqueous and Ethanolic Extracts of *Zingiber officinale* Rhizome in Testosterone Induced Benign Prostatic Hyperplasia Rat Model

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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: To assess the antioxidant effects of the aqueous and ethanolic extracts of Ginger rhizome in testosterone induced prostate hyperplasic male rats.
Study Design: The study was a cross-sectional study.
Place and Duration of Study: The experimental aspect of this study was conducted at the animal house, Department of Pharmacology, University of Port Harcourt between April and September, 2019.
Methodology: Sixty (60) adult albino male wistar rats were used for this study. They were divided into 12 groups of 5 rats each and fed with commercial rat diet and clean drinking water. Aqueous and ethanolic extractions of Ginger rhizome seed were prepared using the maceration method. BPH was induced in rats after they submitted to bilateral orchiectomy by daily injections of

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testosterone propionate (TP) (4 mg/kg b.wt.sc). Rats were treated with 500 or 1500 mg/kg b.wt. of aqueous or ethanol extracts of *Zingiber officinale (Zo)* rhizome, dutasteride or in combination. Administration of extracts was done by gavage. Plasma total oxidant status (TOS), total antioxidant status (TAS), superoxide dismutase (SOD) activity, were analysed using sandwich ELISA Kits by Shanghai Korain Biotech Co., Ltd, China, while oxidative stress indices (OSI) 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 exogenous induction of BPH in rats significantly increased (p=0.000) plasma TOS and OSI while TAS and SOD activities were reduced. However, 500 and 1500 mg/kg b.wt. of *Zo* rhizome administered orally after exogenous induction of BPH had been established for 15 days, significantly decreased (p=0.000) TOS, OSI and significantly improved the activities of antioxidant parameters like SOD and TAS. Non-significantly increased mean TAS and SOD were seen in a combination of both extracts with dutasteride, possibly suggestive of synergistic interaction between the herbs and the drug. Simultaneous administration of aqueous and ethanolic extracts of *Zo* rhizome with TP for 30 days also showed antioxidant qualities, although the effects were statistically not better than values for treatments done when BPH was established before treatment. Ethanolic extracts of *Zo* rhizome produced better antioxidant effects compared to the aqueous extracts.

Conclusion: From the findings, we conclude that *Zo* rhizome can ameliorate oxidative stress and therefore may be beneficial in the management of benign prostatic hyperplasia.

Keywords: Antioxidant; Zingiber officinale; testosterone; benign prostatic hyperplasia; rat.

1. INTRODUCTION

BPH is sometimes referred to as benign prostatic hypertrophy and can either be described based on the clinical or pathological features. Clinical BPH presents as benign enlargement of the prostate, which contributes to an array of voiding difficulties that can range from bothersome to significantly impacting quality of life in older men, [1]. Due to the high prevalence of BPH in elderly men, it has been suggested to be a ubiquitous sign of aging [2]. The concept that androgens are important for the maintenance of prostate disease dictates the standard of care for BPH [3]. Although aging and androgens are two established factors that contribute to the development of BPH, recent novel findings highlight the importance of inflammation and production of reactive oxygen species [4].

Oxidative stress (OS) is considered to be one of the mechanisms that triggers the chain of reactions involved in the development and progression of prostatic hyperplasia. OS is a condition in the cellular environment that occurs when there is an imbalance between the production of reactive oxygen species (ROS) and the ability of biological systems to repair oxidative damage or neutralize the effects of reactive intermediates including peroxides and free radicals. Production of high levels of ROS causes a significant decrease in antioxidant defense mechanisms leading to protein, lipid and DNA damage and subsequent disruption of cellular functions and cell death but at lower levels induce subtle changes in intracellular signaling pathways, [5]. The oxidative damage can be exacerbated by a decreased efficiency of antioxidant defense mechanisms, [6]. Like many different cancer types, OS has been linked with benign prostatic hyperplasia (BPH) and prostate cancer (PCa) development, progression and the response to therapy, [7,8,9,10]. OS and PCa are both associated with increasing age because PCa is more prevalent in older men. Hence, it has been reported that age increases the pro-oxidant antioxidant balance toward a more oxidative state in many tissues [11].

Ginger (*Zingiber officinale*), a member of the Zingiberaceae family, is a popular spice used globally especially in most of the Asian countries [12]. 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 [13]. Ginger spares SOD (superoxide

dismutase)–an important anti-oxidant, catalase which is essential for breaking down potentially harmful hydrogen peroxide in the cells to glutathione peroxidase. SOD also acts on hydrogen peroxide and helps maintain integrity of cell membranes, [14]. The active constituents of ginger have antioxidant properties [15]. The chemical structure of zingerone makes it a potent free radical scavenger. The aim of this study was to assess the antioxidant, effects of the aqueous and ethanolic extracts of Ginger rhizome in testosterone induced prostate hyperplasic male rats.

2. MATERIALS AND METHODS

2.1 Experimental Animals

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 12 hours light and dark cycle and were allowed to acclimatize for fourteen (14) days. They were housed in standard cages and allowed access to feed and clean 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 [16].

2.2 Drugs/Chemicals

Avodart (Dutasteride), Testot (Testosterone Propionate) and Ketalar (Ketamine hydrochloride): Avodart (manufactured by GlaxoSmithKline, UK), Testot (by Laborate Pharmaceuticals India Limited), and Ketalar (by Sular Pharmaceuticals, India) respectively used as anti-BPH, BPH inducing and anesthetic drugs were purchased from Sicone Pharmacy and Stores, No. 2B Evo Road, G.R.A. Phase II Port Harcourt after full explanation of the purpose for procurement.

2.3 Castration of Rats

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 [17]. 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.

2.4 Plant Material

Ginger root (rhizome) was bought at the Fruit Garden Market, Kaduna Street, D-Line, Port Harcourt.

2.4.1 Preparation of plant extracts

2.4.1.1 Preparation of Zingiber officinale Rhizome Plant Extracts (Cold Maceration Extraction Method)

The fresh *Zingiber officinale* rhizome was cut in small bits. They were sun dried for fourteen days. The dried rhizome was ground into fine powder using a blender and poured into a maceration jar, stored and labelled into an air tight container prior to use.

2.4.1.2 Extraction of Powdered Zingiber officinale Rhizome Absolute Ethanol and Distilled Water

About 1.2 kg of finely powdered *Zingiber officinale* Rhizome were separately poured into two beakers and six litres (6L) each of absolute ethanol and distilled water were measured and poured into the beakers. They were intermittently shaken on a shaker and macerated for 48 hours. After 48 hours' storage, they were filtered and the filtrate was separated through a Whatman's Number One filter paper into two clean beakers. 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 50ºC to 60ºC to semi-solid forms. A sticky semi-solid dark brownish substance were obtained. The extracts were stored in well corked universal bottles and were kept in the refrigerator prior to use.

2.5 Dose Calculations

2.5.1 Dutasteride

Human daily dose is 1 capsule (0.5 mg) 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 1kg man would take;

0.5 mg/60kg =0.00833 mg

That is 0.00833mg/kg. Then multiplying by the FDA factor, the AED would be 0.0083 mg/kg X 6.2 = 0.051mg/kg.

This dose was administered mg per kg body weight of the rats dissolved in appropriate volume of normal saline. (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, 2005).

2.5.2 Testosterone propionate

The dose of testosterone propionate (TP) administered was 4mg/kg b.wt.sc which was determined by a pilot study. The pilot study showed that TP at the dose stated above could induce histological BPH and cause significant increases in rat prostate volume, prostate weight, prostatic indices and PSA levels. The changes were sustained throughout the 30-day period of this study.

2.6 Experimental Design

This study was a cross-sectional study. A total of 60 rats were weighed and randomized into twelve (12) groups of five (5) rats each as shown below:

2.6.1 Group 1 (Normal Control Group -NC)

This group contained ten (10) male albino wistar rats, five were used for simultaneous treatment (NC_2), while the remaining five were used for post induction treatment (NC). The rats in this group were not BPH induced. They were subjected to sham bilateral orchiectomy and were allowed rat feed for 30 days.

2.6.2 Group 2 (BPH Control – BPH C)

The group contained ten (10) male albino wistar rats, five were used for simultaneous treatment ($BPHC_2$), while the remaining five were used for post induction treatment (BPHC). The rats in this submitted to bilateral orchiectomy and BPH induced by subcutaneous (s.c.) injection of 4 mg/kg body weight (b.wt.) for the first 15 days of testosterone propionate (BPHC), or daily for 30 days ($BPHC_2$). They were allowed normal rat feed.

2.6.3 Group 3 (Positive Control - PC)

Five (5) male albino wistar rats in this group submitted 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.

2.6.4 Group 4 (500Eth.Zin.)

Five (5) male albino wistar rats in this group submitted 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.

2.6.5 Group 5 (1500EthZin.)

Five (5) male albino wistar rats in this group submitted 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.

2.6.6 Group 6 (500AquZin.)

Five (5) male albino wistar rats in this group submitted 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 500mg/kg b.wt./day aqueous extract of Ginger rhizome from the 16th day for 30 days.

2.6.7 Group 7 (1500Aqu.Zin.)

Five (5) male albino wistar rats in this group submitted 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.

2.6.8 Group 8 (1500EthZin.Dut)

Five (5) male albino wistar rats in this group submitted 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.

2.6.9 Group 9 (1500AquZin.Dut)

Five (5) male albino wistar rats in this group submitted 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.

2.6.10 Group 10 (Positive Control 2 - PC2)

Five male rats in this group submitted 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.

2.6.11 Group 11 (SimAdm1500AquZin)

Five (5) male albino wistar rats in this group submitted 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.

2.6.12 Group 12 (SimAdm1500EthZin)

Five (5) male albino wistar rats in this group submitted 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.

2.7 Sample Collection and Analysis

At the end of the treatments, the rats anaesthetized with chloroform and blood samples collected through cardiac puncture after 8 hours fast. 5 ml of blood was put in lithium heparin container for the determination of total oxidant status (TOS), total antioxidant status (TAS) and superoxide dismutase (SOD) activities.

2.7.1 Determination of Superoxide Dismutase (SOD)

Superoxide dismutase level was measured quantitatively by the sandwich-enzyme linked immunosorbent assay (ELISA) method [18] as described by Shanghai Korain Biotech Co., Ltd, China.

2.7.2 Determination of Rat Total Antioxidant Status (TAS) and Total Oxidant Status (TOS)

Total antioxidant status Total Oxidant Status (TOS) weere measured quantitatively by the sandwich-enzyme linked immunosorbent assay (ELISA) method as described by Shanghai Korain Biotech Co., Ltd, China.

2.8 Calculation of Oxidative Stress Index (OSI)

Oxidative stress index was determined by dividing Total Oxidant Status (TOS) by Total Antioxidant Status (TAS) (TOS/TAS).

2.9 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, we observed significant increases ($p=0.000$) in total oxidant status (TOS), oxidative stress indices (OSI) and a corresponding fall in the activity of superoxide dismuthase (SOD) and total antioxidant status (TAS) in the BPH model group. However, decreased TOS and OSI and a significant rise in SOD activity and TAS were observed in rat groups treated with both higher and lower doses of aqueous and ethanolic extracts of Ginger rhizome, when compared with values for BPH control groups, of which higher doses of both methods of extractions showed better outcomes (Table 1).

Table 1. Oxidant and Antioxidant 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

	TOS (U/ml)	TAS (U/ml)	OSI	SOD(pg/ml)
Grp1(NC (n=5)	1.66 ± 0.20	2.71 ± 0.25	0.62 ± 0.10	41.88 ± 2.98
Grp2(BPHC) (n=5)	3.25 ± 0.50	1.17 ± 0.14	2.81 ± 0.60	23.38 ± 2.09
Grp3(PC1)(n=5)	1.60 ± 0.17	2.13 ± 0.30	0.76 ± 0.15	36.53 ± 2.77
Grp4(500EthZin)n=5	2.15 ± 0.37*	1.99 ± 0.18$^{\#*}$	1.09 ± 0.24*	32.02 ± 0.89*
Grp5(1500EthZin)n=5	1.61 ± 0.41$^{\#*}$+	2.45 ± 0.30*	0.65 ± 0.13*	38.51 ± 1.16^{*+}
Grp6(500AquZin)n=5	2.54 ± 0.44$^{\#}$	1.80 ± 0.16$^{\#*}$	1.42 ± 0.35$^{\#*}$	29.77 ± 4.86$^{\#*}$
Grp7(1500AquZin)n=5	2.19 ± 0.56$^{\#*}$	1.67 ± 0.25$^{\#*}$	1.33 ± 0.38$^{\#*}$	24.02 ± 5.06$^{\#*}$
F value	11.05	22.04	27.17	25.49
P value	0.000	0.000	0.000	0.000
Remark	**S**	**S**	**S**	**S**

*Significant at p<0.05: * compared with BPH model group, # compared with NC, + compared with lower dose*

Oxidative stress has been known to cause damage to the cells, tissues, even to organs by impairing important biomolecules and cells. It is considered as an important factor accounting for the pathogenesis of BPH [19,20]. Conversely, antioxidants, usually exist as compounds or enzymes that could compete with oxidative substrates, thus protecting the cellular structure, [21]. Some studies have demonstrated reductions of antioxidant levels in prostate of BPH animals, while increase in oxidative stress has been known to occur in BPH rat model [22].

Higher dose of ethanolic extract of *Zo* rhizome showed a significantly increased SOD activity when compared with the lower dose (Table 1). The former also showed significant increases in TAS and SOD activity when compared with the higher dose of aqueous extract of *Zo* rhizome. Again, this shows that the ethanolic extract of *Zo* rhizome had more pharmacological effect. The findings of this study is in agreement with several previously conducted studies. Few studies have reported that the presence of oxidative stress is associated with numerous diseases and a common mechanism often put forth to explain the actions and health benefits of ginger is associated with its antioxidant properties [23,24]. In a study conducted by Topic et al. [25], Ginger was reported to decrease age-related oxidative stress markers and was suggested to guard against ethanol-induced hepatotoxicity by suppressing oxidative consequences in rats treated with ethanol [26]. Ginger root was reported to contains a very high level (3.85 mmol/100 g) of total antioxidants, surpassed only by pomegranate and some types of berries [27].

Reactive nitrogen species, such as nitric oxide (NO), have been reported to influence signal transduction and cause DNA damage, which contributes to disease processes. Nitric oxide is produced by inducible nitric oxide synthase (iNOS), which is stimulated in response to various stresses. [6]-gingerol abundantly present in Ginger was reported to dose-dependently inhibit NO production and reduce iNOS in lipopolysaccharide (LPS)-stimulated mouse macrophages [28]. [6]-gingerol also effectively suppressed peroxynitritemediated oxidative damage, [28]. Ippoushi et al in [29] later proposed that [6]-gingerol and peroxynitrite form a symmetric dimer with [6]-gingerol covalently linked at the aromatic ring of peroxynitrite, attenuating peroxynitrite-induced oxidation and nitration reactions [29]. [6]-shogaol, 1-dehydro-[10]-gingerdione, and [10]-gingerdione also decreased LPS-induced NO production, and [6]-shogaol and 1-dehydro-[10]-gingerdione were reported to effectively reduce iNOS expression [30].

As previous stated, there's no known reported study of any combinatorial herbal mixture that involved Ginger and the effects on BPH. However, some studies have reported a combination of Ginger and other herbs. Mashadi and colleagues reported the free radical scavenging properties of a combination of Ginger and Arabic gum [31]. The rich phytochemistry of ginger includes components that scavenge free radicals produced in biological systems. Free radicals are known to be harmfull to cells. However, they still have some vital roles to play during metabolic processes in the body systems. Ramaa and colleagues reported that for the purpose of energy production, some free radicals

which are generated during the process of oxidation are essential [32]. Increased production of free radicals results in oxidative stress that can lead to DNA damage, [33]. In such circumstances of imbalance, extra antioxidant supplementation through dietary modules is essential for organism vitality, [34]. The anti-oxidative properties of ginger and its components have been explored in various *in vitro* and *in vivo* tests. Strengthening the body's defenses by improving the antioxidant status will undoubtedly protect human against many chronic diseases [35]. 6-Shogaol has exhibited the most potent antioxidant and anti-inflammatory properties in ginger, which can be attributed to the presence of alpha, beta-unsaturated ketone moiety [36]. Animal modeling showed that ginger significantly lowered induced lipid peroxidation and raised the levels of antioxidant enzymes, together with serum glutathione, (El-Sharaky et al*.,* 2009). Ahmed and colleagues reported that feeding ginger to rats at 1% w/w during administration of malathion (20 ppm) for 4 weeks significantly attenuated malathion-induced lipid peroxidation, (Ahmed et al*.,* 2000). They further observed that concomitant dietary feeding of ginger (1%w/w) significantly attenuated lindane-induced lipid peroxidation, reduced glutathione (GSH), and the GSH-dependent enzymes glutathione peroxidase, glutathione reductase, and glutathione S-transferase, (Ahmed et al*.,* 2008). *In vitro*, zingerone scavenged O^{2-} and OH^{-} and suppressed lipid peroxidation, so it can possibly value in treatment of Parkinson's disease, (Kabuto et al*.,* 2005).

Treatment with higher doses of ethanolic and aqueous extracts of *Zo* rhizome and the anti-BPH drug dutasteride produced significant decreases in the oxidant and antioxidant systems of rats compared with rats in the BPH group. Whilst, the higher dose of ethanolic extracts of *Zo* rhizome with dutasteride showed significantly increased TAS (p=0.021) and SOD activity (p=0.000), significant decreases were seen TOS (p=0.000) and OSI (p=0.000) when compared with the aqueous extracts of the same plant (Table 2), indicating that there were better drug-herb synergy in the ethanolic extracts with dutasteride than the aqueous extract with same drug.

The ethanolic extracts of the combinations of the drug and the plants had mean TAS and SOD values higher than those of the group treated with dutasteride alone, but statistically not significant. However, the ethanolic extracts of *Zo* combined with dutasteride did not show any statistically significant decrease when mean OSI values were compared with values for the PC_1 group. The aqueous extract also did not show any significant increase in TAS when compared with the PC_1 (Table 2).

There probably may have been synergistic interaction between the ethanolic extracts of *Zo* and the drug. Moreso, Ginger is known to improve blood circulation and bioavailability of some herbs and drugs, (Shoji et al. 1982; Kobayashi et al*.,* 1998) when concurrently administered or co-formulated. It has also been reported to have spasmolytic effect and do cause smooth muscles relaxation (Vishwakarma et al*.,* 2002). These effects may cause a reduction in gastric emptying, gastrointestinal motility and increased the blood circulation to the gastrointestinal tract thereby facilitating the increased absorption of the drug.

Simultaneous administration of 1500 mg/kg b.wt. ethanolic extracts of *Zo* rhizome with TP showed significant decreases in TOS and OSI and a corresponding significantly increased TAS and SOD activity when compared with mean values for the BPH group (Table 3). The aqueous extract did not show any significant increase

Table 2. Oxidant and Antioxidant 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

	TOS (U/ml)	TAS (U/ml)	OSI	SOD(pg/ml)
Grp1(NC (n=5)	1.66 ± 0.20	2.71 ± 0.25	0.62 ± 0.10	41.88 ± 2.98
Grp2(BPHC) (n=5)	3.25 ± 0.50	1.17 ± 0.14	2.81 ± 0.60	23.38 ± 2.09
Grp3(PC_1)(n=5)	1.60 ± 0.17	2.13 ± 0.30	0.76 ± 0.15	36.53 ± 2.77
Grp8(1500EthZinDut)n=4	1.41 ± 0.09*	2.31 ± 0.08*	0.61 ± 0.03*	37.08 ± 2.22#*
Grp9(1500AquZinDut) n=4	1.54 ± 0.08*	1.81 ± 0.46#*	0.90 ± 0.31*	32.83 ± 0.55#*
F value	38.08	21.20	41.20	42.69
P value	0.000	0.000	0.000	0.000
Remark	**S**	**S**	**S**	**S**

*Significant at p<0.05: * compared with BPH model group, # compared with NC*

(except SOD) or decrease. This suggests that the extracts that were simultaneously administered with the inducing agent prevented oxidative stress that would have occurred in BPH.

Oxidative stress markers (TOS and OSI) values for PC, 1500EthZin and 1500AquZin of the groups of rats that BPH was established for 15 days before treatment were lower than that of rats that were simultaneously treated and induced, (Figs. 1 and 2).

This probably shows that the BPH reducing capability of the extracts were better that its inhibitory effect. TAS values also showed that treatment of induction of BPH established after 15 days had higher TAS than those of simultaneous induction and treatment (Fig. 3).

Higher SOD values were however observed for all compared groups (except positive controls) when groups that were simultaneously induced were compared with 15-day induced groups (Fig. 4).

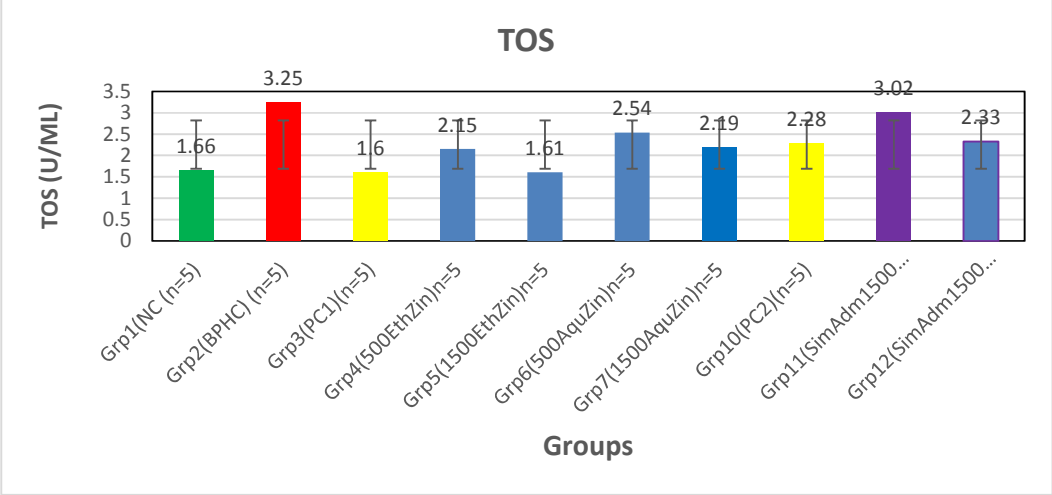

Fig. 1. Comparison of Total Oxidant Status for controls and treated Groups (Simultaneous and BPH established)

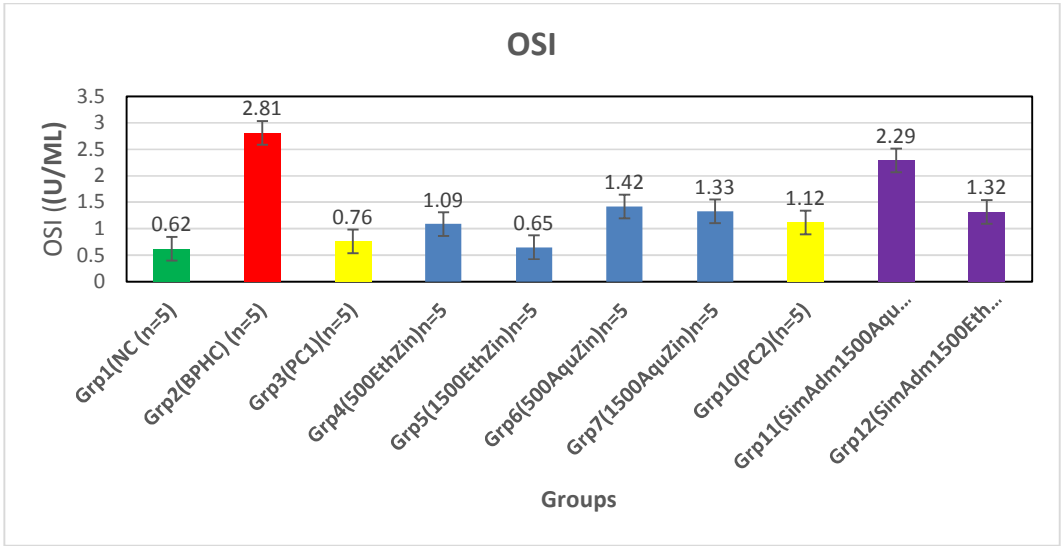

Fig. 2. Comparison of Oxidative Stress Index for controls and treated Groups (Simultaneous and BPH established)

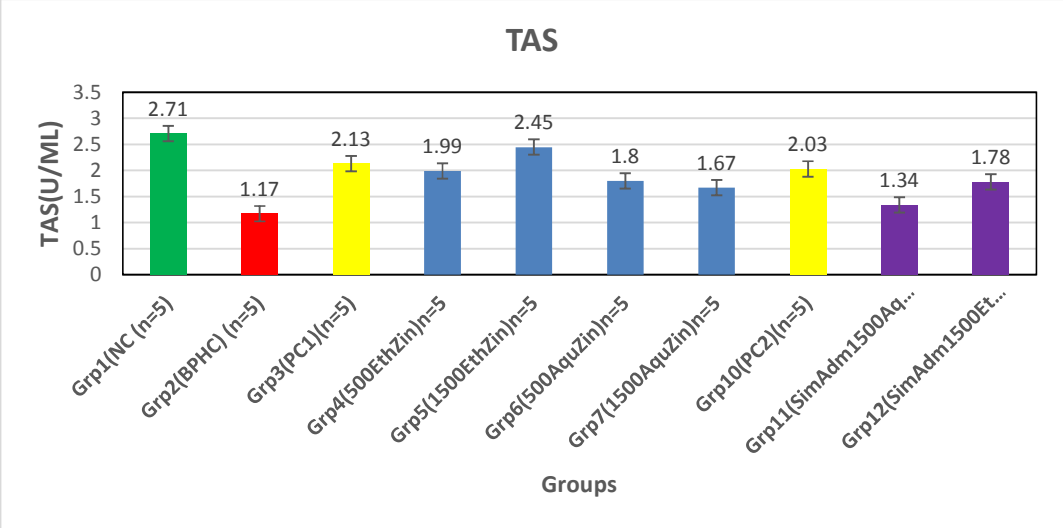

Fig. 3. Comparison of Total Antioxidant Status for controls and treated Groups (Simultaneous and BPH established)

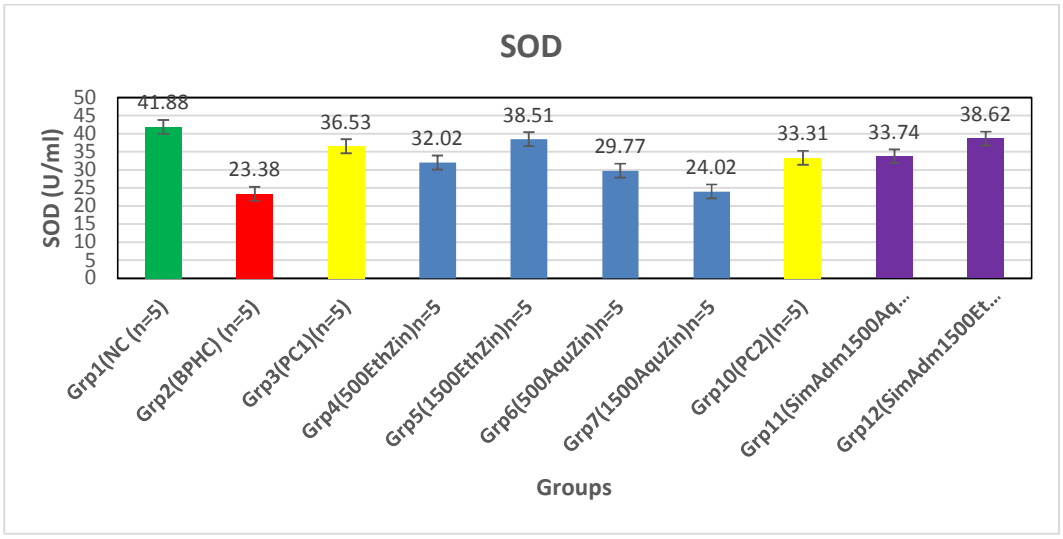

Fig. 4. Comparison of Superoxide Dismutase for controls and treated Groups (Simultaneous and BPH established)

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

	TOS (U/ml)	TAS (U/ml)	OSI	SOD(pg/ml)
Grp1(NC_2) (n=5)	1.69 ± 0.12	2.87 ± 0.47	0.60 ± 0.11	45.08 ± 5.52
Grp2($BPHC_2$) (n=5)	3.34 ± 0.56	1.17 ± 0.12	2.91 ± 0.70	23.00 ± 1.71
Grp10(PC_2)(n=5)	2.28 ± 0.15	2.03 ± 0.10	1.12 ± 0.08	33.31 ± 0.84
Grp11(SimAdm1500AquZin)n=5	3.02 ± 0.18$^{\#}$	1.34 ± 0.17$^{\#}$	2.29 ± 0.39$^{\#}$	33.74 ± 3.87$^{\#*}$
Grp12(SimAdm1500EthZin) n=4	2.33 ± 0.52+*	1.78 ± 0.09$^{\#*}$	1.32 ± 0.35*	38.62 ± 1.49*
F value	17.14	64.10	29.84	37.20
P value	0.000	0.000	0.000	0.000
Remark	**S**	**S**	**S**	**S**

*Significant at p<0.05: * compared with BPH model group, # compared with NC*

4. CONCLUSION

Based on the findings of this study, we conclude that lower and higher doses of aqueous and ethanolic extracts of *Zingiber officinale* can ameliorate BPH induced oxidative stress. The ethanolic extracts of *Zo* also showed better antioxidant capacity. Anti-BPH drug Dutasteride with *Zingiber* officinale extracts showed higher antioxidant parameters and lower mean values in oxidative stress markers compared to Dutasteride administered alone. Oxidative stress markers for simultaneously induced and treated groups were higher than those of groups that were treated after BPH had been induced.

CONSENT

It is not applicable.

ETHICAL APPROVAL

Ethical approval was sought and granted by the ethical committee, Department of Pharmacology, Faculty of Basic Medical Sciences, University of Port Harcourt, Nigeria.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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