Australian Journal of Basic and Applied Sciences
2018 December; 12(12): pages 9-18
DOI: 10.22587/ajbas.2018.12.12.2

Original paper AENSI Publications
Journal home page: www.ajbasweb.com

Effect of ethanol extract of *Zapoteca portoricensis* stem on testosterone-induced benign prostate hyperplasia (BPH) in adult male albino rats

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Received date: 12 August 2018, **Accepted date:** 20 December2018, **Online date:** 31 December 2018

Abstract

The effect of ethanol extract of *Zapoteca portoricensis* stem on testosterone-induced benign prostate hyperplasia (BPH) in adult male albino rats was aimed at in this study. The percentage yield of the extract was determined to be 1.73% and the extract showed the presence of alkaloids, terpenoids, saponins, phenols, flavonoids, tannins, glycosides and carotenoids as phyto constituents. A dose of 5000mg/kg body weight was found to be safe in the LD_{50} study of the extract. A total of 25 adult male albino rats (weighing 231-391g) were selected for this study and randomly divided into five groups (1, 2, 3, 4 and 5) of five animals per group. Group 1 served as the normal control, group 2 as the positive control, group 3 as the standard control and groups 4 and 5 as the test groups. Animals in group 2, 3, 4 and 5 were induced with BPH via daily subcutaneous injection of testosterone propionate (3mg/kgbwt) for 14 days and group 1 received subcutaneous injection of olive oil in place of the hormone for the same duration. After the induction, groups 1 and 2 received oral administration of 2 % v/v tween 80 solution and groups 3, 4 and 5 received oral administration of finasteride (10mg/kg), 100mg/kg extract and 200mg/kg extract respectively for 21 days. The oral administration of the extract showed a non-significant ($P > 0.05$) decrease in the mean body weight of the animals compared to that of the normal control group (group 1) and mean prostate weight decreased significantly ($P < 0.05$) when compared to that of the positive control group (group 2). There were significant ($P < 0.05$) decrease in mean relative prostate weight, lower increase in relative prostate weight and lower percentage increase in relative prostate weight of extract treated group animals compared to those of the positive control animals. However, the extract treated groups (4 and 5) showed higher percentage recovery of relative prostate weight (65.56 and 70 respectively) than that of group 2 (0). Testosterone levels of groups 4 and 5 animals significantly ($P < 0.05$) increased compared to those of group 2 and dihydrotestosterone level in the extract treated groups significantly ($P < 0.05$) decreased compared to that of group 2. The prolactin and prostate specific antigen concentrations of the test groups decreased significantly ($P < 0.05$) compared to those of the positive control group and likewise serum zinc concentration. The result of this study suggests that *Zapoteca portoricensis* stem extract has pharmacological effect in the treatment of induced BPH in animals.

Key words: *Zapoteca portoricensis*, benign prostate hyperplasia, testosterone, dihydrotestosterone

INTRODUCTION

Medicinal plants have been identified and used throughout human history. They have the ability to synthesize a wide variety of bioactive chemical compounds that perform important biological function and have been used to make drugs. At least 12,000 of the bioactive compounds have been isolated so far, a number estimated to be less than 10% of the total number of phytochemicals known. These compounds including alkaloid, tannin, flavonoids, glycosides, saponins and terpenoids are the major basis of pharmacological activities of medicinal plants (Tapsell *et al*., 2006).

Zapoteca portoricensis, commonly called white stickpea, belonging to the family of *Fabaceae* is a perennial shrub with slender unarmed branches and with small oral green leaves. The plant is a native of the West Africa, the West Indies and the Atlantic coast of America and is used in folk medicine in various countries for the treatment of toothaches, tonsilities, external wounds diarheea and convulsion. Different parts of the plants are used in Eastern Nigeria in the treatment of constipation, convulsion, madness, prolonged labour and skin infections (Agbafor, 2014).

Benign prostatic hyperplasia (BPH) is a prostatic disorder that is macroscopically characterized by an enlargement of the prostate gland and histologically caused by the progressive hyperplasia of stromal and glandular epithelial prostatic cells. BPH arises in the periurethral and transition zones (TZs) of the prostate gland and represents an inescapable phenomenon for ageing male population. Although BPH is uncommon before the age of 40, roughly 50% of men develop BPH-related symptoms at 50year of age. The incidence of BPH increases by 10% per decade and reaches 80% at approximately 80 year of age. An estimated 75%

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of men greater than 50 year of age have symptoms arising from BPH and 20-30% of men reaching 80year of age require surgical intervention for the management of BPH. Despite the high impact of BPH on public health, however, the pathogenesis of BPH is still largely unresolved. Although ageing and genetic predisposition represent the central mechanism implicated, recent novel finding also highlighted the key-role of hormonal alterations, metabolic syndromes and inflammation (Parsons and Kashefi, 2008; Alberto *et al*., 2009)

Symptoms of BPH are classified as storage or voiding. Storage symptoms include urinary frequency, urgency (compelling need to void that cannot be deferred), urgency incontinence and voiding at night (nocturia) which may contribute to insomnia. Voiding symptoms include urinary hesitancy (difficulty initiating the stream), straining to void, week or intermittent stream (start and stops), and incomplete bladder emptying. These storage and voiding symptoms are evaluated using the international prostate symptoms score (IPSS) questionnaire, designed to assess the severity of BPH (Black *et al*., 2006).

Over the years, physician advocated the use of lifestyle, voiding position, medications and surgery, for management and control of prostatic disorders especially BPH. The failure of lifestyle and voiding position in management of BPH has made the use of medication an alternative choice for initial therapy as seen in the Europe and in the USA. However there are common side effects associated with medications which include postural hypotension, headache, nasal congestion, weakness, decreased libido, and erectile dysfunction. Surgery, another alternative method of BPH treatment, has been discovered to be associated with dryness of ejaculation leading to sterility (Santillo and Lowe, 2006).

Herbal remedy has become a commonly sought treatment for BPH due to numerous side effects associated with other methods. For instance, saw palmetto extract from *Serenoarepens* and others are approved in European countries and they are available in USA. Other documented herbal medicines for BPH include beta-sitosterol from *Hypeo sisrooperi* (African star grass),*Urtica dioica* (stinging nettle) root and*Telfairi aeccidentalis* Hook F. (fluted pumpkin) seeds (Barry *et al*., 2011; Ejike and Ezeanyika, 2011).

However, there is absence of demonstrated scientific information on the treatment of BPH with stem extracts of *Zapoteca portoricencis* as used by the traditional medicine practitioners in Eastern Nigeria. Hence, the success of this research will help in advancing the search for BPH treatment with bearable or no side effect.

MATERIALS AND METHODS

Plant Materials

The stems of *Zapoteca portoricensis* were collected from Orba, Nsukka, Enugu State of Nigeria. The plant materials were authenticated by Mr. Alfred Ozioko, a taxonomist at the Centre for Ethenomedicine and Drugs Development, a subsidiary of Bioresources Development and Conservation Program (BCDP), Nsukka, Enugu State.

Chemicals and Assay kits

All the chemicals used in this study were of analytical grade and were used as such without further purification. Fresh distilled water was used throughout the experimental period. Assay kits used in all the analysis in this study were products of Bioscience, Cusabio, Magiwel and Randox laboratories.

Laboratory animals

Adult male albino rats (230-400 g) obtained from the animal house of Biochemistry Department, University of Nigeria, was kept under standard environmental condition of 12/12 hours light/dark cycle. They were housed in polypropylene cages (5 animals per cage), and were maintained on mouse chow (Livestock Feeds Nigeria Ltd), provided with water *ad libitum*. They were allowed to acclimatize for 7 days to the laboratory conditions before the experiment. The use and care of the animals, and the experimental protocol were in strict compliance with the Institute of Laboratory Animals Research (ILAR) guidelines on the use and care of animals in experimental studies and approved by the local ethics committee of our institution.

Preparation of ethanolic stem extract of *Zapoteca portoricensis*

Fresh stems of *Zapoteca portoricensis* were collected, cleaned and air dried before being subjected to size reduction to a coarse powder with electric grinder. The stem powder (5kg) was dissolved in 10L of 99.7% ethanol for 72hours to achieve maximum extraction. The mixture was agitated using a magnetic stirrer and filtered with Whatman No.1 filter paper. The filtrate was evaporated to dryness until constant weight of the crude extract was obtained. The concentrated crude extract (86.5g) with a percentage yield of 1.73 was stored in an air tight bottle and kept in a refrigerator at 4 °C till used.

Qualitative phytochemical analysis

Preliminary phytochemical screening was performed to identify the presence of bioactive compounds in crude ethanolic stem extract of the *Zapoteca portoricensis* used in this study. The phytochemicals (such as flavonoids, glycosides, tannins, alkaloids, saponins, steroids and terpinoids) were tested for using the method of Sofowora (2008); modified by Tiwari *et al*. (2011).

Acute toxicity and lethality (LD_{50}) test

The oral acute toxicity of the ethanol extract was determined according to the method described by Lorke (1983).

Determination of Doses

The ethanol extract of *Zapoteca portoricensis was* subjected to acute toxicity studies to determine the dose for the *in vivo* studies according to the Organization for Economic Cooperation and Development guidelines (Deora *et al*., 2010). In all cases, a 3000-mg/kg bwt oral dose of the test extract was found to be tolerable, as no mortality was observed during the study. On the basis of this study, the doses of 100 and 200 mg/kg bwt for the extract were selected.

Preparation of 2 % v/v Tween 80 solution

The 2 % v/v Tween 80 [polyoxyethylene (20) sorbitan monooleate] solution used in dissolving the extract and the drug (finasteride) was prepared by adding 2ml of the Tween 80 in a 100ml measuring cylinder containing 50 ml of distilled water. This was shaken thoroughly and distilled water added to make up the 100 ml mark.

Preparation of testosterone propionate, finasteride and extract solutions

Testosterone propionate solution used in this study was prepared by dissolving 5g of testosterone propionate in 1.25L of olive oil at a stock concentration of 4 mg/ml. Finasteride solution was prepared by dissolving 1g of finasteride in 0.1L of 2 % v/v Tween 80 solution at a stock concentration of 10 mg/ml. The ethanol stem extract was prepared by dissolving 10g of the crude extract in 0.1L of 2 % v/v Tween 80 solution at a stock concentration of 100 mg/ml.

Experimental design for benign prostatic hyperplasia induction and treatment

Animal grouping and BPH induction

A total of 25 adult male albino rats (weighing 230-390g) were selected for this study. They were randomly divided into five groups (1, 2, 3, 4 and 5) of 5 animals each and each group housed in its own cage. Animals in Groups 2, 3, 4 and 5 were induced with BPH by exogenous administration of 10 mg/kg body weight testosterone propionate dissolved in olive oil which served as the vehicle. The administrations which were once a day by subcutaneous injection as outlined by Nandecha *et al*. (2010), Ejike and Ezeanyika (2011) and Surendra *et al*. (2011) lasted for 14 days before commencement of treatment. The normal control group (group 1) received subcutaneous injection of olive oil in place of the hormone for the same duration.

Animal grouping and BPH treatment

At the end of 14 days induction, the animals in Groups 1 (normal controls) and 2 (positive controls) were given oral doses of 2% v/v Tween 80 solution. The animals in Group 3 were given oral dose of 10 mg/kg body weight finasteride, while those in groups 4 and 5 received oral doses of 100 mg/kg and 200 mg/kg body

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weight extracts respectively. The oral administration was done once per day by the use of gavages for 21 days. The animals were weighed prior to the commencement of the experiment and subsequently every week till the end of the experiment as outlined by Nandecha *et al*.,(2010).

Collection of sera and tissue samples for analysis

After 21 days of treatment, the rats were fasted for 12 hours, anesthetized by a brief exposure to trichloromethane vapour, and bled exhaustively by ocular puncture. Blood samples were collected, allowed to clot and centrifuged at 2000 × *g* for 10 min. The sera were carefully separated and used for biochemical analyses. Each rat was promptly dissected and the prostate carefully excised, freed of external fascias, washed in cold normal saline, blotted with filter paper and weighed on a sensitive balance to determine the mean prostate weight and the mean prostate-body weight ratio.

Body and prostate weights

The mean body weight and the mean prostate-body weight ratio (relative prostatic weight) were calculated for each group of the animals as outlined by Ejike and Ezeanyika (2011) with modification. Meanwhile, increase in relative prostatic weight, percentage increase in relative prostatic weight and percentage recovery of relative prostatic weight after treatment were calculated using the method described by Nandecha *et al*. (2010). Relative prostatic weight was calculated as the ratio of the prostate weight to the body weight.

Increase in prostate-body weight ratio (relative prostate weight)

The increase in mean prostate- body weight (P/BW) ratios of the testosterone propionate treated groups (group 2, group 3, group 4, and group 5) were calculated on the basis of mean P/BW ratio of the normal control group (group 1) (Table 2). This was done to compare the increase in P/BW ratio of the induced group after treatment with that of the non-induced group (normal control group). The increase in P/BW ratio of the normal control group was considered to be zero and all other test groups were compared with this reading. Hence, the increase in P/BW ratio of the induced group was obtained from the difference between the mean P/BW ratio of the induced group and that of the non-induced group (normal control group) and can be deduced from the formula shown below:
Increase in P/BW ratio of the induced group after treatment = $I_1 - I_0$,
where I_1 was the mean P/BW ratio of any of the induced groups and I_0 was the mean P/BW ratio of the non- induced group.

Percentage increase in relative prostate weight

The percentage increases in P/BW ratio of the testosterone propionate treated groups (group 2, group 3, group 4, and group 5) were calculated on the basis of the increase in P/BW ratio of the positive control group (group 2). This was done to compare the percentage increase in P/BW ratio of the induced and treated groups (group 3, group 4 and group 5) with that of the induced and non-treated group (positive control group or group 2) while the percentage increase in P/BW ratio of the normal control group (group 1) was negligible. In this case, the percentage increases in P/BW ratio of the induced and non-treated group (group 2) were considered to be 100% while that of the treated group was obtained from the percentage ratio of the increase in P/BW ratio of the treated group and that of the non-treated group. The formula used for calculation of the percentage increase in P/BW ratio was as follows:
Percentage increase in P/BW ratio of the treated group = $T_1 / T_0 \times 100\%$,
where T_1 was the increase in P/BW ratio of any of the treated groups and T_0 was the increase in P/BW ratio of the non-treated group.

Percentage recovery of relative prostate weight

The last calculation on the basis of P/BW ratio was the percentage recovery in the P/BW ratio. The percentage recovery in P/BW ratio of normal control group (group 1) was also negligible as seen in the percentage increase in B/BW ratio. However, the percentage recovery in P/BW ratio of the positive control group (group 2) was considered to be zero and all other test groups were compared with this reading. Calculation of the percentage recoveries in the P/BW ratio by the induced and treated groups (group 3, group 4 and group 5) were obtained by the difference between the percentage increase in P/BW ratio of the positive control group (group 2) and that of the treated groups (group 3, group 4 and group5) using the formula stated below:
Percentage recovery in the P/BW ratio by the test sample = $R_0 - R_1$,
where R_0 was the percentage increase in P/BW ratio of the induced and non-treated group (group 2) and R_1 was the percentage increase in P/BW ratio of the induced and treated groups (group 3, group 4 and group 5).

Biochemical assays

Measurement of serum testosterone concentration

Testosterone level, based on the method of Turkes *et al*. (1979), was measured using testosterone enzyme-linked immunosorbent assay (ELISA) kit and ELISA reader (BIOLINE BPR08). Serum was tested for testosterone content using the procedure supplied with the kit (UBI MAGIWEL Total Testosterone kit purchases from United Biotech Inc., Mountain View, CA, USA). The UBI MAGIWEL testosterone quantitative test is based on the principle of competitive solid-phase enzyme immunoassay. The test sample competes with enzyme-labelled testosterone for a fixed and limited number of antibody sites on the microtitre wells. In the assay procedure, the testosterone standard or test serum is incubated with the testosterone antibody and the testosterone– horseradish peroxidase conjugate in the anti-rabbit IgG-coated well. In this solid-phase system, the antibody bound testosterone will remain on the well while unbound testosterone will be removed by washing. A colour is developed when 3,3', 5,5'-tetramethylbenzidine (TMB) substrate is mixed with the antibody-bound testosterone–horseradish peroxidase enzyme conjugate. After a short incubation, the enzyme reaction is stopped, and the intensity of the colour is measured with micro plate reader at 450 nm.

Mesurement of dihydrotestosterone (DHT) level in the serum

Serum level of dihydrotestosterone (DHT) was measured by the ELISA kits. All protocols were performed following the manufacturer's instruction. The EZ Read 400 Microplate Reader, a product of Biochrom Co. (Cambridge, UK), was used and the values were expressed per ml in the serum.

Estimation of serum prostate specific antigen (PSA)

PSA ELISA kit was utilized for this purpose. The PSA ELISA kit (Cusabio Biotech Co. Ltd.) intended for the quantitative determination of total PSA was determined according to the manual's protocol. The PSA ELISA is a solid-phase, noncompetitive immunoassay based upon the direct sandwich technique. Calibrators, controls and samples were incubated together with biotinylated anti-PSA monoclonal antibody and horseradish peroxidase (HRP)-labelled anti-PSA monoclonal antibody in streptavidin-coated microtitre stripes. After washing, buffered substrate (TMB-HRP substrate) that contains hydrogen peroxide, chromogen reagent (3,3', 5,5' tetra methyl benzidine) was added to each well and the enzyme reaction was allowed to proceed. The colour intensity was determined in the microtitre plate spectrophotometer at 620 nm. Calibration curves were constructed for each assay by plotting absorbance versus the concentration of each calibrator. The concentrations of PSA in samples were then read from the calibration curve. All the protocols were carried out in accordance with the manufacturer's instructions.

Determination of serum prolactin concentration

A solid phase enzyme immunoassay (EIA) quantitative method was employed for determination of prolactin concentration in the serum. The prolactin protocol utilizes 2 antibodies directed against distinct antigenic determinants of the prolactin molecule.

Measurement of serum zinc concentration

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One part serum was mixed with two parts of 15% trichloro acetic acid (TCA) and centrifuged at 1000 x g for 20 min. The supernatants were aspirated directly into a Perkin-Elmer model 2380 (Norwalk, CT) atomic absorption spectrophotometer. Standards containing 0-30 µM zinc were prepared in 10% TCA and used for serum zinc determination. All the protocols were carried out in accordance with the manufacturer's instructions.

Statistical analysis

Data were reported as means ± SEM, where appropriate. One-way analysis of variance (ANOVA) was used to analyse the experimental data and Duncan multiple test range was used to compare the group means obtained after each treatment with control measurements. Differences were considered significant when $P \leq 0.05$.

RESULTS

Preliminary phytochemical screening of*Zapoteca portoricensis* stem extract from aqueous and different organic solvents carried out reported relative presence of various phytoconstituents such as alkaloids, terpenoids, saponins, phenols, flavonoids, tannins, glycosides and carotenoids (Table 1). The LD_{50} result of the ethanol extract of *Zapoteca portoricensis* stem on adult mice showed that the extract was safe at a dose of 5000 mg/kg body weight since there was no record of death during the study.

The data in Fig. 1 show that the mean body weights of the animals in testosterone-treated groups (groups 2, 3, 4 and 5) are non-significantly ($p > 0.05$) lower compared to that of the normal control group (group 1) after 21 days of treatment. The increase in the mean body weight of the group treated with standard drug (group 3) when compared with that of the positive control group (group 2) was statistically non-significant ($p > 0.05$) (Fig. 1). The mean body weight of the ethanol extract treated groups (groups 4 and 5) was also non-significantly ($p > 0.05$) lower when compared with that of the positive control group (group 2) (Fig. 1).

There was a significantly ($p < 0.05$) lower mean prostate weight of the group treated with testosterone propionate alone (positive control group) when compared with those of the normal control group (group 1) and the treated groups (group 3, group 4 and group 5) (Fig. 2). However, Fig. 2 shows a non-significantly ($p > 0.05$) lower mean prostate weight of animals in group 3, group 4 and group 5 when compared with that of the normal control (group 1). The mean prostate weights of the extract treated groups (group 4 and group 5) was non-significantly ($p > 0.05$) lower compared to that of the standard control group (group 3), where as that of group 5 was non-significantly ($p > 0.05$) lower compared to group 4 (Fig. 2). The mean prostate-body weight ratio (relative prostate weight) of animals in the positive control group treated with testosterone propionate only (group 2) is significantly ($p < 0.05$) lower compared to the normal control group receiving only olive oil (group 1). The mean prostate-body weight ratios of the finasteride treated group (group 3) and the extract treated groups (group 4 and group 5) were significantly ($p < 0.05$) higher when compared with that of the normal control (group 1) and significantly ($p < 0.05$) lower when compared with that of the positive control group (group 2) (Fig. 3). Table 2 summarizes the mean body weight, mean prostate weight and mean prostate-body weight (P/BW) ratio as described in Figs. 1, 2 and 3 above. The table shows that the increases in P/BW ratios of group 2 (positive control), group 3 (standard control), group 4 (100mg/kg extract) and group 5 (200mg/kg extract) were calculated to be 0.90, 0.23, 0.31 and 0.27 respectively. This shows that the increases in prostate-body weight ratio of the induced groups (group 2, group 3, group 4 and group 5) are greater than that of the normal control group (group 1). It also shows that the increases in prostate-body weight ratio of the induced and treated groups (group 3, group 4 and group 5) are less than that of the positive control group (group 2). There is increase in prostate-body weight ratio of the extract treated groups (group 4 and group 5) compared with that of the finasteride treated group (group 3) (Table 2).

The percentage increase in P/BW ratios of group 2 (positive control), group 3 (standard control), group 4 (100mg/kg extract) and group 5 (200mg/kg extract) were calculated to be 100%, 25.56%, 34.44% and 30% respectively. This shows that there was reduction in percentage increase in P/BW ratio of the treated groups (group 3, group 4 and group 5) compared with that of the non-treated group (group 2). It also shows that there were high percentage increases in P/BW ratio of the groups treated with the extract (group 4 and group 5) compared with that of the finasteride treated group (group 3) and the percentage increase in P/BW ratio thus calculated for group 4 is greater than that calculated for group 5 (Table 2).

The percentage recoveries in P/BW ratios thus calculated for the treated groups were as follows: the group treated with finasteride (group 3) was 74.44% while the group treated with 100mg/kg body weight of the extract (group 4) was 65.56% and the group treated with 200mg/kg body weight of the extract (group 5) was 70% (Table 2). The result shows that group 3 has the greatest percentage recovery in P/BW ratio among the treated groups followed by group 5 while group 4 has the least percentage recovery in relative prostate weight. It was observed that the mean serum testosterone concentrations of animals in the induced groups (group 2, group 3, group 4 and group 5) were significantly ($p<0.05$) higher compared to the mean serum testosterone concentration of animals in group 1. There was also significantly ($p<0.05$) higher mean serum testosterone concentration of animals in the treated groups (groups 3, 4 and 5) compared to the mean serum testosterone concentration of animals in the non-treated group (group 2). The mean serum testosterone concentrations of the extract treated groups was non-significantly ($p>0.05$) lower compared to the mean serum concentration of the finasteride treated group (group 3). There was non-significantly ($p>0.05$) lower concentration of mean serum testosterone in the extract treated groups compared to that of group 3 animals and mean serum testosterone concentration of group 5 animals was non-significantly ($p>0.05$) higher compared to that of group 4 animals (Fig. 4).

The mean serum dihydrotestosterone (DHT) concentrations of animals in the induced groups (groups 2, 3, 4 and 5) were observed to be significantly ($p<0.05$) higher compared to the concentration of mean serum dihydrotestosterone in the normal control group animals (group 1) (Fig. 5). However, there were significantly ($p<0.05$) lower concentrations of mean serum DHT in the induced and treated group animals (groups 3, 4 and 5) compared to the mean serum DHT concentration of animals in the induced and non-treated group (group 2). Non-significantly ($p>0.05$) higher concentrations of mean serum dihydrotestosterone were observed in the extract treated groups (groups 4 and 5) compared to the mean serum dihydrotestosterone concentration of animals in the finasteride treated group (group 3) (Fig. 5). The mean serum prolactin concentrations of animals in the induced groups (groups 2, 3, 4 and 5) were significantly ($p<0.05$) higher compared to the mean serum prolactin concentrations of animals in the non-induced group (group 1) (Fig. 6). However, there was significantly ($p<0.05$) lower mean serum prolactin concentrations of animals in the induced and treated groups (groups 3, 4 and 5) compared to the mean serum prolactin concentration of animals in the induced and non-treated group (group 2). It was also observed that the mean serum prolactin concentrations of animals in the extract treated groups (groups 4 and 5) were non-significantly ($p>0.05$) higher compared to the mean serum prolactin concentration of animals in the finasteride treated group (group 3). Animals in group 5 showed non-significantly ($p<0.05$) lower concentration of mean serum prolactin compared to that of group 4 animals (Fig.6).

The mean serum PSA levels of animals in the induced groups (groups 2, 3, 4 and 5) were significantly ($p<0.05$) higher compared to the mean serum PSA level of the non-induced and non-treated (normal control) animals (group 1). However, there was significantly ($p<0.05$) lower mean serum concentrations of PSA in the induced and treated groups (groups 3, 4 and 5) compared to the concentration of mean serum PSA observed in the induced and non-treated (positive control) animals (group 2). The PSA levels of the extract treated groups (groups 4 and 5) were non-significantly ($p>0.05$) higher compared to that observed in finasteride treated (standard control) animals (group 3) and there was non-significantly ($p>0.05$) lower mean serum PSA level of animals in group 5 compared to that observed in group 4 animals (Fig. 7).

There were non-significantly ($p>0.05$) higher concentrations of mean serum zinc observed in the induced and treated animals (group 3, group 4 and group 5) compared to the mean serum zinc concentration of animals in the non-induced and non-treated (normal control) group (group 1). However, the mean serum zinc concentrations of the induced and treated groups (groups 4 and 5) were significantly ($p<0.05$) lower compared to the mean serum zinc concentration of animals in the induced and non-treated (positive control) group (group 2). There was no significant difference observed in the mean serum zinc concentration of animals in finasteride treated group (group 3) and 100 mg/kg body weight extract treated group (group 4) but the mean serum zinc concentration of animals in 200 mg/kg body weight extract treated group (group 5) was non-significantly ($p<0.05$) lower compared to that of group 4 animals (Fig. 8).

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Table 1: Preliminary analysis of phytochemicals in aqueous and different organic stem extract of *Zapotecaportoricensis*

S. No.	Phytochemicals	Inference in different extracts				
		A	B	E	EA	M
1	Alkaloids	+	++	++	+++	++
2	Anthracminone	-	-	-	-	-
3	Carotenoids	+	+	++	+	+
4	Flavonoids	-	-	++	+	-
5	Glycosides	++	+	++	+	++
6	Phenols	+	+	+	-	+
7	Reducing sugar	-	-	-	-	-
8	amnia	++	+	+++	+	++
9	Steroids	-	+	-	-	-
10	Tannins	-	-	+	-	+
11	1.emillp.la	-	++	++	-	++

Key: A = Aqueous extract, B = Butanol extract, E = Ethanol extract, EA = Ethyl acetate extract, M = Methanol extract, − = Not detected, + = Present in low concentration, + + =Present in moderate concentration and + + + = Present in high concentration.

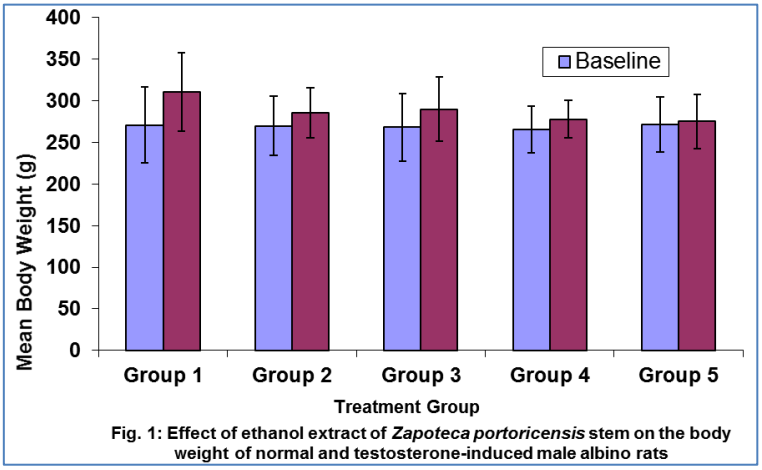

Fig. 1: Effect of ethanol extract of *Zapoteca portoricensis* stem on the body weight of normal and testosterone-induced male albino rats

Group 1 = Normal control
Group 2 = Positive control (Testosterone-induced BPH non-treated)
Group 3 = Standard control (BPH + finasteride-treated)
Group 4 = BPH + extract treated (100mg/kg body weight)
Group 5 = BPH + extract treated (200mg/kg body weight)

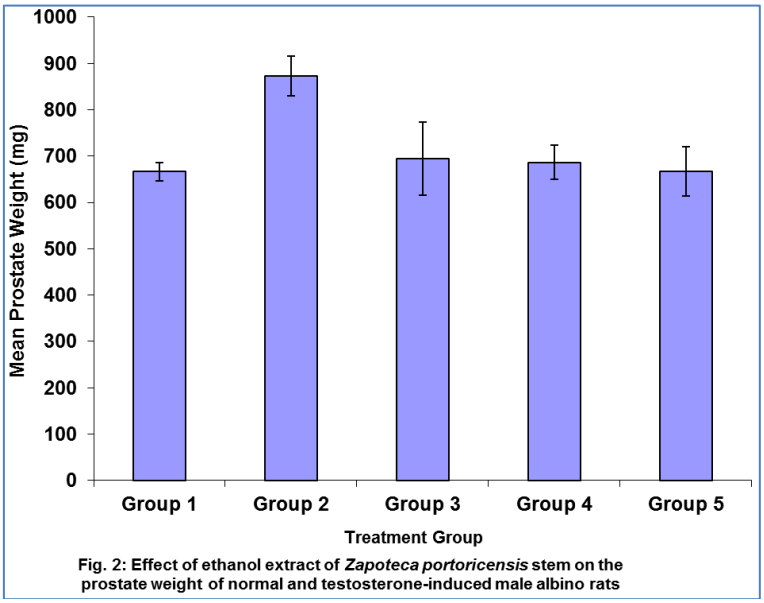

Fig. 2: Effect of ethanol extract of *Zapoteca portoricensis* stem on the prostate weight of normal and testosterone-induced male albino rats

Group 1 = Normal control
Group 2 = Positive control (Testosterone-induced BPH non-treated)
Group 3 = Standard control (BPH + finasteride-treated)
Group 4 = BPH + extract treated (100mg/kg body weight)
Group 5 = BPH + extract treated (200mg/kg body weight)

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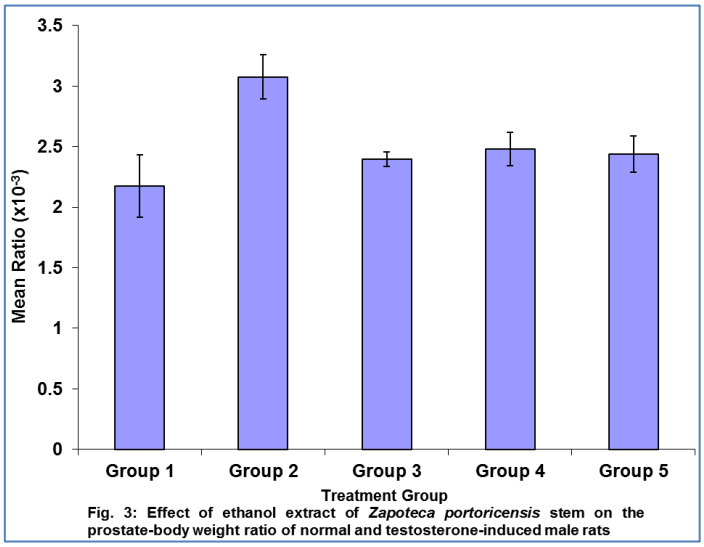

Fig. 3: Effect of ethanol extract of *Zapoteca portoricensis* stem on the prostate-body weight ratio of normal and testosterone-induced male rats

Group 1 = Normal control
Group 2 = Positive control (Testosterone-induced BPH non-treated)
Group 3 = Standard control (BPH + finasteride-treated)
Group 4 = BPH + extract treated (100mg/kg body weight)
Group 5 = BPH + extract treated (200mg/kg body weight)

Table 2: Effect of ethanol extract of *Zapotecaportoricensis* stem on the body weight, prostate weight, relative prostate weight, increase in relative prostate weight, percentage increase in relative prostate weight and percentage recovery in relative prostate weight of male albino rats

Mean BW (g)			Mean prostate weight (mg)	Mean P/BWratio (10^{-3})	Increase in P/BW ratio(I_1-I_0)	% increase in P/BW ratio$T_1/T_0\times100$) (%)	% recovery in B/PW ratio(R_0-R_1) (%)
Group	Baseline	Day 35					
1	27028 (45.34)	310.89 (4132)	666.32 (20.11)	2.17 (0.26)	0		
2	269.93 (3537)	285.57 (2930)	872.94 (42.92)	3.07 (0.18)	0.90	100	0
3	268.11 (40.46)	290.07 (3835)	694.61(79/7)	/40 (0.06)	0.23	25.56	74.44
4	26533 (28.54)	277.92 (22.50)	686.55 (3622)	/48 (0.14)	0.31	34.44	65.56
5	271.61 (33.97)	275.17 (3235)	667.06 (53.00)	2A4 (0.15)	0.27	30.00	70.00

Key: Group 1 = Normal control; Group 2 = Positive control (Testosterone-induced BPH non-treated); Group 3 = Standard control (BPH + finasteride-treated); Group 4 = BPH + extract treated (100mg/kg body weight); Group 5 = BPH + extract treated (200mg/kg body weight); P/BW = prostate weight / body weight; I_1 = mean P/BW ratio of the induced group; I_0 = mean P/BW ratio of the normal control group; T_1 = increase in P/BW ratio of the induced and treated group; T_0 = increase in P/BW ratio of the induced and non-treated group; R_0 = percentage increase in P/BW ratio of the induced and non- treated group; R_1 = percentage increase in P/BW ratio of the induced and treated group. Data are mean SD, unless otherwise indicated.

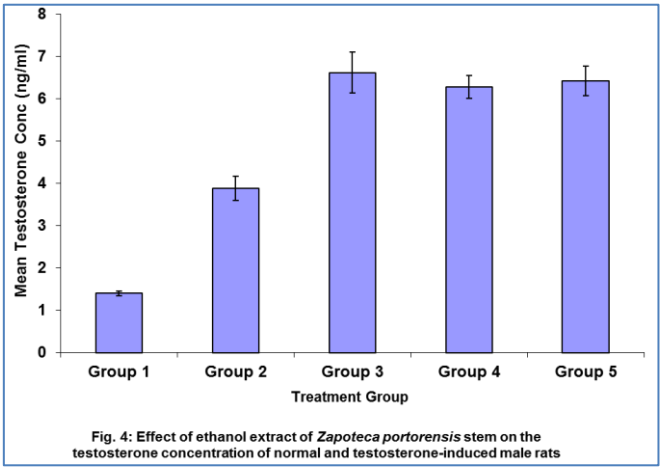

Fig. 4: Effect of ethanol extract of *Zapoteca portorensis* stem on the testosterone concentration of normal and testosterone-induced male rats

Group 1 = Normal control
Group 2 = Positive control (Testosterone-induced BPH non-treated)
Group 3 = Standard control (BPH + finasteride-treated)
Group 4 = BPH + extract treated (100mg/kg body weight)
Group 5 = BPH + extract treated (200mg/kg body weight)

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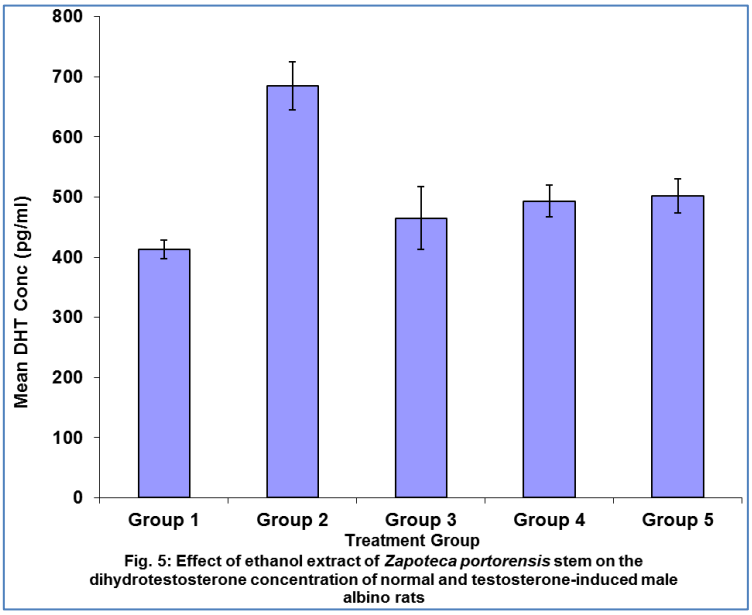

Fig. 5: Effect of ethanol extract of *Zapoteca portorensis* stem on the dihydrotestosterone concentration of normal and testosterone-induced male albino rats

Group 1 = Normal control
Group 2 = Positive control (Testosterone-induced BPH non-treated)
Group 3 = Standard control (BPH + finasteride-treated)
Group 4 = BPH + extract treated (100mg/kg body weight)
Group 5 = BPH + extract treated (200mg/kg body weight)

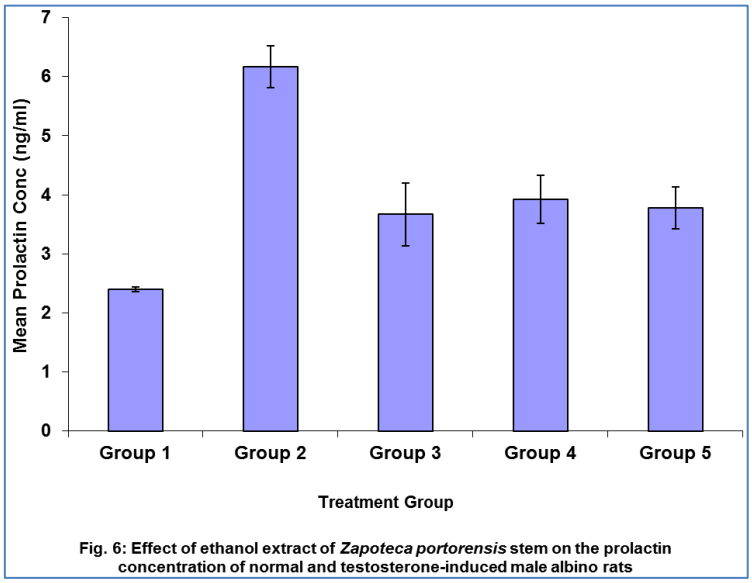

Fig. 6: Effect of ethanol extract of *Zapoteca portorensis* stem on the prolactin concentration of normal and testosterone-induced male albino rats

Group 1 = Normal control
Group 2 = Positive control (Testosterone-induced BPH non-treated)
Group 3 = Standard control (BPH + finasteride-treated)
Group 4 = BPH + extract treated (100mg/kg body weight)
Group 5 = BPH + extract treated (200mg/kg body weight)

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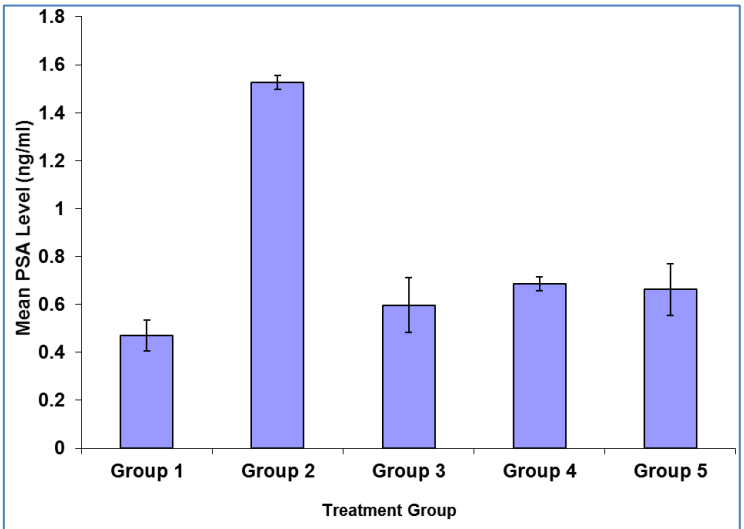

Fig. 7: Effect of ethanol extract of *Zapoteca portorensis* stem on the prostate specific antigen level of normal and testosterone-induced male albino rats

Group 1 = Normal control (Vehicle only)
Group 2 = Positive control (Testosterone-induced BPH only)
Group 3 = Standard control (BPH + 10 mg/kg finasteride treated)
Group 4 = BPH + extract treated (100mg/kg body weight)
Group 5 = BPH + extract treated (200mg/kg body weight)

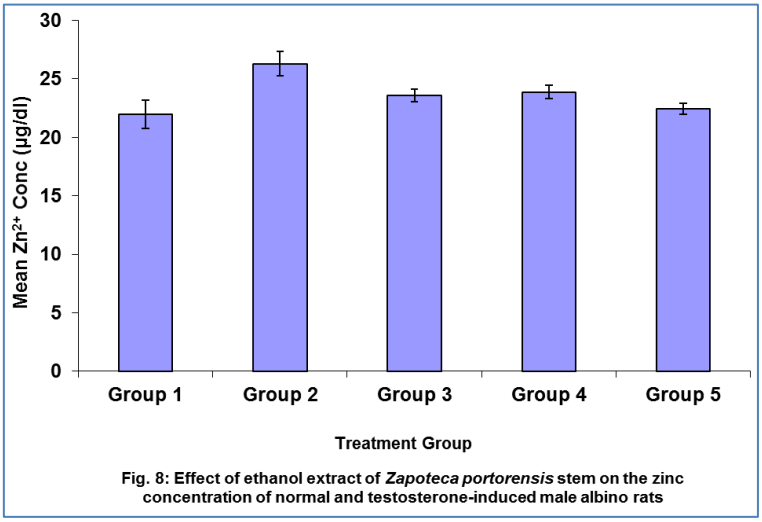

Fig. 8: Effect of ethanol extract of *Zapoteca portorensis* stem on the zinc concentration of normal and testosterone-induced male albino rats

Group 1 = Normal control
Group 2 = Positive control (Testosterone-induced BPH non-treated)
Group 3 = Standard control (BPH + finasteride-treated)
Group 4 = BPH + extract treated (100mg/kg body weight)
Group 5 = BPH + extract treated (200mg/kg body weight)

DISCUSSION

Benign prostatic hyperplasia (BPH) and prostate cancer are the most common proliferative disorders affecting older men. BPH is known to be nonexistent in eunuchs or hypogonadal individuals and in men who were castrated prior to puberty (Nandecha *et al.* 2010; Ejike and Ezeanyika, 2011). Also, in the rare disorders of androgen resistance like testicular feminization and 5 α-reductase deficiency, only a remnant prostate is present. Androgens and oestrogens significantly influence the development of benign prostatic hyperplasia. However, the prostate needs androgens for normal physiological functioning and concentration of these hormones in excess of the physiological threshold may result in aberrant growth of the prostate (Lee *et al*., 2012). An alternative pharmacological approach in patients with BPH is to inhibit the androgens responsible for prostatic hyperplasia.

The findings in this study showed a non-significantly ($p>0.05$) lower mean body weight exhibited by animals that were experimentally induced with BPH when compared with that of the normal control group. This could be due to differences in the initial whole body weights and unknown physiological processes that might have occurred during prostate enlargement and during metabolism of finasteride and plant extract components. This finding is in contrast to the studies by Mbaka *et al*. (2013) and Ramani *et al*. (2015) which stated an increase in the mean body weight of the BPH induced animals compared to that of the normal control animals though, their induction and treatment were simultaneously for 28 days and 45 days respectively.

The effects of testosterone and DHT on prostatic growth in rodents have previously been documented and used to assess the effects of drugs used for the treatment of prostatic hyperplasia, including saw palmetto fruit lipid extract (Nandecha *et al*., 2010). The absence of significant differences ($p>0.05$) in the mean prostate weights of animals in the normal control group and treated groups may be due to differences in the final whole body weights of the animals (Ejike and Ezeanyika, 2011). However, the significantly ($p<0.05$) lower mean prostate weights of animals in group 3, group 4 and group 5 compared to that of the positive control animals after three weeks of treatment suggest that ethanol extract of *Zapoteca portoricensis* stem is associated with the significant attenuation of prostatic

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hyperplasia in rats. This result is in agreement with the studies carried out by Surendra *et al*. (2011) and Ramani *et al*. (2015) where there was a significant increase in the mean prostate weight of animals in the BPH induced non-treated group. This increase may be attributed to the effect of exogenous hormone administration on the non-treated group.

The relative prostate weight (prostate-body weight ratio) is used as one important marker of BPH development (Arruzazabala*et al*., 2006; Veeresh *et al*., 2010). In previous studies, animals with BPH have shown an increased relative prostate weight (Ejike and Ezeanyika, 2011; Surendra *et al*. 2011; Lee *et al*., 2012; Ramani *et al*., 2015). Finasteride or other agents used to treat BPH decrease relative prostate weight. These confirm the significantly ($p<0.05$) higher mean relative prostate weight of animals in the induced groups compared to that of animals in the non-induced found in this study. However, the significantly ($p<0.05$) lower mean relative prostate weights of induced and treated groups compared to that of the induced and non-treated (positive control) group (group 2) may be attributed to the attenuating effect of the finasteride and the plant extract on the aberrant growth of the prostate after three weeks of treatment.

The present study showed that the mathematical expression results in remarkable differences in the increase in relative prostate weight, the percentage increase in relative prostate weight and the percentage recovery in relative prostate weight when comparing the induced groups to the non-induced group (normal control) and the induced and treated groups to the induced and non-treated group (positive control). These results are in tandem with those obtained in the studies carried out by Nandecha *et al*. (2010) and Ramani *et al*. (2015).

Testosterone is also an important agent considered in benign hyperplasia because of its involvement in prostate cell proliferation. In this study, it was observed that benign prostate hyperplasia led to increase in testosterone level of group 2, group 3, group 4 and group 5 animals (the induced-BPH groups) compared to that of the normal control (group 1) animals (Fig. 4). In this study, the high testosterone level may be attributed to exogenous hormone administration, while the significantly ($p<0.05$) lower testosterone level of group 2 (non-treated) animals (positive control) compared to that of animals in treated groups (group 3, group 4 and group 5) may be due to conversion of the testosterone to DHT during the treatment period (Fig. 4). Conversely, the elevated level of testosterone in the treated groups (group 3, group 4 and group 5) compared to that of the non-treated group (group 2) suggested inhibitory effects of the finasteride and the *Zapoteca portoricensis* stem extract on steroid 5α-reductase activity. This result was in contrast to the reports of Ejike and Ezeanyika (2011), Lee *et al*. (2012) and Mbaka*et al*. (2013) that stated increase in testosterone level of animals in the induced and non-treated group but had simultaneous induction and treatment of animals in the induced and treated groups. There was dose dependent decrease in the mean testosterone concentration of the extract treated group (group 4 and group 5) which suggest that increasing the dosage could return the concentration of the testosterone to that of the normal control group (group 1) (Fig. 4).

Steroid 5α-reductase converts testosterone to DHT, an active form of androgen, in the prostate and high level of DHT results in the development of prostatic hyperplasia (Pais, 2010). It has already been reported that increased level of testosterone and DHT are responsible for increased cell proliferation. DHT has a 10 times higher affinity for the androgen receptor than testosterone and easily binds to androgen receptor, which stimulates the transcription of growth factors that are mitogenic for the epithelial and stromal cells for prostate (Carson and Rittmaster, 2003). The importance of DHT in prostatic hyperplasia was demonstrated by previous studies in which an inhibitor of 5α-reductase was administered to experimental animals with BPH (Roehrborn, 2011). These may be the processes that resulted in significantly ($p<0.05$) lower mean sera DHT concentrations of animals in BPH-induced groups (group 2, group 3, group 4 and group 5) compared to that of the normal control animals (group 1) (Fig. 5). However, the reduced DHT level in the treated groups (group 3, group 4 and group 5) compared to the non-treated (positive control) group (group 2) (Fig. 5) also suggested the inhibitory potential of finasteride and the plant extract on steroid 5α-reductase activity. This report was in agreement with the study by Lee *et al*. (2012) which reported a significant ($p<0.05$) decrease in the DHT level of BPH-induced animals treated with *Melandrium firmum* methanoic extract compared with the BPH-induced non treated group. Unlike testosterone concentration, decrease in DHT level of extract treated groups was not dose dependent (Fig. 5).

Although an indispensable role of androgens in prostate biology is undisputed, the molecular mechanisms underlying benign prostatic hyperplasia (BPH) and prostate cancer remain largely uncharacterized. Prolactin (PRL) is one of the non-androgenic hormones and growth factors that have been implicated during development of these disorders. Prolactin provides an additional growth regulatory mechanism for the prostate and its overexpression can result in prostatic enlargement. Both the prolactin ligand and its receptors are normally expressed in human and rodent prostate. According to studies, general overexpression of a rat prolactin transgene (Mt-PRL) resulted in hyperprolactinemia and a dramatic enlargement of the murine prostate gland in older transgenic males with a concomitant elevation of circulating testosterone. An increase in mean serum prolactin level, found in this study, of the animals induced with BPH compared to the non-induced animals (Fig. 6) supports the above report on prostatic enlargement. However, in this study it was found that the mean serum prolactin level of the treated groups lowered significantly ($p<0.05$) suggesting antiproliferative effect of *Zapoteca portoricensis* stem extract (Fig. 6). This result is in tandem with the report of Wennbo *et al.* (1997) but in contrast to the report of Ejike and Ezeanyika (2011) where there was decrease in prolactin level of animals with estradiol velerate-induced BPH compared to that of normal control animals.

The prostate specific antigen (PSA) level, elevated after the animals were induced with benign hyperplasia, was observed to have decreased significantly after treatment with *Zapoteca portoricensis* stem extract for twenty one days (Fig. 7). PSA, a glycoprotein produced by cells of the prostate gland and present in serum, is usually elevated in prostate disorders and is a reliable marker for BPH and prostate cancer. A decrease in PSA is associated with reduced prostate hyperplasia as a direct consequence of 5α-reductase inhibition or anti-inflammatory actions (Roehrborn*et al*., 2007). The results obtained indicate that this plant extract has protective effects against the development of BPH as seen in the reduction in PSA levels (Fig. 7). These results are in agreement with the reports of Roehrborn*et al*. (2007) and Mbaka*et al*. (2013) which stated an increase in serum PSA level of induced and non-treated BPH animals and a decrease in PSA level of the induced and treated BPH animals.

Zinc is an important regulator of prostate function and plays a unique role in prostate health. The prostate gland accumulates zinc at higher concentration than other body tissues. Zinc helps prostate cells resist malignant transformation by creating an intracellular environment toxic to cancerous cells and normal prostate cells have evolved powerful mechanisms to protect themselves against zinc toxicity. There are reported cases of increased zinc concentration in BPH patients and in prostatic carcinoma (Park *et al*., 2013). The increase in prostatic and serum zinc concentration associated with testosterone-induced prostatic hyperplasia in rats has also been documented (Gonzales *et al*., 2012). These support the increase in mean serum zinc concentration observed in the animals with testosterone-induced BPH compared to that of the non-induced (normal control) animals (Fig. 8). However, the plant extract used in this study reduced the concentration of serum zinc, in a dose dependent manner, to a level very close to that of the normal control animals and this corroborates the study carried out by Gonzales *et al*. (2012) where there was a decrease in serum zinc concentration of rats with testosterone-induced BPH treated with red maca (*Lepidium meyenii*) extract.

CONCLUSION

The beneficial effects of *Zapoteca portoricensis* in various common ailments have been suggested through various studies. Recent literatures have explored antimicrobial, hepatoprotective, antioxidant and anti-inflammatory activities of this herb and our findings in this study, such as reduction in prostate and relative prostate weights; lower concentrations of DHT, prolactin, PSA and zinc ion of the treated animals, suggest possible beneficial effects of ethanol extract of the *Zapoteca portoricensis* stem in the treatment of testosterone-induced benign prostate hyperplasia in male albino rats.

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