EUROPEAN JOURNAL OF BIOMEDICAL AND PHARMACEUTICAL SCIENCES
http://www.ejbps.com

ISSN 2349-8870
Volume: 6
Issue: 13
141-147
Year: 2019

DETERMINATION OF EXOGENOUS TESTOSTERONE PROPIONATE DOSE FOR INDUCTION OF BENIGN PROSTATIC HYPERPLASIA IN RAT MODEL

Obisike U. A.*[1], Nwachuku E. O.[1], Boisa N.[2] and Nduka N.[1]

[1]Department of Medical Laboratory Science, Rivers State University, Port Harcourt.
[2]Department of Chemistry, Rivers State University, Port Harcourt, Nigeria.

***Corresponding Author: Obisike U. A.**
Department of Medical Laboratory Science, Rivers State University, Port Harcourt.

Article Received on 10/10/2019 **Article Revised on 01/11/2019** **Article Accepted on 21/11/2019**

ABSTRACT
Benign prostatic hyperplasia (BPH) is one of the major public health concerns, which has a high prevalence rate and causes significant decline in men's quality of life. There are currently no drug that can completely cure BPH without causing adverse side effects in which sexual dysfunction is chief. As a result, there is great interest in research using animal models. BPH can basically be induced in rats by subcutaneous injection of testosterone propionate. However, there is controversy in the specific concentration of TP that can comfortably induce BPH and for how long the biochemical, clinical and histological manifestations last. The aim of this study was to determine the concentration of TP that could induce BPH in albino wistar rats and to determine how long TP induced changes could last. A total of 24 adult wistar albino rats were used for the study. They were housed in standard cages under 12 hours light and dark cycle and allowed access to feed and water *ad libitum*. The rats were allowed to acclimatize for fourteen (14) days. Castration of rats were done to avoid interference from endogenous testosterone. The rats were divided into four groups of 6 rats each: Orch only: rats received only olive oil as vehicle for TP; Orch + 2 received 2mg/kg b.wt. sc. TP/day; Orch + 4 received 4mg/kg b.wt. sc. TP/day and Orch + 6 received 6mg/kg b.wt. sc. TP/day. All rats were castrated before treatment of TP, though the Orch only group received sham castration where the testes were not excised during the process. The first three sets of rats were sacrificed after 15 days of treatment, while the remaining 3 rats in each cage were sacrificed 30 days after. The rats were euthanized after 15/30 days, blood for the determination of PSA (by ELISA) was collected through cardiac puncture and prostate glands were harvested, rinsed, measured (length, height and breadth) with a ruler calibrated in centimeter, weighed and fixed in 10% formol saline for histological examination which was done and slides stained with H & E staining method. The prostatic indices - prostate weight to body weight (PW/BW) ratio were calculated. SPSS version 22.0 was used to statistically analyse data and p values less than 0.05 were considered significant. Results showed that there were no significant difference in body weights of rats for both 15 and 45-day studies. Benign prostatic hyperplasia was confirmed in the group of rats injected with 4mg/kg testosterone for 15 days. Histological changes in the group of rats treated with 4mg/kg b.wt.sc. corroborated with the biochemical findings. Both biochemical and histological changes that occurred after BPH was established were sustained for 30 days. Though the rat prostate does not surround the urethra, it is believed that rats are unlikely to develop the LUTS seen in humans. However, there is evidence that the overgrowth of the prostate located at the anterior urethra can affect the free passage of urine during micturition.

KEYWORDS: Testosterone propionate, benign prostatic hyperplasia, induction, albino wistar rats.

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, 1996; Berry *et al.*, 2014). 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, 2003; Wasserman, 2006).

It has been estimated that 50% of men have histologic evidence of BPH by age 50 and 75% by age 80. It has also been reported that in about 40–50% of these men, BPH becomes clinically significant, Guess *et al.*, 1999). It increases the risk of lower urinary tract symptoms (LUTS), which can be categorized into filling/irritative symptoms (increased urinary urgency and frequency, painful urination, and excessive passage of urine at night) and obstructive symptoms in voiding (poor stream, hesitancy, incomplete voiding, terminal

dribbling, and overflow incontinence, (Chapple and Roehrborn, 2006; Rosini *et al.*, 2007).

There is no known anti-BPH drug that can completely cure BPH without serious adverse effect. Therefore efforts to develop new drugs have been on the increase. In order to test new anti-BPH drugs, BPH animal models are necessary for *in vivo* efficacy studies. The concept that androgens are important for the maintenance of prostate disease dictates the standard of care for BPH (Huggins and Hodges, 2002).

The rat models of BPH have been induced by hormones, including androgenic, estrogenic, and progestational hormones, (Turner, and Brading, 1997; Irwin *et al.*, 2009). Xenograft models have also been established with cells derived from human BPH tissue culture and primary surgical specimens, which were implanted subcutaneously in immune-deficient rats or mice (Otto *et al.*, 1992). Transplants displayed histologically typical BPH acini and stroma, (Debiec-Rychter *et al.*, 1994).

Using transgenic mice, androgen receptor, insulin-like growth factor-1 and a number of other growth hormones have been found to play roles in BPH, (Ruan *et al.*, 1999). These models are typically used to test drugs that can prevent hyperplasia or reduce the size of the prostate. Therefore, the aim of this study was to determine the concentration of exogenous TP that could induce BPH in albino wistar rats and to determine how long TP induced changes could last.

MATERIALS AND METHODS

Experimental Animals

A total of twenty four 20 week-old male albino wistar rats weighing between 170-200g 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 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 (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, 1985).

Drug/Chemicals

Testosterone (Testosterone Propionate)

Testosterone propionate (brand name **Testost** – manufactured by Laborate Pharmaceuticals India Limited) was 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.

Ketamine Injection (Ketamine hydrochloride)

Ketamine is a medication mainly used for starting and maintaining anaesthesia (brand name **Ketalar** – manufactured by Sular Pharmaceuticals, India) was purchased from Sicone Pharmacy and Stores, No. 2B Evo Road, G.R.A. Phase II Port Harcourt and was used to anaethesize the rats prio to bilateral orchiectomy.

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 (Coppenolle *et al.*, 2000). 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.

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.

Calculation of Prostatic Indices

Prostate Index was calculated using the formula below:

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

Arbitrary unit = mg/g.

Experimental Design

After fourteen days of acclimatization, twenty four (24) rats were weighed and ramdomly divided into four groups (6 rats per group) and used for the determination of the dose of testosterone (testosterone propionate) that could induce benign prostatic hyperplasia. Eighteen rats submitted to bilateral orchiectomy (castrated) as described above, while the remaing six rats grouped as control were subjected to sham operation.

They were placed into the following groups:

- **Control Group** – Orch only (submitted to sham orchiectomy and given olive oil only as vehicle),
- **Orch + 2** – Rats submitted to orchiectomy and injected with 2mg/kg b.wt s.c. of testosterone propionate
- **Orch + 4** - Rats submitted to orchiectomy and injected with 4mg/kg b.wt. s.c. of testosterone propionate) and
- **Orch + 6** - Rats submitted to orchiectomy and injected with 6mg/kg b.wt. s.c. of testosterone propionate

The weights of the rats were recorded weekly. After 15 days, three rats were randomly selected from each group. They were euthanized, blood for the determination of PSA was collected through cardiac puncture and prostate

glands were harvested, rinsed, measured (length, height and breadth) with a ruler calibrated in centimeter, weighed and fixed in 10% formol saline for histological examination. The prostate index - prostate weight to body weight (PW/BW) ratio was calculated. The remaining three rats in each group were euthanized after 30 days from then. They were not given further treatments prior to that. The reason was to check if BPH would be sustained for up to 30 days.

Sample Collection and Storage
At the end of the treatments, the rats anaesthetized with chloroform and blood samples collected through cardiac puncture after 8 hours fast. 5ml of blood was put in lithium heparin container for the determination of plasma PSA.

Laboratory Methods and Principles
Estimation of Rat Plasma Prostate Specific Antigen (McCarthy *et al.*, 1983 as modified by Shanghai Korain Biotech Co., Ltd, China.

Method
The method used was sandwich enzyme-linked immunosorbent assay (ELISA).

Principle of Test
The PSA ELISA test is based on the principle of a solid phase enzyme-linked immunosorbent assay. The assay system utilizes a rabbit anti-PSA antibody directed against intact PSA for solid phase immobilization (on the microtiter wells). A monoclonal anti-PSA antibody conjugated to horseradish peroxidase (HRP) is in the antibody-enzyme conjugate solution. The test sample is allowed to react first with the immobilized rabbit antibody at room temperature for 60 minutes. The wells are washed to remove any unbound antigen. The monoclonal anti-PSA-HRP conjugate is then reacted with the immobilized antigen for 60 minutes at room temperature resulting in the PSA molecules being sandwiched between the solid phase and enzyme-linked antibodies. The wells are washed with water to remove unbound-labeled antibodies. A solution of TMB (Human tubular basement membrane antibody) Reagent is added and incubated at room temperature for 60 minutes, resulting in the development of a blue color. The color development is stopped with the addition of Stop Solution changing the color to yellow. The concentration of PSA is directly proportional to the color intensity of the test sample. Absorbance was measured spectrophotometrically at 450 nm.

Histological Analysis
The prostate gland, kidneys and liver were harvested for histological analysis, and were fixed in 10% formal saline solution. The tissues were dissected and representative tissue blocks were taken for histological processing each with identifying label in a tissue cassette. The fixed tissue blocks were dehydrated through ascending grades of alcohol, de-alcoholised in xylene, infiltrated and embedded in molten paraffin wax. Sections were cut at 3µm on a rotary microtome. Deparaffinised sections were then stained with the standard haematoxylin and eosin staining technique and the slides mounted in DPX. Sections on slide were examined and photomicrographs captured with X40 objective lens using the ScopeTek™ device and software v1.3.

Staining Procedure
Paraffin wax was removed by dipping slide into xylene 1 and 2 for 1 minute each. The slide was immersed in absolute alcohol for 30 second and hydrated in descending grades of alcohol (90%, and 70%) for 30 seconds each. It was rinsed in tap water for 1 minute, stained with Erlich's hematoxylin for 30 minutes and rinsed in running tap water for 5 minutes until colour turns blue. It was counterstained with 1% aqueous eosin for 5 minutes, and rinsed with tap water for 30 seconds.

The slide was dehydrated by passing through ascending grades of alcohol (70%, and 90%) for 30 seconds each and immersed in absolute alcohol twice for 30 seconds each. Finally it was cleared in xylene for one minute.

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.

RESULTS
Table 1: Body Weights of Rats used for 15 Days Pilot Study for the Determination of Dose of Testosterone Propionate Effective for BPH Induction.
Orch – Ochiectomy
NS – Non significant (at $P<0.05$)

Table 4.2: Prostatic Prameters of Rats used for 15 Days Pilot Study for the Determination of Dose of Testosterone Propionate Effective for BPH Induction.

Groups	Prostate Volume (cm^3)	Prostate Weight(mg)	PSA (pg/ml)	LBW(g)	Prostate Index
Orch only	0.34 ± 0.07	215.6 ± 7.51	82.97 ± 10.50	179.3 ± 13.05	1.20 ± 0.08
Orch + 2	0.37 ± 0.08	279.3 ± 8.08	131.17 ± 4.63	173.0 ± 11.35	1.61 ± 0.06
Orch + 4	1.01 ± 0.12*	577.6 ± 91.85*	638.77 ± 124.82*	178.3 ± 7.09	3.22 ± 0.38*
F value	47.851	566.590	54.308	0.298	65.281
P value	0.000	0.000	0.000	0.752	0.000
Remark	S	S	S	NS	S

***** Significant (p<0.05) post hoc (compared with Orch only)
S – Significant (at p<0.05)
NS – Non-significant (at p<0.05)

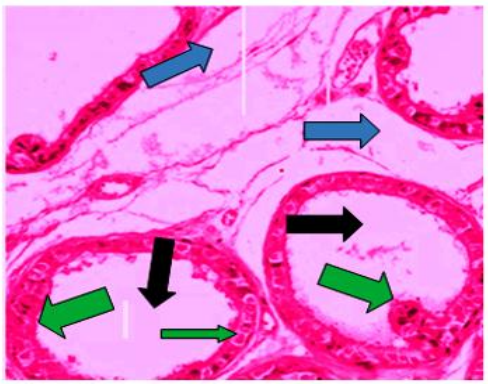
Table 4.3: Prostatic Prameters of Rats used for 45 Days Pilot Study for the Determination of Dose of Testosterone Propionate Effective for BPH Induction.

Groups	Prostate Volume (cm^3)	Prostate Weight(mg)	PSA (pg/ml)	Last BW (g)	Prostate Index
Orch only	0.29 ± 0.06	230.33 ± 3.21	91.97 ± 6.68	182.3 ± 10.97	1.26 ± 0.05
Orch + 2	0.32 ± 0.10	290.66 ± 12.74	127.70 ± 11.77	176.6 ± 11.93	1.64 ± 0.08
Orch + 4	0.86 ± 0.14*	546.66 ± 42.85*	673.43 ± 156.17*	180.3 ± 7.02	3.03 ± 0.23*
F value	27.238	126.364	38.897	0.238	122.269
P value	0.001	0.000	0.000	0.795	0.000
Remark	S	S	S	NS	S

***** Significant (p<0.05) post hoc (compared with Orch only)
S – Significant (at p<0.05)
NS – Nonsignificant (at p<0.05).

Table 4.2: Body Weights of Rats used for 45 Days Pilot Study for the Determination of Dose of Testosterone Propionate Effective for BPH Induction.

		Rat Weight (g)					
Groups	**Day 1**	**Day 7**	**Day 14**	**Day 21**	**Day 28**	**Day 35**	**Day 45**
Orch only	183.3±11.01	167.6±7.23	168.6±8.14	171.0±13.89	170.6±7.63	173.0±6.24	182.3±10.96
Orch + 2	181.3±11.01	175.6±14.36	184.0±9.53	183.0±13.52	186.3±9.07	180.6±12.89	176.6±11.93
Orch + 4	182.0±7.55	177.6±16.65	172.3±8.08	182.0±7.54	178.6±8.73	179.0±7.81	180.3±7.02
F value	0.031	0.470	2.591	0.921	2.545	0.549	0.238
P value	0.969	0.646	0.154	0.448	0.158	0.604	0.795
Remark	NS	NS	NS	NS	NS	NS	NS



Blue – Normal glandular stroma

Black – Clear lumen with no infiltrations

Green – Thick intraglandular epithelia convolution (**thick arrow**) and cuboidal epithelia cells of regular sizes (**thin arrow**)

Plate 4.1: Photomicrograph of Cross Section of the Prostate Gland of 15 Days Pilot Study Rat (H &E) Submitted to Sham Orchiectomy Only (Orch Only) 400X.

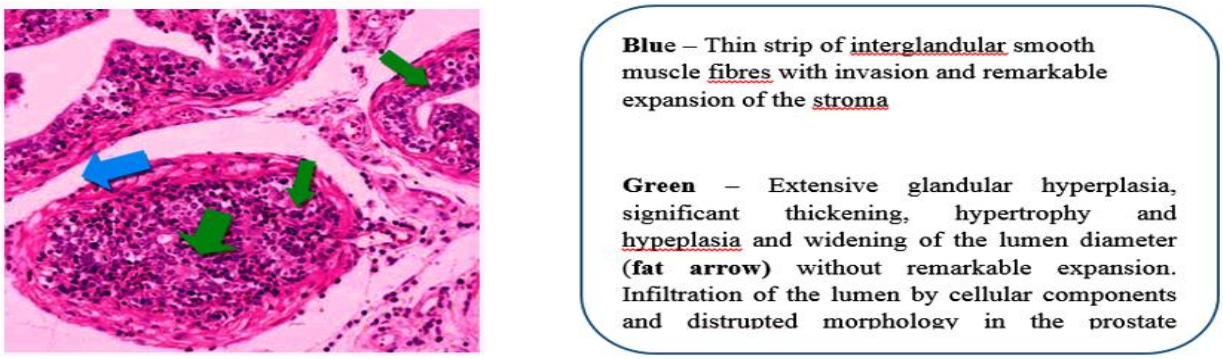


Plate 4.2: Photomicrograph of a Cross Section of the Prostate Gland of 15 Days Pilot Study Rat (H &E) Submitted to Orchiectomy and treated with 4mg/kg b.wt. sc. Testosterone Propionate. 400X.

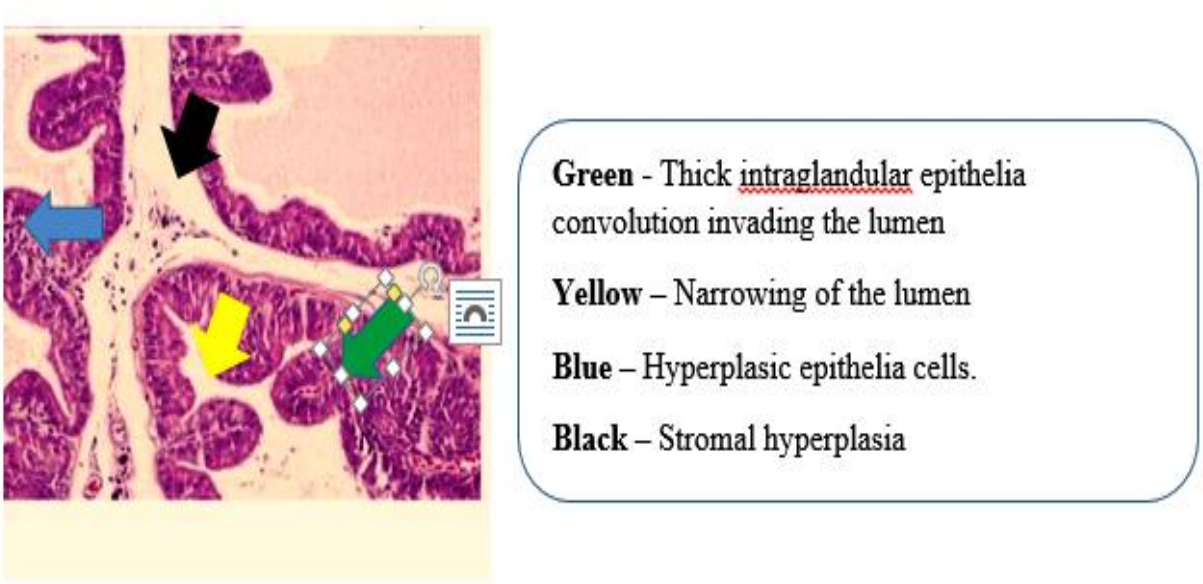


Plate 4.3: Photomicrograph of a Cross Section of the Prostate Gland of 45 Days Pilot Study Rat (H&E) submitted to Orchiectomy and treated with 4mg/kg b.wt. sc. Testosterone Propionate. 400X.

DISCUSSION

The aim of this study was to determine the concentration of TP that could induce BPH in albino wistar rats and to determine how long TP induced changes could last. In this study, testosterone propionate was used to induce benign prostatic hyperplasia (BPH) in adult male rats. Rat weights measured at weekly intervals during the 15-day pilot study showed that there were slight non-significant increases in the mean body weight of the rats in the three groups, (table 4.1).

Mean rat weight for rats groups used for 45 days pilot study were statistically non-significant when the three groups (Orch only, Orch +2 and Orch + 4) were compared (table 4.4). The administration of 4mg/kg b.wt. sc. daily for 15 days caused a significant increase (P=0.000) in mean prostate volume (PV) of rats when compared with the mean PV values of rat groups in Orch only and Orch + 2. There were also significant increases (P=0.000) in mean prostate weight (PW), mean prostate index (calculated by dividing PW with last body weight) and prostate specific antigen (PSA) values when the mean values for the three groups in the pilot study were compared and when values for Orch + 4 were compared with mean values for the group of rats that were not induced but underwent bilateral orchiectomy (table 4.3). The results above showed that testosterone propionate at a concentration of 4mg/kg b.wt. administered daily through subcutaneous route for 15 days could induce BPH in male rats. In summary, BPH induced by testosterone propionate at 4mg/my b.wt. sc. daily markedly increased the prostate volume, prostate weight, PSA and prostate index which is consistent with previous studies (Veeresh *et al.*, 2010; Yang *et al.*, 2014). Although, they used 3mg/kg b.wt. Thirty days after induction, mean PV, PW, PSA and PI were still significantly increased (table 4.4). This shows that BPH induction was sustained for up to 30 days.

Histological examination of prostatic tissues of rats in Orch + 4 group for both 15 and 45 days study showed extensive glandular hyperplasia and significant

thickening and hypertrophy. Widening of the diameter of the lumen without remarkable expansion in stroma was also seen. There were thickening of inter and intra glandular epithelial linings, (plates 4.2 and 4.3). Those features show rapid multiplication and enlargement of cells as seen in BPH. The histological findings of this study showed that the rat ventral lobes that were used responded to treatment with testosterone. This is line with a study by Nevalainen et al in 1991 where they reported that rat prostate can respond to hormone treatment. However, the noted that only the dorsal lobe of the rodent prostate was ontogenetically comparable to the human prostate, (Nevalainen *et al.*,1991). It has been reported that hormonal treatment not only induces prostate growth but also hardens the ventral lobe of the prostate, (Constantinou, 1996), and such hardening. Our results have shown that the epithelial cell layer and stromal cell space in both the ventral lobe of the rat prostate were induced by testosterone.

The study to determine the concentration of testosterone propionate that could induce clinical and histological BPH showed that 4mg/kg b.wt. sc. daily for 15 days was enough for BPH induction. Results from the groups of rats that were allowed for additional 30 days after induction (making it a total of 45 days) also showed that the features that characterize BPH were sustained throughout the period (30 days) of the main study.

CONCLUSION

Benign prostatic hyperplasia was confirmed in the group of rats injected with 4mg/kg testosterone for 15 days. Histological changes in the group of rats treated with 4mg/kg b.wt. corroborated with the biochemical findings. Both biochemical and histological changes that occurred after BPH was established were sustained for 30 days. Though the rat prostate does not surround the urethra, it is believed that rats are unlikely to develop the LUTS seen in humans. However, there is evidence that the overgrowth of the prostate located at the anterior urethra can affect the free passage of urine during micturition.

REFERENCES

1. Oesterling, J.E. (1996). Benign prostatic hyperplasia: a review of its histogenesis and natural history, *The Prostate*, 29(6): 67–73.
2. Berry, S. J. Coffey, D. S. Walsh, P. C. & Ewing, L. L. (2014). The development of human benign prostatic hyperplasia with age, *The Journal of Urology*, 132(3): 474–479.
3. O'Leary, M. P. (2003). Lower urinary tract symptoms/benign prostatic hyperplasia: maintaining symptom control and reducing complications, *Urology*, 62(3): 15–23.
4. Wasserman, N. F. (2006). Benign prostatic hyperplasia: a review and ultrasound classification, *Radiologic Clinics of North America*, 44(5): 689–710.
5. Guess, H.A., Arrighi, H.M., Metter, E.J. & Fozard, J.L. Cumulative prevalence of prostatism matches the autopsy prevalence of benign prostatic hyperplasia. *Prostate*, 17(3): 241-246.
6. Rosini, M., Bolognesi, M.L., Giardina, D., Minarini A, Tumiatti V, Melchiorre C. (2007). Recent advances in alpha1-adrenoreceptor antagonists as pharmacological tools and therapeutic agents. *Current Tropical Medicinal Chemistry*, 7(2): 147-162.
7. Chapple, C.R & Roehrborn, C.G. (2006). A shifted paradigm for the further understanding, evaluation, and treatment of lower urinary tract symptoms in men: focus on the bladder. *European Journal of Urology*, 49(4): 651-658.
8. Huggins, C. & Hodges, C.V. (2002). Studies on prostatic cancer. The effect of castration, of estrogen and of androgen injection on serum phosphatases in metastatic carcinoma of the prostate. 1941. *Journal of Urolology*, 168: 9-12.
9. Irwin, D.E., Milsom, I., Kopp, Z., Abrams, P., Artibani, W., Herschorn, S. (2009). Prevalence, severity, and symptom bother of lower urinary tract symptoms among men in the EPIC study: impact of overactive bladder. *European Journal Urology*, 56(1): 14-20.
10. Turner, W.H. & Brading, A.F. (1997). Smooth muscle of the bladder in the normal and the diseased state: pathophysiology, diagnosis and treatment. *Pharmacological Therapy*, 75(2): 77-110.
11. Otto, U., Wagner, B., Becker, H., Schroder, S., & Klosterhalfen, H. (1992). Transplantation of human benign hyperplastic prostate tissue into nude mice: first results of systemic therapy. *Urology International*, 48(2): 167-170.
12. Debiec-Rychter, M., Asgari, K., Jones, R.F., Grignon, D.J., Haas, G.P., Wang, C.Y. (1994). Xenografts of human benign prostatic hyperplasia tissues in the nude mouse. *In Vivo*., 8(3): 449-150.
13. Ruan, W., Powell-Braxton, L., Kopchick, J.J., Kleinberg, D.L. (1999). Evidence that insulin-like growth factor I and growth hormone are required for prostate gland development. *Endocrinology*, 140(5): 1984-1989.
14. National Institute of Health (1985). Guide for the Care and Use of Laboratory Animals prepared by the National Institute of Health.
15. Van Coppenolle F., Le Bourhis, X., Carpentier, F., Delaby, G, Cousse, H., Raynaud, J.P., Dupouy, J.P. & Prevarskaya, N. (2000). Pharmacological effects of the lipidosterolic extract of Serenoa repens (Permixon) on rat prostate hyperplasia induced by hyperprolactinemia: comparison with finasteride. *Prostate*, 43(1): 49-58.
16. McCarthy, U., Distaburd, R.T. & Larry, D.G. *(*1983). Novel Estimation of Plasma Protein. *Journal of Diagnostic Medicine*, 232: 345-241.
17. Veeresh, S.V., Veeresh, B. Anup, A. Patil, Y. & Warke, B. (2010). Lauric acid and myristic acid prevent testosterone induced prostatic hyperplasia in

rats Author links open overlay panel. *European Journal of Pharmacology*, 626: 2–3.

18. Yang, X., Yuan, L., Xiong, C. Yin, C. & Ruan, J. (2014). Abacopteris meningioma exerts testosterone-induced benign prostatic hyperplasia protective effect through regulating inflammatory responses, reducing oxidative stress and anti-proliferative, *Journal of Ethnopharmacology*, 157: 105–113.
19. Nevalainen, M.T, Valve, E.M., Makela, S.I., Blauer, M., Tuohimaa, P.J. & Harkonen, P.L. (1991) Estrogen and prolactin regulation of rat dorsal and lateral prostate in organ culture. *Endocrinology*, 129(2): 612-622.
20. Constantinou, C.E. (1996). Influence of hormone treatment on prostate growth and micturition characteristics of the rat. *Prostate*, 29(1): 30-35.