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Protective effects of *Chromolaena odorata* extract on experimental benign prostatic hyperplasia in rats

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Abstract

Benign prostatic hyperplasia (BPH) is an age-related disease in dogs and man leading to prostate enlargement which impinges on the urethra causing urinary outflow obstruction. Due to the side effects of surgery and chemotherapy used for the treatment of this disease, attention is now focused on phytotherapeutics for its management. Thus, we investigated the inhibitory effect of hydro-methanol extract of *Chromolaena odorata* (HMECO) on testosterone propionate (TP)-induced BPH rat model. A total of forty-two 10-12 weeks old male Sprague-Dawley outbred albino rats (*Rattus norvegicus*) weighing 200 - 250 g were randomly divided into six equal groups of seven rats each based on body weight as follows: A) Control group given phosphate-buffered saline orally and corn oil subcutaneously (SC) once daily, B) TP at a dose of 3.00 mg kg^{-1} SC once daily, C) TP at a dose of 3.00 mg kg^{-1} SC and finasteride at a dose of 10.00 mg kg^{-1} orally once daily, D) TP at a dose of 3.00 mg kg^{-1} SC plus 200 mg kg^{-1} HMECO orally once daily, E) TP at a dose of 3.00 mg kg^{-1} SC plus 400 mg kg^{-1} HMECO orally once daily and F) TP at a dose of 3.00 mg kg^{-1} SC plus 800 mg kg^{-1} HMECO orally once daily for 28 days. Results showed that HMECO significantly reduced prostate weight, prostatic index; serum levels of testosterone and prostatic epithelial thickness and increased luminal diameter in BPH induced rats. Thus, the results of this study suggest that *C. odorata* is a potential pharmacological candidate for the management of BPH.

Introduction

Benign prostatic hyperplasia (BPH) is a benign and uncontrolled growth of the prostate gland which may lead to bladder outflow obstruction, lower urinary symptoms, sepsis, irreversible bladder damage, renal failure or even death.[1] It is a major health issue in both aging men and dogs. Presently, BPH is known to be caused by the enzymatic conversion of testosterone into its more active metabolite, dihydrotestosterone (DHT), by 5-alpha reductase enzyme in the prostate cells.[2] This enzyme is the target for drug therapy aimed at reducing the size of prostate. Thus, the two main classes of conventional therapeutic agents used in managing BPH are the 5-alpha reductase inhibitors (5-ARIs) and alpha-adrenergic receptor blockers[3]; while, minimally invasive surgical therapies are also used as an option if chemotherapy does not alleviate urinary symptoms.[4] These conventional methods of BPH management have adverse side effects. Some alpha blockers cause dizziness, headache, weakness, asthenia, retrograde ejaculation and nasal congestion; while, 5-ARIs cause ejaculatory dysfunction, erectile dysfunction and gynaecomastia.[5-7] Minimally invasive surgical procedures may also lead to permanent sexual side effects and urinary incontinence.[8,9] As a result, some patients seek phytotherapy for the management of BPH.[10] Thus, it is worthwhile for local plants to be assessed for possible therapeutic activity; thus, phyto-therapeutics can be developed locally to aid this common condition management.

Chromolaena odorata is a fast-growing perennial shrub native to South and Central America. It was introduced into the tropical regions of Asia and Africa where it is an invasive weed.[11] The plant is reported to have notable pharmacological activities including anti-androgenic effects.[12] However, there is no report in the literature regarding the effects of *C. odorata* on BPH. Therefore, the objective of this study was to evaluate the patho-

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morphology and histochemical changes associated with experimental BPH in rats administered hydro-methanol extract of *C. odorata* (HMECO).

Materials and Methods

Drugs. Finasteride, the standard drug used, and testosterone propionate (TP) used to induce BPH were based on previous reports[13] and purchased from Sigma Chemical Co. (St. Louis, USA).

Preparation of plant extract. The fresh leaves of *C. odorata* were collected from the wild (with the voucher number of H113827). The leaves were washed gently with tap water to remove dust and other impurities and dried under shade for about 10 days; after which they were ground to powder using a grinding machine. The dried ground powder of the leaves was soaked in 70.00% methanol (Sigma) for three days; after which the extract was evaporated using a rotary evaporator (ML Rotary Evaporator, Heidolph Instruments Inc., Schwabach, Germany). The HMECO obtained was stored in a container at 4.00˚C and reconstituted with Tween 80 (0.20% v/v) prior to administration.[14]

Acute toxicity study of the extract. The acute toxicity study of HMECO was done using the Organization for Economic Co-operation and Development guidelines.[15] Fifteen healthy male rats were used for the acute toxicity study. They were acclimated for two weeks during which the free access to feed and water was provided. The animals were randomly assigned into three groups of five rats each. They were fasted overnight prior to extract administration. Group A served as control and the rats were administered 2.00 mL distilled water once. Two doses of the hydro-methanol extract (2,000 and 5,000 mg kg^{-1} body weight) were constituted respectively each in 2.00 mL distilled water. These extracts were orally given as a single dose and only once to groups B and C using gavages, respectively. Food was withheld for further 3 hr after extract administration. Thereafter, they were observed for signs of toxicity and deaths respectively for 24 hr. Based on the absence of signs of toxicity or mortality at these dose levels, the oral doses of 200, 400 and 800 mg kg^{-1} body weight were selected for this study.

Experimental design and animal treatment. The experiment was performed in compliance with the Ethics and Regulations Guiding the Use of Research Animals as approved by the Institutional Animal Ethical Committee (UNN/FVM/2020/1106). A total of forty-two male 10 - 12 weeks old healthy Sprague-Dawley outbred albino rats (*Rattus norvegicus*) weighing 200 - 250 g were used for the study. The rats were housed (five *per* cage) in standard polypropylene cages having size of 60.00 cm × 45.00 cm × 45.00 cm with wood shavings as a bedding in the Experimental Laboratory Animal Unit and acclimatized at a temperature of 25.00 ± 4.00 ˚C and relative humidity of 65.00 ± 5.00% with an alternating 12 hr light and dark cycle for two weeks. They were fed commercial growers' diet and given water *ad libitum.* After an acclimatization period of two weeks, the rats were randomly divided into six groups of seven rats each based on body weight in a completely randomized design as follows: A:Control group given phosphate-buffered saline (PBS) orally and corn oil subcutaneously (SC) once daily for 28 days, B:TP at a dose of mg kg^{-1} SC once daily for 28 days, C:TP at a dose of 3.00 mg kg^{-1} SC and finasteride at a dose of 10.00 mg kg^{-1} orally once daily for 28 days, D:TP at a dose of 3.00 mg kg^{-1}SC plus 200 mg kg^{-1} HMECO orally once daily for 28 days, E:TP at a dose of 3.00 mg kg^{-1}SC plus 400 mg kg^{-1} HMECO orally once daily for 28 days and F: TP at a dose of 3.00 mg kg^{-1} SC plus 800 mg kg^{-1} HMECO orally once daily for 28 days. The technologist and histopathologist analyzing testosterone concentration and prostate histology respectively were blinded to the group allocation. The effective doses of finasteride for the treatment of BPH and testosterone used to induce BPH were determined from a previous study.[13] The volume for oral administration of PBS, finasteride and HMECO was 5.00 mL kg^{-1} and 2.00 mL kg^{-1} for SC injection of corn oil and TP, respectively.[13]

Sample collection. At the end of the experiment, the rats were fasted overnight and 2.00 mL of blood was collected in the morning via retro-orbital plexus into tubes without ethylenediaminetetraacetic acid in order to obtain serum after anesthesia by intra-peritoneal injection of 100 mg kg^{-1} body weight ketamine hydrochloride (Laborate Pharmaceutical India Limited, Bengaluru, India) and 5.00 mg kg^{-1} body weight xylazine (Kepro, Deventer, Netherlands). The samples were centrifuged (model 5702; Eppendorf, St. Louis, USA) at 1,000 *g* for 10 min for serum collection being used for hormonal assay. Thereafter, the animals were dissected and the prostates were removed and fixed in 10.00% buffered formalin after weighing. The body and prostate weights of the individual rat in a group were determined in each case using a high precision electronic balance (model HL, Fuzhou Huake Electronics Instrument Co. Ltd., Fujian, China) at the end of the experiment; while, the prostatic index was calculated as:

$$\textit{Prostatic index} = \textit{Organ weight} / \textit{Body weight} \times 100$$

Serum testosterone determination. The enzyme-linked immunosorbent assay kits (Shanghai Yi Li Biological Technology, Shanghai, China) were used for the quantitative determination of testosterone concentration according to the manufacturer's instructions. In this technique, the samples were added to microplate wells and absorbance was read in duplicates at 405 nm. The results were expressed as ng mL^{-1}.[16]

Histological investigations. The ventral prostate lobes of the prostate glands were manually processed for histopathological studies after fixing in 10.00% neutral

buffered formalin. Five-micrometer thick sections were cut, mounted on glass slides and stained with Hematoxylin and Eosin as well as periodic acid Schiff (PAS) for light microscopy (Nikon, Tokyo, Japan).[17,18] Photomicrographs of the sections were captured using Motic Images plus 2.00 digital cameras (Motic, Xiamen, China).

Statistical analysis. Statistical analysis of the data was done by SPSS Software (version 23.00; IBM Corp, Armonk, USA). Multiple comparisons were performed using one-way ANOVA followed by post-hoc test ($p < 0.05$). Values were expressed as means ± standard error of the mean.

Results

Effects of HMECO on organ and body weights of BPH induced rats. The mean body weights of the experimental groups were not significantly ($p > 0.05$) different from each other; but, the mean body weight of testosterone (BPH) group was higher than that of the control and treated groups after 28 days of treatment; as treatment with both finasteride and the three different doses of HMECO led to a lower mean body weight being shown in Table 1. The mean prostate weight and prostatic index values of the untreated testosterone propionate (BPH) group (B) were significantly ($p < 0.05$) higher compared to the control and finasteride- and extract-treated groups after 28 days of treatment.

Effects of HMECO on serum testosterone of BPH induced rats. The mean serum testosterone levels of testosterone (BPH) group B were significantly ($p < 0.05$) higher than all experimental groups except the finasteride-treated group C. The administration of HMECO led to a significant ($p < 0.05$) decrease in serum testosterone levels of groups D, E and F in a dose-dependent manner (Fig. 1).

Effects of HMECO on gross and histopathology of BPH induced rats. Grossly, the size of the prostate gland of group B (TP at a dose of 3.00 mg kg^{-1} SC) was larger compared to group A (control) and finasteride- or HMECO-treated groups (Fig. 2). The histological features of the prostate gland from control rats showed normal histological architecture characterized by secretory acini of variable diameter and luminal pinkish secretions. The acini were separated by a fibromuscular stroma, blood vessels and lymphatics (Fig. 3A). All these were surrounded by a capsule comprising connective tissue and a thick layer of smooth muscles. However, the group B (TP at a dose of 3.00 mg kg^{-1} SC) showed hyperplastic acinar epithelium with projections into the lumen in-variably reducing the acinar lumen of the gland compared to the control (Fig. 3B).

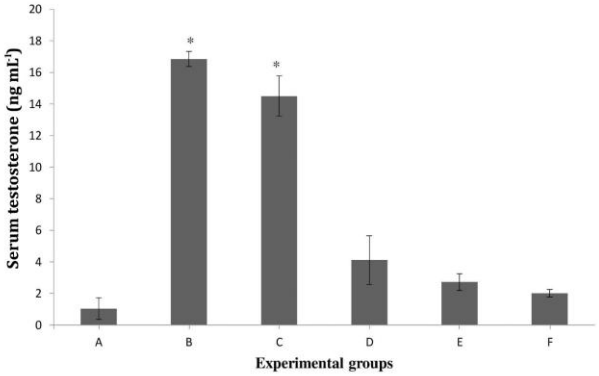

Fig.1. A graph showing the mean serum testosterone concentration of rats (n = 5) from the experimental groups: **A)** control; **B)** testosterone propionate (TP) at a dose of 3.00 mg kg^{-1} SC only; **C)** TP + finasteride; **D)** TP and 200 mg kg^{-1} of hydro-methanol extract of *Chromolaena odorata* (HMECO); **E)** TP and 400 mg kg^{-1} of HMECO and **F)** TP and 800 mg kg^{-1} of HMECO.
* Significantly different from the control and extract-treated groups D, E and F.

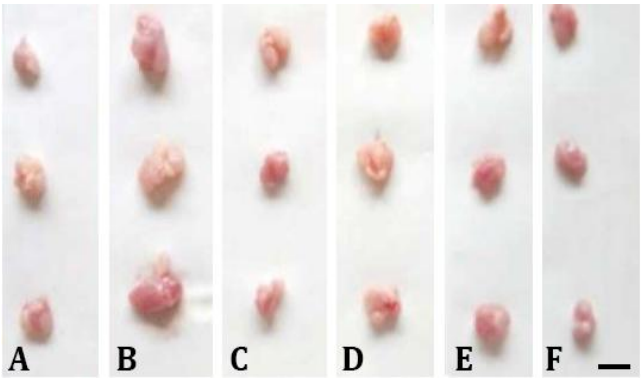

Fig. 2. Gross pictures of the prostate of rats (n = 3) from the experimental groups: **A)** control; **B)** testosterone propionate (TP) at a dose of 3.00 mg kg^{-1} SC only; **C)** TP + finasteride; **D)** TP and 200 mg kg^{-1} of hydro-methanol extract of *Chromolaena odorata* (HMECO); **E)** TP and 400 mg kg^{-1} of HMECO and **F)** TP and 800 mg kg^{-1} of HMECO. Note that the prostate in group B is larger in size than groups A, C, D, E and F. (Bar = 10.00 mm).

Table 1. The mean body and prostate weights and prostatic index of the experimental rats (n = 5).

Groups	Body weight (g)	Prostate weight (g)	Prostatic index (%)
A (control)	213.60 ± 7.80	0.39 ± 0.03^{a}	0.18 ± 0.01^{a}
B (TP only)	230.00 ± 17.50	1.09 ± 0.09^{b}	0.47 ± 0.02^{b}
C (TP + finasteride)	210.00 ± 10.10	0.59 ± 0.09^{cde}	0.27 ± 0.03^{c}
D (TP + 200 mg kg^{-1} HMECO)	205.60 ± 11.10	0.64 ± 0.04^{d}	0.31 ± 0.02^{c}
E (TP + 400 mg kg^{-1} HMECO)	210.60 ± 6.50	0.63 ± 0.03^{dc}	0.30 ± 0.01^{c}
F (TP + 800 mg kg^{-1} HMECO)	211.80 ± 9.90	0.45 ± 0.06^{ac}	0.21 ± 0.02^{a}

TP: Testosterone propionate; and HMECO: Hydro-methanol extract of *Chromolaena odorata.*
$^{a-e}$ different superscripts in the same column indicate significant differences at $p < 0.05$.

The amount of secretions observed in the lumen was also increased in comparison with control group and there were areas of lymphocytic infiltrates within the fibro-muscular stroma (Fig. 3B). Treatment with finasteride (group C) led to a reduction in epithelial cell projections and lymphocytic infiltration of the stroma (Fig. 3C). However, treatment with the various doses of the extracts in groups D (TP and 200 mg kg^{-1} of HMECO), E (TP and 400 mg kg^{-1} of HMECO) and F (TP and 800 mg kg^{-1} of HMECO) caused a dose-dependent restoration of glandular structures (Figs. 3D-3F) along with mild epithelial projections into the lumen and lymphocytic infiltration in the stroma of group D (Fig. 3D). Histochemical staining with PAS showed a strong positivity for neutral mucin in the intra-luminal secretions of the prostate in group B (TP at a dose of 3.00 mg kg^{-1} SC) compared to the normal rats of groups A (control). However, the positivity for neutral mucins in the secretions reduced significantly following treatment with finasteride and medium (400 mg kg^{-1} of HMECO) and high (800 mg kg^{-1} of HMECO) doses of the extract compared to the low dose group (Fig. 4).

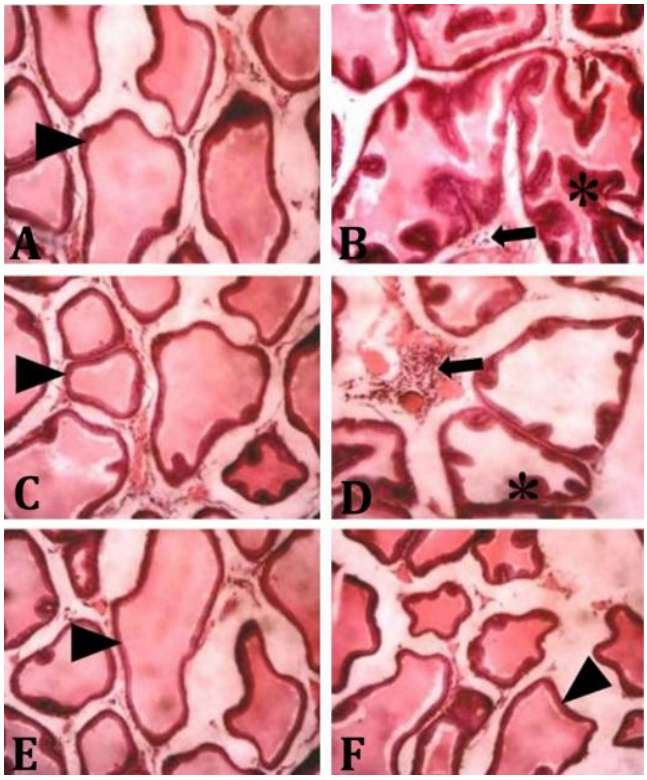

Fig. 3. Representative photomicrographs of the cross-section of prostate of rats (n = 5) from **A)** control group; **B)** testosterone propionate (TP) at a dose of 3.00 mg kg^{-1} SC only, **C)** TP + finasteride, **D)** TP and 200 mg kg^{-1} of hydro-methanol extract of *Chromolaena odorata* (HMECO), **E)** TP and 400 mg kg^{-1} of HMECO and **F)** TP and 800 mg kg^{-1} of HMECO. Note the areas of lymphocytic infiltrations in the connective tissue of stroma (arrows) and epithelial projections (asterisks) in B and D; while, A, C, E and F show thin epithelial lining cells (arrowheads), (Hematoxylin and Eosin staining, 100×).

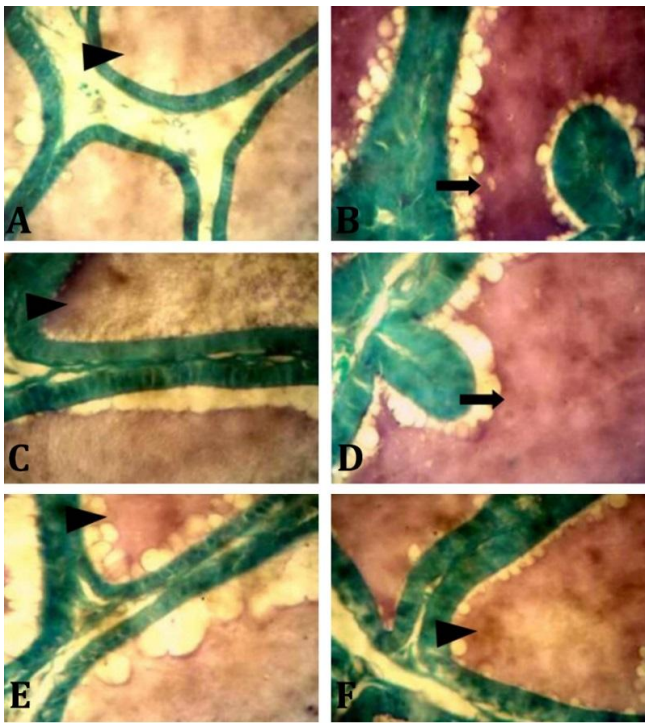

Fig.4. Representative photomicrographs of the cross-section of prostate of rats (n = 5) from **A)** control group; **B)** testosterone propionate (TP) at a dose of 3.00 mg kg^{-1} SC only, **C)** TP + finasteride, **D)** TP and 200 mg kg^{-1} of hydro-methanol extract of *Chromolaena odorata* (HMECO), **E)** TP and 400 mg kg^{-1} of HMECO and **F)** TP and 800 mg kg^{-1} of HMECO. Note the strong periodic acid Schiff positivity in B and D (arrows) compared to the weak reactions in A, C, E and F (arrowheads), (Periodic acid Schiff staining plus fast green counterstaining, 400×).

Discussion

In this research, we investigated the effects of HMECO on testosterone-induced BPH in rats. The significant increase in prostate weight and prostatic index in testosterone-treated group compared to the control animals is suggestive of the development of BPH. Prostate weight is used as one of the crucial markers of BPH.[19] In previous studies, animals with BPH had a significant increase in prostate weights compared to the control animals; whereas, those of animals treated with finasteride or other herbal remedies for the management of BPH meaningfully reduced compared to BPH animals.[19,20] For these reasons, several studies have evaluated the inhibitory effects of various compound on the development of BPH by measuring prostate weight.[21,22] In this study, animals treated with HMECO showed a significant reduction in prostate weight and prostatic index compared to the control and finasteride-treated groups. Histological examination of the prostate tissue of testosterone-induced BPH rats in this study revealed thick epithelial layers and glandular hyperplasia with focal areas of lymphocytic infiltration. However, BPH rats treated

with HMECO showed a marked decrease in epithelial layer thickness and increased luminal diameter with the absence of inflammation, suggesting that HMECO was an effective treatment for BPH. The mechanism of the effect may be attributed to some endogenous and exogenous androgens activity inhibition. In our experiments, the exogenous testosterone-induced increase in expression of serum testosterone was significantly inhibited by oral administration of HMECO in a dose-dependent manner. This shows that the inhibitory effect of HMECO in our BPH animal model was due to down-regulation of serum testosterone level. The findings are similar to those being previously reported by Yakubu *et al*.[12] showing that the leaves of *C. odorata* have an androgen deprivation effect along with the alkaloids as the active constituent. Interestingly, testosterone level in finasteride-treated group was lower but not significantly different from that of BPH group. Many researchers have conducted studies on the relationship between drug treatment and testosterone level in BPH condition. In most of the previous studies, administration of finasteride led to increased serum testosterone level due to the inhibition of the transformation of testosterone to DHT by the drug.[23] Inflammation is emerging as a critical factor in the etiopathogenesis of BPH.[24] The focal areas of lymphocytic infiltration in BPH tissues in this study suggest a progressing immune response in BPH [25] being ameliorated following treatment with HMECO. It has been reported that HMECO has anti-inflammatory properties.[26] The strong positivity for neutral mucins in the intra-luminal secretions of the BPH group compared to the control and finasteride- and HMECO-treated groups as observed in the histochemical evaluation using PAS in this study is similar to earlier report in humans showing that benign hyperplasia of the prostate has up to 93.30% positivity for neutral mucins.[27,28]

The results of this study suggest that *C. odorata* may be effective in the treatment of prostatic hyperplasia through its androgen deprivation effect. Thus, HMECO may be considered as a potentially efficacious therapeutic strategy to alleviate benign hyperplasia of the prostate in man and dogs. This will reduce the cost of treatment and adverse effects associated with the application of conventional management practices such as surgery and chemotherapy.

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Conflict of interest

Authors declare that there are no known conflicts of interest associated with this publication.

References

1. Roehrborn CG. Pathology of benign prostatic hyperplasia. Int J Impot Res 2008; 20(Suppl 3): S11-S18.
2. McConnell JD, Roehrborn C, Bautista OM, et al. The long-term effects of doxazosin, finasteride and the combination on the clinical progression of benign prostatic hyperplasia. N Engl J Med2003; 349(25): 2387-2398.
3. Gacci M, Ficarra V, Sebastianelli A, et al. Impact of medical treatments for male lower urinary tract symptoms due to benign prostatic hyperplasia on ejaculatory function: a systematic review and meta-analysis. J Sex Med 2014; 11(6): 1554-1566.
4. Rassweiler J, Teber D, Kuntz R, et al. Complications of transurethral resection of the prostate (TURP)--incidence, management, and prevention. Eur Urol2006; 50(5): 969-979.
5. Gormley GJ, Stoner E, Bruskewitz RC, et al. The effect of finasteride in men with benign prostatic hyperplasia. N Engl J Med1992; 327(17): 1185-1191.
6. Bullock TL, Andriole GL Jr. Emerging drug therapies for benign prostatic hyperplasia. Expert OpinEmerg Drugs2006; 11(1): 111-123.
7. Traish AM, Hassani J, Guay AT, et al. Adverse side effects of 5*α*-reductase inhibitors therapy: persistent diminished libido and erectile dysfunction and depression in a subset of patients. J Sex Med2011; 8(3): 872-884.
8. Kortt MA, Bootman JL. The economics of benign prostatic hyperplasia treatment: a literature review. Clin Ther 1996; 18(6): 1227-1241.
9. Preuss HG, Adderly B. The prostate cure. 1st ed. New York, USA: Crown Publishers Inc.1998; 1-251.
10. Park HS, Seo CS, Wijerathne CU, et al. Effect of *Veratrum maackii* on testosterone propionate-induced benign prostatic hyperplasia in rats. Biol Pharm Bull2019; 42(1): 1-9.
11. Rouw A. The invasion of *Chromolaena odorata* (L.) King Robinson (ex *Eupatorium odoratum*), and competition with the native flora in a rain forest zone, south-west Cote d'Ivoire. J Biogeogr 1991; 18: 13-23.
12. Yakubu MT, Akanji MA, Oladiji AT. Evaluation of antiandrogenic potential of aqueous extract of *Chromolaena odoratum* (L.) K. R. leaves in male rats. Andrologia 2007; 39(6): 235-243.
13. Ko JW, Park SW, Shin NR, et al. Inhibitory effects of Pycnogenol®, a pine bark extract, in a rat model of testosterone propionate-induced benign prostatic hyperplasia. Lab Anim Res 2018; 34(3): 111-117.
14. Daud D, Fekery NFM, Hashim N. Reproductive health of rats with benign prostatic hyperplasia following *Cosmos caudatus* ethanolic extract consumption. J App Pharm Sci 2017; 7(6): 202-205.
15. OECD Guidelines for the testing of chemicals, test No.

423: Acute oral toxicity-acute toxic class method, 2001: pp1-14. Available at: https://ntp.niehs.nih.gov/iccvam/suppdocs/feddocs/oecd/oecd_gl423.pdf. Accessed April 12, 2020.
16. Ekins RP. Ligand assays: from electrophoresis to miniaturized microarrays. Clin Chem 1998; 44(9): 2015-2030.
17. Suvarna KS, Layton C, Bancroft JD. Bancroft's theory and practice of histological techniques. 8th ed. London, UK: Elsevier Health Sciences 2018; 40-183.
18. Gholami S, Ansari-Lari M, Khalili L. Histologic and histomorphometric changes of testis following oral exposure to methyl tertiary-butyl ether in adult rat. Iran J Vet Res 2015; 16(3): 288-292.
19. Pais P. Potency of a novel saw palmetto extract, SPET-085, for inhibition of 5alpha-reductase II. Adv Ther2010; 27(8): 555-563.
20. Bisson JF, Hidalgo S, Rozan P, et al. Therapeutic effect of ACTICOA powder, a cocoa polyphenolic extract on experimentally induced prostate hyperplasia in Wistar-Unilever rats. J Med Food 2007; 10(4): 628-635.
21. Jang H, Ha US, Kim SJ, et al. Anthocyanin extracted from black soybean reduces prostate weight and promotes apoptosis in the prostatic hyperplasia-induced rat model. J Agric Food Chem 2010; 58(24): 12686-12691.
22. Veeresh Babu SV, Veeresh B, Patil AA, et al. Lauric acid and myristic acids prevents testosterone induced prostatic hyperplasia in rats. Eur J Pharmcol 2010; 626(2-3): 262-265.
23. Gasco M, Villegas L, Yucra S, et al. Dose-response effect of Red Maca (*Lepidium meyenii*) on benign prostatic hyperplasia induced by testosterone enanthate. Phytomedicine 2007; 14(7-8): 460-464.
24. Kramer G, Mitteregger D, Marberger M. Is benign prostatic hyperplasia (BPH) an immune inflammatory disease? Eur Urol 2007; 51(5): 1202-1216.
25. Theyer G, Kramer G, Assmann I, et al. Phenotypic characterization of infiltrating leukocytes in benign prostatic hyperplasia. Lab Invest 1992; 66(1): 96-107.
26. Umukoro S, Ashorobi RB. Evaluation of the anti-inflammatory and membrane stabilizing effects of *Eupatorium odoratum*. Int J Pharmacol 2006; 2(5): 509-512.
27. Mathur SK, Gupta S, Marwah N, et al. Significance of mucin stain in differentiating benign and malignant lesions of prostate. Indian J Pathol Microbiol 2003; 46(4): 593-595.
28. Agrawal DN, Zawar MP, Deshpande NM, et al. The study of mucinhistochemistry in benign and malignant lesions of prostate. J Sci Soc 2014; 41(1): 38-40.