Journal of Infertility and Reproductive Biology

J Infertil Reprod Biol, 2021, Volume 9, Issue 1, Pages: 22-26. https://doi.org/10.47277/JIRB/9(1)/22

Ethanolic Extract of Combined *Cynodon dactylon* and *Mimosa pudica* Ameliorated Experimentally Induced Benign Prostatic Hyperplasia in Wistar Rats

Adesanya Olamide Adewale[1]*, Suleiman Isa Adedeji[1], Odubela Olukayode Olusola[1], Sheu Oluwadare Sulaiman[2,3], Imade Oluwatosin Victoria[2]

[1] Department of Anatomy, Faculty of Basic Medical Sciences, Olabisi Onabanjo University-Ikenne Campus, Ikenne, Nigeria
[2] Physiology Department, Faculty of Biomedical Sciences, Kampala International University - Western Campus, P.O. Box 71, Ishaka, Uganda
[3]Cell Biology Department, Universidade Federal de Minas Gerais, Belo Horizonte, Brazil

Received: 05/10/2020 Accepted: 27/01/2021 Published: 20/03/2021

Abstract

Cynodon dactylon (CD*)* and *Mimosa pudica* (MP) have been known in folk medicine to relieve urinary troubles, and scientific studies have demonstrated their anti-inflammatory, diuretic, nerve regenerative, antispasmodic, pain alleviation, and muscle relaxant potentials. This study attempted to screen extract of CD and MP combination (CDMP) for its ability to ameliorate experimentally induced prostatic hyperplasia in the rat model, which could serve as possible management of benign prostatic hyperplasia (BPH). Twenty adult male Wistar rats were divided randomly into four groups. Groups A (normal control) and B (induced BPH control) received oral administration of distilled water, while IBPH groups C, and D received oral administration of low dose (LD) 0.46 g/ml and high dose (HD) 0.92 g/ml of CDMP extract per Kg BW, respectively for four weeks. Blood samples were collected from each rat to determine prostate-specific antigen (PSA) concentrations. Caudal epididymal sperm count, and testicular weight and histological examination were also evaluated. The CDMP ethanolic extract caused a significant ($p<0.05$) reduction in PSA levels and relative weight of the prostate in the treated rats. The relative weight of testis was significantly higher in CDMP treated groups. The sperm count in all IBPH groups was significantly ($p<0.05$) reduced. Evidence of recoveries was observed in prostates of CDMP treated groups with an increase in the fibromuscular, interacinar tissue, thinning of the prostate epithelium, and stroma. The findings suggest that ethanolic extracts of *Cynodon dactylon* and *Mimosa pudica* can serve as a potential candidate in the management of benign prostatic hyperplasia and its symptoms.

Keywords: Prostatic hyperplasia, *Cynodon dactylon, Mimosa pudica*, Prostate-specific antigen, Sperm count

Introduction

Benign prostatic hyperplasia (BPH) is a common disease in older men, with lower urinary tract symptoms caused by hyperplasia of the prostatic epithelium and stromal cells (1,2). Androgens that are essential for the growth of the prostate during early development, puberty, and aging have been documented to play a role in hypertrophy of the prostate along with other factors such as aging, diet, an increase in inflammatory mediators, hormones, and oxidative stress (2). At present, the main therapeutic drugs for BPH are 5 alpha-reductase inhibitors and beta-blockers (3, 4), such as finasteride and terazosin. However, these drugs caused adverse reactions such as fatigue, hypotension, ejaculation disorders, sexual dysfunction, and an increased risk of prostate fibrosis (4, 5). Surgical treatment is another potent remedy for BPH.

Meanwhile, this option poses a lot of complications and the possibility of recurrence. Thus, the number of patients undergoing surgery for BPH is gradually decreasing (6). Therefore, it is crucial to find a curative drug that can effectively treat BPH with few adverse reactions.

Phytotherapeutic agents have attracted tremendous attention for the treatment of BPH and prostate cancer because they are thought to be safer, cost-effective, and have fewer side effects than conventional alternatives especially in developing countries (7-9). New theories hold that many of the potent herbs may inhibit the action of an essential hormone-regulating enzyme that converts testosterone into dihydrotestosterone, a process believed to be important in the development of both an enlarged prostate and prostate cancer. Some medicinal products, like saw palmetto, *Cucurbita pepo* (pumpkin) have been recognized to suppress prostate-specific antigen (PSA) levels in the blood (10).

One of the few plants that have been used over some time with a potential reputation in ethnomedicine to treat urinary troubles and inflammation of the urinary bladder efficiently is *Cynodon dactylon* (CD) known as Bermuda grass (11, 12). In Iraq, a decoction of this plant is reported as a diuretic (13), anti-anasarca, genito-urinary disorder control (14), and anti-cystitis (15). Besides, *Mimosa pudica* (MP) is recognized worldwide for its pharmacological activities such as

***Corresponding author:** Adesanya Olamide Adewale**,** Department of Anatomy, Faculty of Basic Medical Sciences, Olabisi Onabanjo University-Ikenne Campus, Ikenne, Nigeria. Email: oaesanya@gmail.com

antidiabetic, antitoxin, antioxidant, wound healing (16), and aphrodisiac (15) activities. All these medicinal and phytotherapeutic potencies are attributed to the plants' natural chemicals such as alkaloid, glycoside, flavonoid, and tannin (17, 18). Therefore, this study investigated the effects of *Cynodon dactylon* and *Mimosa pudica* ethanolic extract mixture on induced benign prostate hyperplasia (IBPH), testes, and caudal epididymal sperm count to find medicinal plant substances that can ameliorate enlarged prostatic hyperplasia using a male Wistar rat animal model.

Materials and methods

Animals

Wistar male rats (150±30g) used for this study were obtained from the Animal Colony of the Department of Animal Sciences, University of Ibadan, Nigeria. The rats were acclimatized in the wooden cages for two weeks before the experiment with unrestricted access to feed and water.

Plants and extract preparation

Fresh leaves of *Cynodon dactylon (CD)* and *Mimosa pudica (MP)* were collected from Ikenne and were air-dried in a well-ventilated room. About 50 g each of the air-dried leaves were blended together and then soaked into 200 ml of 95% ethanol. The solution was left for 48 hours, after which it was filtered. A semi-solid grayish dark oily extract (CDMP ethanolic extract) was obtained by evaporating ethanol with a rotary evaporator. The extract was diluted with corn oil to get the stock solution used for animal treatment which was kept in the refrigerator until further use. Ethical approval was obtained from the Olabisi Onabanjo University, Faculty of Basic Medical Sciences ethical committee (OOU/FBMS/ANA/O1/19/VOL.II/08).

Hormone treatment

Testosterone propionate (T), brand name: Ricostrone (25 mg/ml vial) and estradiol valerate (E2), brand name: Puregynon Depot (10 mg/ml vial) were purchased from Greenfield Pharma, Jiangsu Co Ltd., China and Medipharm Ltd., 108-Kotlakhpat Industrial Estate; Lahore, India, licensed by Schering Ag, Germany, respectively. These two synthetic hormones were diluted with corn oil to get the desired concentrations which were later used for prostate enlargement induction at a dose of 3mg T and 0.8mg E2 per Kg BW as described by Adesanya et al. (10). This was administered to the rats for three weeks subcutaneously in the inguinal region after which a few rats were sacrificed and inspected for the gross examination of prostate enlargement.

Experimental procedure and data collection

Five untreated Wistar male rats were used as normal control (group A). Fifteen IBPH Wistar male rats were randomized into three groups (n=5 rats in each group) of IBPH control (group B), low dose (LD) CDMP-treated IBPH (group C) and high dose (HD) CDMP- treated IBPH (group D) groups. Groups A and B received oral administration of distilled water, while IBPH rats in groups C, and D received oral administration of 0.46 g/ml (LD) and 0.92 g/ml (HD) stock solution of CDMP extract per Kg BW, respectively for four weeks. After the 4-week experimental period, the animals were anesthetized using chloroform and were dissected. Blood samples were collected from each animal through the cardiac puncture into plain collection bottles. Each blood sample was centrifuged at 1000g for 10 mins and decanted to obtain a clear serum. The serum was kept in the refrigerator, after which it was taken to the laboratory for analysis. The caudal epididymis was carefully dissected out and minced in 1ml of normal saline solution for sperm count evaluation. The prostate was excised, blotted, weighed, and transferred into a universal bottle containing 10% neutral formalin solution prior to the histological assessment.

Assays for prostate-specific antigen (PSA)

Prostate-specific antigen levels were determined with the PSA ELISA kit (Rapid Labs Limited, Colchester Essex, UK) according to the manufacturer's instructions. The absorbance was measured at 450 nm using a microplate ELISA reader (BIO-Rad Laboratories Inc). The values were expressed as ng/ml.

Tissue processing

Each prostate fixed in 10 per cent neutral formalin solution was trimmed, washed, and dehydrated in ascending alcohol grades. After the complete fixation, the blocks were embedded in paraffin and sectioned in the form of a ribbon at 5µm. The sectioned tissue was then stained with Hematoxylin-eosin dye. The slides were mounted in Canada balsam. The microscopic examination of the sections was then carried out under a light microscope.

Epididymal sperm count

The caudal epididymis was dissected out and minced in 1ml of normal saline solution. Then, the Neubauer's chamber was charged with a drop of the mixture and the sperms counted using 10x magnification.

Statistical analysis

Data were expressed as mean ± standard deviation (SD). Statistical analysis is done by one-way analysis of variance using GraphPad prism 6, followed by a Tukey's test for multiple comparisons. p-Values $\leq$ 0.05 were considered statistically significant.

Results

PSA decreased significantly ($p<0.05$) in both low dose (LD) and high dose (HD) CDMP treated IBPH groups (groups C and D; 2.40 and 2.20 ng/ml), compared to IBPH untreated group (group B; 8.75 ng/ml). The normal control group (group A) value was 3.75ng/ml (Figure 1).

The prostate's relative weight was high in the IBPH untreated control group, but oral administration of CDMP ethanolic extract significantly reduced the prostate's relative weight at low and high doses (Figure 2). Furthermore, the relative weight of the testis was significantly ($p<0.05$) increased in both LD- and HD-treated groups compared to groups A and B (Figure 3).

Figure 4 shows that the epididymal sperm count was high in the control group (49.4 million/ml) compared to IBPH groups. A significant ($p<0.05$) high sperm count was recorded in LD, and HD CDMP treated groups (5.1 and 9.6 million/ml respectively) compared with the IBPH-control group (2.7 million/ml).

The prostate of rats in the normal control group is shown in figure 5. There is an appearance of normal epithelial and stromal cells. Also, the presence of corpora amylacea is visible (PC). Figure 5B shows the prostate of group B rat with hypertrophy of the epithelium and inter-acinar fibromuscular tissue, and absence of corpora amylacea in the enlarged glandular acinar. BPH induction led to the thickening of the stromal cells and narrowing of the lumen. In contrast, oral

Journal of Infertility and Reproductive Biology
J Infertil Reprod Biol, 2021, Volume 9, Issue 1, Pages: 22-26. https://doi.org/10.47277/JIRB/9(1)/22

CDMP ethanolic extract caused thinning of the stromal and further narrowing of the prostate lumen (Figure 5C and 5D). Considerable prostate stromal cell degeneration (FT) and attenuation of BPH were observed in the low dose group (group C).

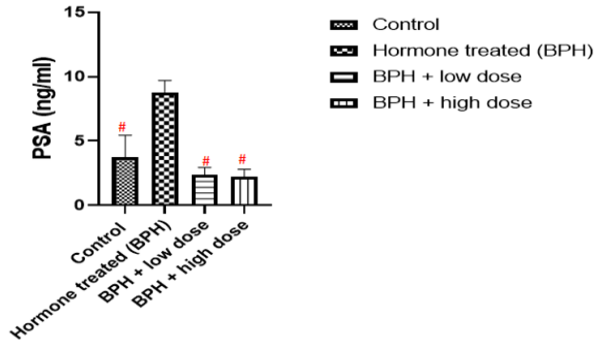

Figure 1. Effects of oral administration of CDMP ethanolic extract on prostate PSA levels (ng/ml) in control and BPH rats

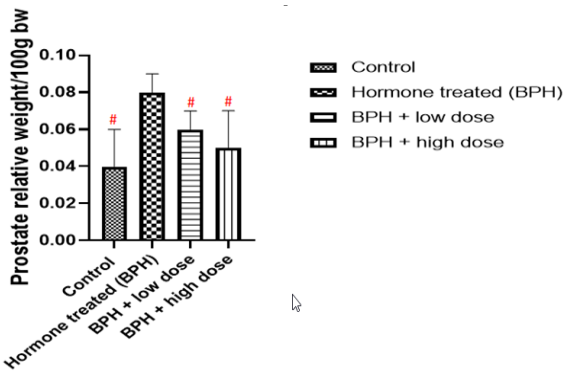

Figure 2. Effects of oral administration of CDMP ethanolic extract on relative weight prostate in control and BPH rats

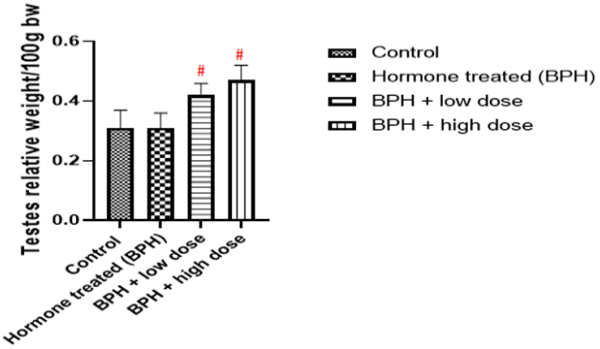

Figure 3. Effects of oral administration of CDMP ethanolic extract on the relative weight of testes in control and BPH rats

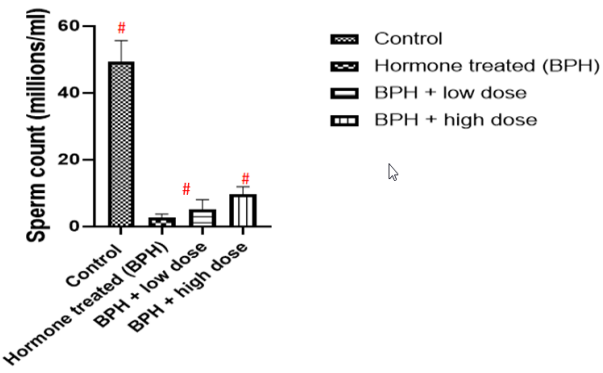

Figure 4. Effects of oral administration of CDMP ethanolic extract on caudal epididymal sperm count (millions/ml) in BPH rats

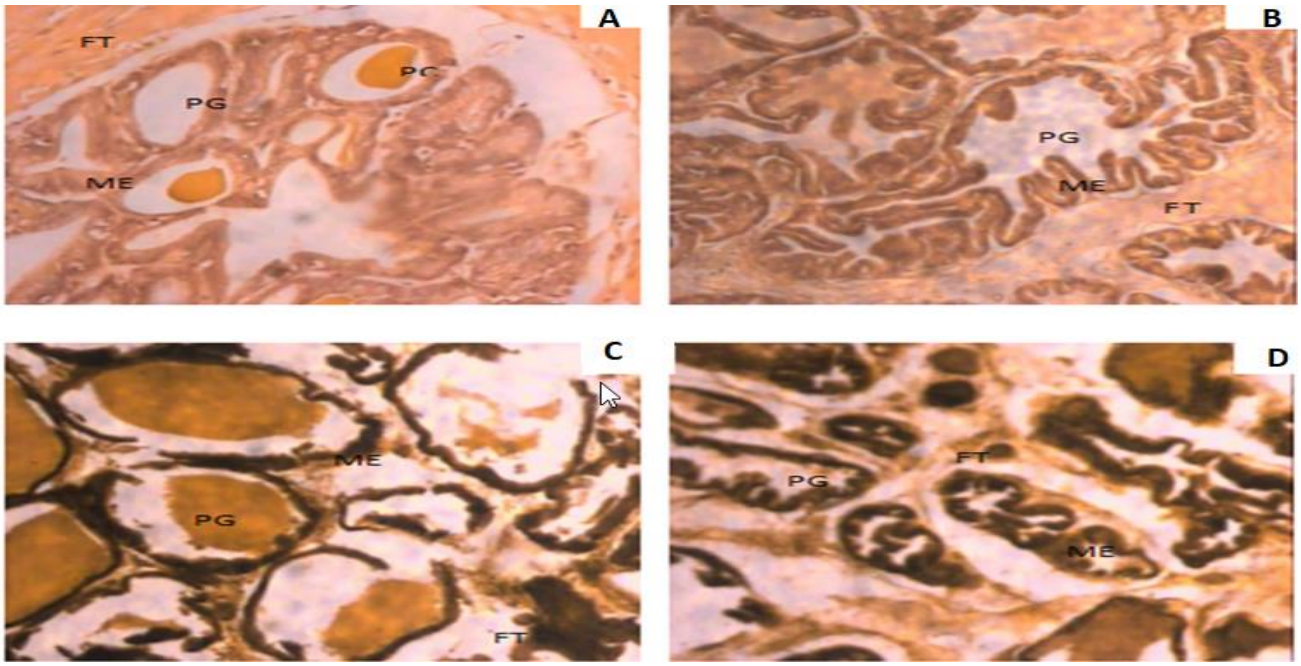

Figure 5. Micrograph of the prostate (H&E -X720). A: Control, B: Induced BPH, C: BPH treated with a low dose of CDMP ethanolic extract, D: BPH treated with a high dose of CDMP ethanolic extract. PG-Prostatic gland; PC-Prostatic concretion; FT-Fibromuscular tissue; ME-Mucosa Epithelium

Discussion

The use of plant-derived non-nutritive compounds with protective or disease-preventive properties for urinary symptoms with BPH has gained widespread interest, majorly because of reduction in side effects, and desire to maintain control over their treatment (8, 19, 20). Phytochemicals such as antioxidant present in some medicinal plants play a huge role in countering the process of oxidative stress (21). Inhibition of 5α-reductase enzymes was hypothesized as part of the mechanisms that may affect BPH. In the present study, the amount of PSA which is the standard test for BPH, prostate cancer, and other prostate conditions was significantly lowered by the CDMP ethanolic extract, thus, confirms the potency of this herbal mixture in the management of induced BPH. A decreased in PSA level may be associated with improved BPH conditions via the inhibition of the 5α-reductase enzyme. The 5α-reductase enzyme is responsible for the conversion of testosterone (T) to the active form dihydrotestosterone (DHT). The blockage of this enzyme indirectly put a check on the abnormal growth of the prostate (20). Increased prostate relative weight can be used as a vital indicator of successful BPH induction (21, 10). The relative prostate weights or prostate indices are often used as reliable markers of BPH development (22, 23). The significant increase in the relative weight of the prostate in the IBPH model group compared to the normal control observed in this study confirmed the successful induction of BPH. Moreover, a significant increase in the weight of the prostate in the IBPH untreated group compared with the CDMP treated IBPH groups affirmed that the CDMP extract was able to reduce the enlargement of induced prostate. The observed effects may be due to the presence of antioxidants in the CP, which counteracts the oxidative stress caused by the hormone of BPH induction. Also, phytosterol present in MP may be the cause of the effects seen. Plant sterols are chemically related to cholesterol, and they are also inhibitory to the 5α-reductase enzyme in the prostate, thereby reducing the prostate weight (24).

The relative weight of the testis was not affected by the hormone administration used in the induction of BPH; however, the CDMP treatment caused a significant increase in testicular weight of the treated induced BPH animals. This testicular weight gain is due to the positive effect of the CDMP herb on the testicular activity, which may be due to increased fluid secretion by the Sertoli cell and increase in tubular fluid content that is usually accompanied by an increase in the diameter of the tubular lumen (25).

The exogenous supply of testosterone and estrogen used for the induction of BPH caused the suppression of gonadotropins (LH and FSH) to levels below that required to maintain spermatogenesis. As spermatogenesis is dependent on high intra-testicular testosterone concentration and the action of FSH on the Sertoli cells, the outcome was a significant reduction in the sperm count as observed in all groups treated with hormones. The decrease in LH leads to marked suppression of testosterone production by the Leydig cells; the decrease in intra-testicular testosterone coupled with suppression of FSH leads to a decrease in Sertoli cell function required for germ cell maturation and survival (26). Low intratesticular testosterone levels result in decreased proliferation of spermatogonia, accelerated apoptosis, defects in spermiation and disorganization of steps leading to the production of mature spermatozoa by Sertoli cells (27). However, the administration of the CDMP herb counteracts the effect of the hormone used in the induction of BPH by increasing the sperm count at both high and low doses, although the increase was not significant compared to normal control.

BPH animals showed epithelial hyperplasia and fibromuscular tissue with an increase in epithelial thickness compared with the normal control animals. In contrast, CDMP-treated animals showed a reduction in epithelial hyperplasia with a decrease in epithelial thickness. Based on these results, CDMP treatment effectively inhibited the prostatic hyperplasia induced by testosterone and estradiol. Several medicinal plants have been investigated for their ability to shrink prostates in experimentally induced BPH. The mechanisms of their action range from blocking the 5-alpha-reductase enzyme to possessing phytoestrogens and phytosterols that have been reported to block the action of 5-alpha-reductase enzymes, required for the conversion of T to DHT. DHT is the major culprit in the development of this condition (28, 29).

Conclusion

The findings of this research show that oral administration of ethanolic extract of *Cynodon dactylon* and *Mimosa pudica* combination in a BPH rat model may effectively inhibit the BPH development and may be useful in the treatment of BPH patients. Further studies are required to carefully investigate the metabolites responsible for the observed effects and mechanism for the actions.

Acknowledgement

We wish to appreciate our chief technology in the Department of Anatomy, Mr Onafowora Bola, for his assistance in preparing the slides used in this study.

Funding

No organizations have funded this research.

Conflict of interest

None declared

References

1. Saito M, Tsounapi P, Oikawa R, et al. Prostatic ischemia induces ventral prostatic hyperplasia in the SHR; possible mechanism of development of BPH. Scientific Reports. 2014;4:3822.1-7.
2. Bostanci Y, Kazzazi A, Momtahen S, et al. Correlation between benign prostatic hyperplasia and inflammation. Current Opinion in Urology. 2013;23:5-10
3. Sáez C, González-Baena AC, Japón MA, et al. Regressive changes in fnasteride-treated human hyperplastic prostates correlate with an upregulation of TGF-beta receptor expression. Prostate. 2011;37:84-90.
4. Tract Symptoms/Benign Prostatic Hyperplasia Guidelines Committee. Singapore urological association clinical guidelines for male lower urinary tract symptoms/benign prostatic hyperplasia. Singapore medical journal. 2017;58(8):473-480.
5. Kim HK, Zhao C, Choi BR, et al. Is transforming growth factor-beta signaling activated in human hypertrophied prostate treated by 5-alpha reductase inhibitor? Disease Markers. 2013;35(6):679-685.
6. Minutoli L, Rinaldi M, Marini H. et al. Apoptotic pathways linked to endocrine system as potential therapeutic targets for benign prostatic hyperplasia. International Journal of Molecular Sciences. 2016;17:1311.
8. Arora RP, Nayak RL, Malhotra V, Mohanty NK. Role of herbal drugs in the management of benign prostatic hyperplasia: Clinical trial to evaluate the efficacy and safety of Himplasia. Med Update. 2003;11:55-58.

9. Caesar LK, Cech NB. Synergy and antagonism in natural product extracts: when 1+ 1 does not equal 2. Natural product reports. 2019;36(6):869-888.
10. Adesanya AO, Olaseinde OO, Oguntayo OD, et al. Effects of Methanolic Extract of *Citrullus lanatus* Seed on Experimentally Induced Prostatic Hyperplasia. European Journal of Medicinal Plants. 2011;1(4):171-179.
11. KOBASI H. Dictionary of Herbs and Medicinal plants. Scientific Books House, Beirut, Lebanon, (1993):Pp 566.
12. Rizk AM, El-ghazaly GA. Medicinal and poisonous plants of Qatar. Scientific and applied research centre, university of Qatar. 1995;50-150.
13. Boulos L. Medicinal plants of North Africa. Reference publication Inc. Algonac, Michigan USA (1983).
14. Al-Rawi A, Chakravarty HL. Medicinal plants of Iraq. National herbarium of Iraq. Ministry of agriculture. Baghdad-Iraq. 1964.
15. Duke JA, Wain KK. Medicinal plants of the world. Computer index with more than 85000 entries. Handbook of Medicinal Herbs. 1981:96.
16. Ahmad H, Sehgal S, Mishra A, Gupta R. Mimosa pudica L.(Laajvanti): an overview. Pharmacognosy reviews. 2012;6(12):115.
17. Muhammad G, Hussain MA, Jantan I. A high-value medicinal plant as a source of bioactives for pharmaceuticals. Comprehensive Reviews in Food Science and Food Safety. 2016;15(2):303-315.
18. Thompson IM. : Pharmacologic agents in complementary medicine in prostatic. *Drugs Today*. 2001;37:427-433.
19. Roehrborn CG. The epidemiology of acute urinary retention in benign prostatic hyperplasia. Reviews in urology. 2001;3(4):187.
20. Nozhat F, Alaee S, Behzadi Kh, Azadi Chegini N. Evaluation the possible toxic effects of spearmint (*Mentha spicata*) on the reproductive system, fertility and number of offspring of adult male rats. Avicenna Journal of Phytomedicine. 2014;4(6):420-429.
21. Adesanya OA, Shittu LAJ, Awobajo FO, et al. Knowledge and Practice of Prostate Health of Men in the Work Place. Journal of Infertility and Reproductive Biology. 2013;1(2):54-57.
22. Pais P. Potency of a novel saw palmetto extract, SPET-085, for inhibition of 5 alpha-reductase II. Advances in Therapy. 2010;27:555-563.
23. Arruzazabala ML, Ma R, Molina V, et al. Effect of D-004, alipid extract from the fruits of Cuban royal palm, on the atypical prostate hyperplasia induced with phenylephrine in rats. Drugs in R&D. 2006;7:233-241.
24. Veeresh BSV, Veeresh B, Patill AA, et al. Lauric acid and myristic acid prevent testosterone induced prostatic hyperplasia in rats. European Journal of Pharmacology. 2010;625:262-265.
25. Bent S, Kane C, Shinohara K, et al. Saw Palmetto for Benign Prostatic Hyperplasia. New England Journal of Medicine. 2006;354:557-566.
26. Creasy DM, Chapin RE. Male reproductive system. Haschek and Rousseaux's handbook of Toxicologic Pathology. 2013;1:2493-598.
27. Wang C, Swerdloff RS. Hormonal Approaches to Male contraception. Current Opinion Urology. 2010;20(6):520-524.
28. Shin IS, Lee MY, Ha HK, et al. Inhibitory effect of Yukmijihwang-tang, a traditional herbal formula against testosterone-induced benign prostatic hyperplasia in rats. BMC Complementary and Alternative Medicine. 2012;12(1):1-7.
29. Afriyie DK, Asare GA, Bugyei K. Treatment of benign prostatic hyperplasia with Croton membranaceus in an experimental animal model. Journal of Ethnopharmacology. 2014;157:90-98