**Effects of hydromethanolic leaf extract of *Englerina* on Benign Prostatic hyperplasia in Experimental Animal Models**

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**Abstract**

This study was aimed at investigating the effects of hydromethanolic leaf extract of *Englerina* *Gabonensis* (Mistletoe) on Benign Prostate hyperplasia (BPH) in experimental animal models. Hydromethanolic extraction was done. 46 adult male *wistar* rats were used and assigned into 6 groups. Group1 received 1ml of distilled water daily for 45days, Group2 received 0.32mls of Testosterone and 0.2mls Estradiol thrice weekly, Group 3 received 300mg/kg of extract daily, Group 4 received 0.32mls Testosterone and 0.2mls Estradiol + 150mg/kg of extract, Group5 received 0.32mls Testosterone and 0.2mls Estradiol + 300mg/kg of extract, Group 6 received 0.32mls Testosterone and 0.2mls Estradiol + 5mg/kg Finasteride. BPH was induced 3times weekly for 21 days and extract was administered daily for 24 days. At day45 of the experiment, rats were anaesthetized using chloroform, and blood sample and prostate tissue were collected for analysis. Serum from the blood was used for analysis of Prostate specific antigen (PSA) and Testosterone, while prostate tissue was used for analysis of Oxidative Stress markers (Catalase enzyme (CAT), Glutathione reductase (GSH), Superoxide dismutase (SOD), Malondialdehyde (MDA) and histology. The result showed no significant decrease (p >0.05) in body weight, a significant decrease (p <0.05) in prostate weight, PSA, MDA, and a significant increase (p <0.05) in testosterone, SOD, GSH and CAT (p <0.05) in the BPH group due to extract administration.

**Introduction**

Benign prostatic hyperplasia (BPH) also known as benign prostatic hypertrophy has commonly been diagnosed among aged population with increasing prevalence (Chughtai ***et al,*** 2016). This disease causes lower urinary tract symptoms (LUTS) which may be categorized to be either obstructive or irritative symptoms. Obstructive symptoms such as prolonged micturition, feeling of incomplete bladder emptying, dribbling and irritative symptoms such as urgency, incontinence and nocturia (Herbert, 2005). So many studies have been carried out by researchers to mitigate the effect of this disease.

For example, A Study which was aimed at investigating the effect of saw palmetto on benign prostatic hyperplasia was carried out by Bent, ***et al,*** (2009). In his research, He considered assigning 25 men over the age of 49 years who had moderate to severe symptoms of benign prostatic hyperplasia to one year of treatment with saw palmetto extract (160 mg twice a day) of placebo. Primary outcome measures were changes in the scores on the American Urological Association Symptom Index (AUASI) and the maximal urinary flow rate were assessed and Secondary outcome measures such as changes in prostate size, residual urinary volume after voiding, quality of life, laboratory values and rate of reported adverse effects were also assessed. It was documented that there was no significant difference between the saw palmetto and placebo groups in the change in AUASI score, prostate size, residual volume after voiding, quality of life, or serum prostate specific antigen levels during the one-year study. He concluded that saw palmetto did not improve symptoms of benign prostate hyperplasia.

Another study carried out by Zerafatjou ***et al,*** (2021). which was aimed at investigating the effect of Pumpkin seed oil (*Cucurbita pepo*) versus tamsulosin for benign prostatic hyperplasia symptom relief. He considered evaluating patients with BPH 50 years. In his method, Patients were also randomized into two groups. One group received 0.32 mg tamsulosin every night at bedtime and the other received 360 mg pumpkin seed oil twice a day. Patients age, height, weight and body mass index (BMI) were recorded. International prostate symptom score (IPSS) was filled out by the patients at baseline and then 1 month and 3 months after initiation of treatment. BPH associated quality of life (QoL), serum prostate specific antigen, prostate and post void residual volume, and maximum urine flow were also assessed at baseline and 3 months later. He documented that there was no significant decrease in IPSS and a significant improvement in QoL in both groups. He concluded that pumpkin seed oil relieved BPH symptoms with no side effects but was not as effective as tamsulosin.

Many of these scientists further recommends that more research should be carried out to find a better and more effective way of treating benign prostatic hyperplasia.

**Materials and Methods**

**Collection of Plant Materials**

150kg of fresh Leaves of *Englerina Gabonensis* was obtained from Khana Local Government in Port Harcourt, River State Nigeria and authenticated with hebarium number UPH/P/278 in the department of Plant Science and Biotechnology, Faculty of Science, University of Port Harcourt, Nigeria.

**Preparation of plant material and extraction**

Leaves were washed to remove dirt, and dried using ultraviolent light. The plant was later grinded into powdered form using a machine.

**Method of extraction of plant materials**

This was done using Maceration Method. Active ingredients using Hydromethanolic solvent (30:70) ratio (H20: Methanol).

**Study Design**

A total of forty six adult male *wistar* rats weighing from 150-205g were used. The animals were obtained from animal house in the Department of Physiology Faculty of Basic Medical Sciences, College of Health Science, University of Port Harcourt, Nigeria.

The 46 *wistar* rats were grouped into 6, with 7 rats in group1 and 6, and 8 rats in group2 to group5.

Study1 : One animal each from the induced group (2,4,5,6) were used to determine prostate weight and all the animals in the group were used to determine body weight after induction of Benign prostatic hyperplasia.

Study2: Six animals each from group1 and group6, and seven animals each from group2 to group5 were used in determination of body weight and prostate weight, oxidative stress biomarkers (SOD, MDA, GSH, CAT), testosterone and Prostate Specific Antigen and Histology after treatment with extract.

**Testosterone and Estradiol administration and preparation**

Rats were induced with benign prostatic hyperplasia by subcutaneous injection of testosterone and estradiol in staggered doses (three times a week respectively) for three weeks (Mbaka ***et al,*** 2017).

The table below shows the induced groups and the extract treated group

**Table1**

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
| **Treatment Groups** | **Group Name** | **Number of rats** | **Administration** | **Dose/day** |
| 1 | Control | 6 | Distilled water | 1ml daily |
| 2 | BPH induced | 7 | Testosterone and Estradiol | 0.32ml and 0.2ml |
| 3 | Extract Only | 7 | *EnglerinaGabonensis* | 300mg/kg daily |
| 4 | BPH + Extact | 7 | Testosterone and Estradiol + *Englerina Gabonensis* | 0.32ml and 0.2ml + 150mg/kg daily |
| 5 | BPH + Extract | 7 | Testosterone and Estradiol + *Englerina Gabonensis* | 0.32ml and 0.2ml + 300mg/kg daily |
| 6 | BPH + Finasteride | 6 | Testosterone and Estradiol +Finasteride | 0.32ml and 0.2ml + 5mg/kg daily |

**Determination of body weight and prostate weight of the animals after induction of benign prostatic hyperplasia**

The body weight and prostate weight of animals were determined on the final day (Day 21) of benign prostatic hyperplasic induction between 10:00a.m to 12:00pm.

Animals were placed on a weighing balance and their weights were recorded. Animals were anaesthetized with chloroform and then dissected with surgical blade. The prostate gland was excised and weighed and values were recorded.

**Extract administration and preparation**

Extract was administered once daily for 24days by oral route according to the method of (Ofem ***et al,*** 2014).

**Sacrificing of the animals and sample collections**

Rats were sacrificed after 24 days of extract administration after anaesthesia with chloroform. Blood samples and prostate gland were collected and preserved for analysis.

**Determination of testosterone and prostate specific antigen**

Enzyme linked immunoassay technique was used for the quantitative determination of testosterone concentration and PSA evaluation.

**Determination of malondialdehyde (MDA)**

Lipid peroxidation was determined by the method of Ohkawa and Ohishi (1979).

**Determination of catalase**

Catalase enzyme (CAT) was determined by the method of Goth, (1991)

**Determination of gluthathione**

Reduced glutathione (GSH) was determined by the method of (Ellman, 1959)

**Determination of superoxide dismutase**

Superoxide dismutase(SOD) was determined by the method of (Kakkar ***et al,*** 1984)

**Tissue histology and preparation**

The prostate tissue histology was done using the method of (Mbaka ***et al,*** 2014).

**Time frame**

This research work was done in the animal house Department of Human physiology, University of Port Harcourt from November 2020 to February 2021

**Ethical and environmental consideration**

Ethical approval was done by the University of Port Harcourt Research Ethics Committee at its 78th meeting held on Thursday, 15th July, 2021. UPH/CEREMAD/REC/MM78/035.

**Statistical analysis**

Statistical analysis was carried out using statistical package for social science software (SPSS version 20.0) Difference between groups was evaluated using ANOVA test (analysis of variance) with mean and standard.

**Results and Discussion**

**The Effects of *Englerina gabonensis* on Prostate weight in BPH induced male *wistar* rats.**

The results for the effect of *Englerina gabonensis* on prostate weight (Table2) showed significant decrease (p < 0.05) in the test group 3, 4, 5 and 6 compared to the induced group. There was a significant decrease (p < 0.05) in test group2 and 6 compared to control group. No significant decrease ( p > 0.05) in test group3 and 5 compared to control group and non significant increase (p > 0.05) in test group4 compared to control group

**Table2 The Effects of *Englerina gabonensis* on Prostate weight in BPH induced male *wistar* rats.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment Groups** | **Group name** | **Prostate weight (Day1) (g)** | **Prostate weight (Day25) (g)** |
| Group 1 | Control | 0.4 | 0.4±0.03 |
| Group 2 | BPH induced | 0.8 | 0.74±0.05a |
| Group 3 | Extract (300mg/kg) | 0.3 | 0.27±0.03b |
| Group 4 | BPH+Extract (150mg/kg) | 0.6 | 0.321±0.03b |
| Group 5 | BPH+Extract (300mg/kg) | 0.5 | 0.29±0.03b |
| Group 6 | BPH+Finasteride | 0.7 | 0.25±0.02a,b |

Values are Mean ± SEM, n=6 and n=7,a= Significant difference compared to control at p<0.05, BPH = Benign prostate hyperplasia,b=Significant difference compared to BPH group at p<0.05.

**The Effects of *Englerina gabonensis*on Prostate specific antigen and Testosterone in BPH induced male *wistar* rats.**

The results for the effect of *Englerina gabonensis* on Testosterone (Table3) showed significant increase (p < 0.05) in test group4, 5 and 6 compared to the induced group and control group.There was no significant increase (p > 0.05) in test group 3 compared to the control group and induced group. There was no significant increase (p > 0.5) in test group4 compared to control group and non significant decrease (p > 0.05) in test Group2 compared to control group.

The result for the effect of *Englerina gabonensis* on prostate specific antigen showed significant decrease (p < 0.05) in the test group 3, 4, 5compared to the induced group and control group. There was no significant increase ( p > 0.05) in test group 6 compared to control group.

**Table3 The Effects of *Englerina gabonensis* on Prostate specific antigenand Testosterone in BPH induced male *wistar* rats.**

|  |  |  |  |
| --- | --- | --- | --- |
| **Treatment Groups** | **Group name** | **Testosterone (Day25) ng/ml** | **Prostate specific antigen**  **(Day 25) ng/ml** |
| Group 1 | Control | 4.78±1.40 | 0.79±0.03 |
| Group 2 | BPH induced | 0.324±0.07 | 1.81±0.23a |
| Group 3 | Extract (300mg/kg) | 5.27±0.34 | 1.26±0.06a,b |
| Group 4 | BPH+Extract (150mg/kg) | 7.10±0.53b | 1.15±0.04a,b |
| Group 5 | BPH+Extract (300mg/kg) | 15.98±3.41a,b | 1.19±0.05a,b |
| Group 6 | BPH+Finasteride | 19.11±3.50a,b | 0.90±0.06b |

Values are Mean ± SEM, n=6 and n=7, a= Significant difference compared to control, BPH = Benign prostate hyperplasia, b=Significant difference compared to BPH group at p<0.05

**The Effects of *Englerina gabonensis*on Oxidative stress markers in BPH induced male *wistar* rats.**

The results for the effect of *Englerina gabonensis* on Malondialdehyde (Table4) showed a significant decrease (p < 0.05) in the test group 4, 5 and 6and non significant decrease in test group3 (p > 0.05) compare to the induced group. There was a significant increase (p < 0.05) in test Group2, 3 and 6 compared to control group and non significant increase (p > 0.05) in test group 4 and 5 compared to control group.

The results for the effect of *Englerina gabonensis* on Catalase enzyme (Table4) showed no significant increase in test group 4, 5 and 6 (p > 0.05) compare to the induced group and no significant decrease in group4 and 5 compared to the control group. There was a significant increase (p < 0.05) in test Group2, 3 and 6 compared to control group and non significant increase (p > 0.05) in test group 4 and 5 compared to control group. There was a significant decrease (p < 0.05) in Group2 and Group6 compared to control group

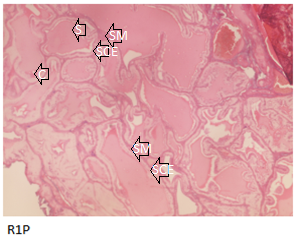
The results for the effect of Englerina gabonensis on Glutathione reductase (Table4) showed significant increase (p < 0.05) in test group 3, 4, 5 and 6 compared to induced group and non significant decrease (p > 0.05)in test group4 compared to the control group. There was a significant decrease (p < 0.05) in test Group2,3,5 and 6 compared to control group.

The results for the effect of *Englerina gabonensis* on Superoxide dismutase (Table4) showed no significant increase (p < 0.05) in test group 3 and 4 compared to induced group and a significant increase (p < 0.05) in test group 5 and 6 compared to induced group and control group. There was no significant decrease in test Group2 and 3 compared to control group.

**Table4 The Effects of *Englerina gabonensis* on Oxidative stress markers in BPH induced male *wistar* rats.**

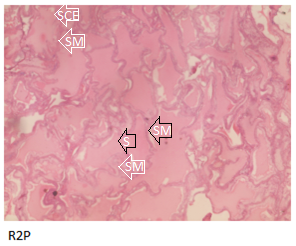
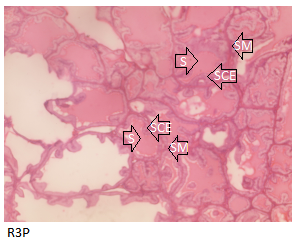
|  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- |
| **Treatment Groups** | **Group name** | **MDA**  **(Day25) /ml** | **CAT**  **(Day 25) U/ml** | **GSH**  **(Day25) red(nmol/L)** | **SOD**  **(Day 25) U/ml** |
| Group 1 | Control | 4.30±0.29 | 52.28±17.25 | 108.99±6.25 | 80.00±3.36 |
| Group 2 | BPH induced | 11.67±0.07a | 13.01±1.47a | 40.64±4.08a | 68.49±5.52 |
| Group 3 | Extract (300mg/kg) | 9.41±0.86a | 51.29±4.57b | 91.90±3.05a,b | 71.19±5.52 |
| Group 4 | BPH+Extract (150mg/kg) | 6.05±0.60b | 32.24±10.36 | 94.45±4.08b | 84.24±4.17 |
| Group 5 | BPH+Extract (300mg/kg) | 6.25±0.61b | 26.24±7.53 | 89.11±1.60a,b | 90.00±2.52b |
| Group 6 | BPH+Finasteride | 7.29±0.58a,b | 51.46±14.36a | 90.56±2.89a,b | 90.56±2.81b |

Values are Mean ± SEM, n=6, n=7,a= Significant difference compared to control, BPH = Benign prostate hyperplasia, b=Significant difference compared to BPH group at p<0.05, CAT=Catalase enzyme, MDA= Malondialdehyde, SOD=Superoxide dismutase, GSH=Glutathione reductase.

**The Effects of *Englerina gabonensis* on Prostate tissue histology in BPH induced *wistar* rats** 

BPH induced (Group2)

Extract Only(300mg/kg) Group3

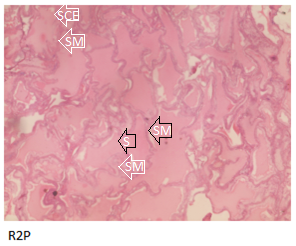
 

Photomicrograph of cross section of normal prostate gland indicating interglandular smooth muscle fibers (SM), interglandular simple columnar epithelium (SE) and glandular stroma (S). (H&E stain). Mag x100.

Photomicrograph of cross section of prostate gland of test Group2 indicating a thin strip of interglandular smooth muscle fibers (SM), extensive glandular hyperplasia (S) and a thin simple columnar epithelia lining (SCE)(H&E stain). Mag x100 compared to the control group. While test Group3 indicating reduced glandular hyperplasia (S), thick interglandular simple columnar epithelial (SCE) and thick interglandular smooth muscle fibers (SM)(H&E stain). Mag x100 compared to control group.

**Plate2 Effects of *Englerina Gabonensis in* on Prostate tissue histology in BPH induced *wistar* rats.**

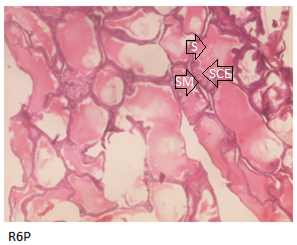
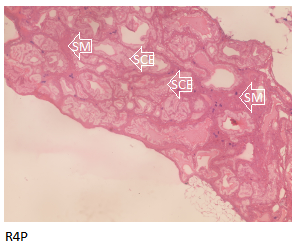
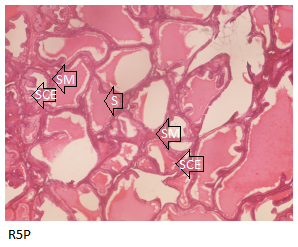
BPH induced (Group2)



BPH + Finasteride (Group6)

BPH + Extract (300mg/kg) Group5

BPH + Extract (150mg/kg) Group4



Photomicrograph of Prostate gland. Mag. X100 H&E. Simple columnar epithelium (SCE), Smooth muscles (SM), Secretions (S), and concretions (C). BPH = Benign prostate hyperplasia.

The image shows a decrease in the number of SM and SCE in group4, 5 and 6 due to extract and finasteride treatment compared to the BPH induced group2.

Photomicrograph of cross section of prostate gland of test Group2 indicating a thin strip of interglandular smooth muscle fibers (SM), extensive glandular hyperplasia (S) and a thin simple columnar epithelia lining (SCE). (H&E stain). Mag x100.

Photomicrograph of cross section of prostate gland of test Group4 indicating reduced glandular hyperplasia (S), large interglandular simple columnar epithelial (SCE) and thick interglandular smooth muscle fibers (SM) (H&E stain). Mag x100. Compared to induced Group2. Test Group5 indicating reduced glandular stroma (S), thick interglandular simple columnar epithelial (SCE) and thick interglandular smooth muscle fibers (SM) (H&E stain). Mag x100 compared to induced Group2. test Group6 indicating reduced glandular stroma (S), largeinterglandular simple columnar epithelial (SCE) and thick interglandular smooth muscle fibers (SM) (H&E stain). Mag x100 compared to induce Group2.

**Discussion**

**Effects of *Englerina gabonensis* on Prostate weight in BPH induced *wistar* rats.**

**Prostate weight**

Prostate weight in benign prostatic hyperplasia is very important to note as Benign prostatic hyperplasic conditions have been characterized with prostate hypertrophy. The result from this study showed that the extract *Englerina gabonensis* caused a significant decrease (p < 0.05) in prostate weight which agrees with the study of (Kavya, ***et al,*** 2021). The decrease may be associated with the Phytochemical constituent (Polyphenols) of the extract. The mechanism of action may be due to the preventive ability of Polyphenols against prostate inflammation. Clinical study of 5α-reductase inhibitors reported that patients with inflammatory findings on prostate biopsy specimens had higher prostate volumes. Cytokines derived from inflammatory cells also induce growth factors. Among them, interleukin (IL)-1α induces fibroblast growth factor (FGF)-7, and the IL-1α-related system is involved in proliferation (Kensuke***et al****,* 2021). Polyphenols inhibits prostate inflammation which may result to a decrease in prostate volume, leading to a decrease in prostate weight.

**Effects of *Englerina gabonensis* on Testosterone and Prostate specific antigen in BPH induced *wistar* rats**

**Testosterone**

Testosterone is an important steroid hormone required in the development of benign prostatic hyperplasia. In benign prostatic hyperplasic conditions, testosterone has been documented to be low as more of it has been converted to dehydrotestosterone (DHT). From the result of this study, the extract *Englerina gabonensis* caused a significant increase (p < 0.05) in testosterone which agrees with the study of (Ofem ***et al,*** 2014). This result can be associated to their Phytochemical (Polyphenols) constituent as Polyphenols have been documented to increase the level of testosterone ((Beigip ***et al,*** 2017). The mechanism of action can be linked to Polyphenols having the ability to inhibit steroidogenic enzymes. In the case of benign prostatic hyperplasia, they inhibit 5alpha reductase enzyme, which is an enzyme that catalyzes the conversion of testosterone to dehydrotestosterone (DHT). DHT accumulation in the prostate gland leads to hyperplasic or hypertrophic conditions. Therefore Polyphenols act by increasing testosterone level and decreasing dehydrotestosterone level.

**Prostate specific antigen**

Prostate specific antigen test have been used as an important biomarker for prostate cancer. In benign prostatic hyperplasic conditions, prostate specific antigen also plays an important role as more PSA are produced in this case. The result from this study showed that the extract *Englerina gabonensis* caused a significant decrease (p < 0.05) in Prostate specific antigen which is a new finding from this study. This result may not directly be associated with the Phytochemical constituent (Polyphenols) of the extract as other studies have documented that Polyphenols increases the level of PSA in men (Clin ***et al,*** 2013). Therefore, there may be other factors which could lead to the result obtained, which is open for further investigations. The mechanism of action that led to the result may be explained by any factors that have the potential of inhibiting the production of extra proteins within the prostate gland during benign prostatic hyperplasic conditions. In Benign prostatic hyperplasia, extra proteins such as killekreins are produced in the prostate gland which may be leaked into the blood resulting into hyperplasic or hypertrophic condition of the prostate.

**Effects of *Englerina gabonensis*on Oxidative stress markers (MDA, CAT,GSH, SOD) inBPH induced *wistar* rats.**

Oxidative stress markers such as malondialdehyde (MDA), catalase enzyme (CAT), glutathione reductase (GSH) and Superoxide dismutase (SOD) are important biomarkers in the development of benign prostatic hyperplasia. An increase in oxidative stress which results to an increase in MDA and decrease in CAT, GSH and SOD could result to benign prostatic hyperplasic condition. The result from this study showed that the extract *Englerina gabonensis* caused a decrease in oxidative stress as shown by the significant decrease in MDA (p<0.05) and significant increase (p<0.05) in CAT, GSH and SOD. This result agrees with the findings of (Zheng ***et al****,* 2016).The result may be due to the Phytochemical constituent (Polyphenols) of the extract, as Polyphenols have been documented by (Ring  ***et al,*** 2010)to exhibit antioxidative properties. The mechanism of action can be narrowed to the ability of Polyphenols in suppressing the generation of free radicals, thus reducing the rate of oxidation by inhibiting the formation of or deactivating the active species and precursors of free radicals. More frequently, they act as direct radical scavengers of the lipid peroxidation chain reactions (chain breakers). Chain-breakers donate an electron to the free radical, neutralizing the radicals and themselves becoming stable (less reactive) radicals, thus stopping the chain reactions.

**Effects of *Englerina gabonensis* on Prostate tissue histology in BPH induced *wistar* rats.**

The histology of the prostate gland is very important to note in benign prostatic hyperplasic conditions as benign prostatic hyperplasia have been characterized as an hypertrophy and hyperplasia of the stroma and epithelial cells of the prostate gland.The result from this study showed that the extract *Englerina gabonensis* caused a decrease in the hyperplasia of the glandular stroma of the prostate gland. This also may be due to the Phytochemical constituent (Polyphenols) of the extract, as Polyphenols have been documented by Mbaka ***et al,*** (2014) to have antiproliferative properties. The mechanism of action can be associated to the inhibitory ability of polyphenols in increasing the number of nodules in the prostate cell thereby preventing hyperplasia of the cells.

**Conclusion**

From this research, hydromethanolic leaf extract of *Englerina gabonensis* showed therapeutic potential against benign prostatic hyperplasia (BPH), by decreasing oxidative stress and prostate specific antigen and increasing the level of testosterone in benign prostatic hyperplasic induced rats. And its active chemical component may be used for BPH treatment.

Hence, Further studies should be carried out to confirm if the decrease in prostate specific antigen showed by the extract was as a result of their phytochemical composition or not.

**References**

Beigip M, Shahrokhi SS, Birjandi M, Abbaszadeh A, Beyranvand F, Hamoleh S, Zandbaf Z, Gholami M (2017). Effects of pomegranate peel extract on histopathology, testosterone levels and sperm of testicular torsion-detorsion induced in adult Wistar rats. J Complement Integr Med.14.

Bent S, Kane C, Shinohara K, Neuhaus J, Hudes ES, Goldberg H, Avins AL (2009). Saw palmetto for benign prostatic hyperplasia. N Engl J Med. 354(6):557-66.

Chughtai, B., Forde, J., Thomas, D. et al (2016). Benign prostatic hyperplasia. Nat Rev Dis Primers 2, 16031.

Zheng W, Wang SY (2001) Antioxidant activity and phenolic compounds in selected herbs. J Agric

Food Chem 49:5165–5170

Clin J. Oncol RJ Thomas, MMA Williams, H Sharma (2013). A Chaudry, P Bellamy. A Double-Blind, Placebo RCT Evaluating the Effect of a Polyphenol-Rich Whole Food Supplement on PSA Progression in Men With Prostate Cancer: The U.K. National Cancer Research Network (NCRN) Pomi-T Study

Ellman, G.L (1959). Tissue sulphydryl groups. Arch BiochemBiophys, 82: 70-77.

Góth, L. (1991) A Simple Method for Determination of Serum Catalase Activity and Revision of Reference Range. Clinica Chimica Acta, 196, 143-152.

Herbert L (2005). Pathophysiology of benign prostatic hyperplasia in the aging male population. Rev Urol. Suppl 4(Suppl 4):S3-S12.

Kakkar PS, Das BB, Viswanathan PN (1984) A modied spectrophotometric assay of superoxide dismutase. Indian J Biochem Biophys 21:130–132

Kavya B, Rajendra K, Lillian W, Yuhan Y, Deven T, Keerti S, Eleni P, Ivelisse P, Jennifer D, Michael C, Channing P and Sushant K (2021). Effect of mistletoe extract on growth and proliferation of prostate cancer cells. Proceedings: AACR Annual Meeting. 17-21.

Kensuke M, Yasuyoshi M, Tomohiro M, Yuta M, Asato O, Junki H, Tsubasa K, Tsuyoshi M, Kojiro O, and Hideki S (2021). Pharmacological Effects and Potential Clinical Usefulness of Polyphenols in Benign Prostatic Hyperplasia. Molecules Multidisciplinary Digital Publishing Institute (MDPI). 26(2): 450

Mbaka G, Ogbonnia S, Sulaiman A, Daniel Osiagwu (2017). Histomorphological effects of the oil extract of Sphenocentrumjollyanum seed on benign prostatic hyperplasia induced by exogenous testosterone and estradiol in adult Wistar rats. Avicenna Journal of Phytomedicine, 9(1):21-33.

Mbaka, John. (2014). Supplemanetary information. J. Mbaka et al.2014. Methane derived carbon in the benthic food web in stream impoundments. PLoS ONE 9 (10). e111392.

Ofem, O. E., Antai, A. B., Essien, N. M., & Oka, V. O. (2014). Enhancement of some sex hormones concentrations by consumption of leaves extract of Viscum album (mistletoe) in rats. Asian Journal of Medical Sciences, 5(3), 87–90.

Ohkawa, H., Ohishi, N. and Yagi, K. (1979) Assay for lipid peroxide in animal tissues by thiobarbituric acid reaction. Annal Biochem. 95, 51-55.

Perera D, Soysa , and Wijeratne S (2016). Polyphenols contribute to the antioxidant and antiproliferative activity of Phyllanthusdebilis plant in-vitro. BMC Complement Altern Med 16, 339.

Ring T (2010). Chemistry and Biochemistry of Dietary Polyphenols. Nutrients. 2(12): 1231–1246.

Zerafatjou, N, Amirzargar, M., Biglarkhani, M. et al (2021). Pumpkin seed oil (Cucurbita pepo) versus tamsulosin for benign prostatic hyperplasia symptom relief: a single-blind randomized clinical trial. BMC Urol 21, 147.

Zheng W, Wang SY (2001) Antioxidant activity and phenolic compounds in selected herbs. J Agric

Food Chem 49:5165–5170

Zheng W, Wang SY (2001) Antioxidant activity and phenolic compounds in selected herbs. J Agric

Food Chem 49:5165–517

Zheng W, Wang SY (2001) Antioxidant activity and phenolic compounds in selected herbs. J Agric

Food Chem 49:5165–517

Zheng W, Wang SY (2001) Antioxidant activity and phenolic compounds in selected herbs. J Agric

Food Chem 49:5165–517

Zheng W, Wang SY (2001) Antioxidant activity and phenolic compounds in selected herbs. J Agric

Food Chem 49:5165–5170

Zheng W, Wang SY (2001) Antioxidant activity and phenolic compounds in selected herbs. J Agric Food Chem 49:5165–5170

Zheng W, Wang SY (2001) Antioxidant activity and phenolic compounds in selected herbs. J Agric

Food Chem 49:5165–5170