ORIGINAL ARTICLE

Inhibitory Effects of *Pueraria mirifica* Aqueous Extracts on 5α-reductase and Prostate Histomorphometry in Testosterone-induced Benign Prostatic Hyperplasia Sprague Dawley Rats

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ABSTRACT

Introduction: Benign prostatic hyperplasia (BPH) is the most prevalent prostatic disease in ageing men, characterised by an excessive proliferation of the prostatic epithelial and stromal cells. Despite the extensive choices of pharmaceutical therapies, the current treatments possess side effects, necessitating the search for new alternative options, including herbal substances such as *Pueraria mirifica*. This tuberous root of *P. mirifica* is a medicinal plant that contains numerous phytoestrogens, traditionally used for health rejuvenation in aged men and women. This study was carried out to access the inhibitory effect of 5α-reductase of *P. mirifica* and its histoprotective effect in a rat model of testosterone-induced BPH. **Methods:** Adult Sprague Dawley (12 weeks) were subcutaneously injected with testosterone propionate (3 mg/kg) daily to induce BPH. Rats (n=6) in all groups (aqueous extract of *P. mirifica* (APM): 10, 100, and 1000 mg/kg, p.o.; finasteride: 2mg/kg, p.o., BPH model, and sham groups) were treated for 30 days. The determination of serum dihydrotestosterone (DHT) level, prostatic index and prostate structural changes were investigated. **Results:** APM and finasteride-treated groups showed significantly lesser prostatic weight and prostatic index, serum DHT levels compared to the model group ($p<0.05$). Furthermore, there was a significantly lower prostate score with improved prostate histomorphology, demonstrating fewer epithelial involutions of glandular tissues and improved stromal and epithelial cells. **Conclusion:** In conclusion, the aqueous extract of *P. mirifica* tuberous root mitigates the development of BPH and it can be inferred that aqueous extract of *P. mirifica* tuberous root may possess the active agents for anti-BPH treatment.
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Keywords: Benign prostatic hyperplasia; Dihydrotestosterone; 5α-reductase ; *Pueraria mirifica*; Histology

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INTRODUCTION

Benign prostatic hyperplasia (BPH) is a condition where the prostate gland enlarges due to benign and uncontrolled proliferation of the prostatic cells. It is a non-malignant condition, meaning that the prostate grows in size without being malignant. As the prostate enlarges, it can compress the bladder and urethra, leading to unfavourable clinical signs such as bladder outlet obstruction, urine storage issues, difficulties with urination (voiding), nocturia (frequent nighttime urination), and dysuria (painful urination) [1-4]. BPH is the most prevalent problem among ageing men, with its incidence increasing with age, becoming a significant cause of adverse effects on quality of life and mortality [5,6,7]. It is predicted to affect up to 40% of older men over 50 [8]. Although the precise aetiology of BPH is still obscure, age and androgens are significant contributors to the growth and progression of BPH in ageing men.

BPH development has been linked to hormonal changes in ageing men and various other factors such as androgen-oestrogen imbalance [9]. Specifically, by the stimulation of prostatic androgen dihydrotestosterone (DHT), an active metabolite produced by the enzyme 5α-reductase during the enzymatic conversion of testosterone [7]. BPH is also associated with the activity of 5α-reductase [10].

Ageing and increasing DHT production and accumulation in the prostate will cause hyperplasia to be induced, as well as enhanced cell proliferation [11]. BPH development is not a transformational mediator, but rather a more permissive mediator considering DHT activity is higher in BPH tissue than in normal prostate gland tissue [12].

Commonalities practice in clinical care, the treatment for BPH patients involves surgical intervention as well as the use of pharmacotherapeutic medication such as 5α-reductase inhibitors (dutasteride and finasteride), α-blocker, α-adrenoreceptor antagonists [3,13-16]. Among the available medication for BPH, 5α-reductase inhibitors have been shown to efficiently decrease prostate volume, thus reducing the risk of complication and surgery [14]. Hence, 5α-reductase inhibitors are the preferred option for treating BPH, even though they have been associated with undesired effects like ejaculatory dysfunction, decreased libido and dizziness [15,16], prompting the search for alternative therapies in managing BPH.

Pueraria mirifica (PM) is a perennial climber plant with tuberous roots, belonging to the Leguminosae family, specifically the Papilionoideae subfamily, which includes soy, beans and pea [17,18]. The tuberous root of *P. mirifica* contains a high number of potent phytoestrogens, including isoflavones (daidzein, daidzin, genistein, genistin, and puerarin), lignans and coumestans [18]. Traditionally, *P. mirifica* has been consumed for various purposes, such as an anti-wrinkle agent, appetite promoter, hair-blackening agent, memory enhancer, and longevity booster [19,20]. It exerts an oestrogenic effect on hormone-sensitive tissues and organs, contributing to its antioxidant properties [17], prevention of bone loss [21,22], and prevention of breast cancer in rats [23]. Moreover, studies have demonstrated its ability to downregulate the expression of interleukin-6, androgen eceptor and oestrogen receptors in prostatic tissue, leading to anti-inflammatory effects that ameliorate BPH [24]. As BPH is primarily driven by the growth of prostatic tissue in response to the presence of androgens, particularly DHT, which bind to and activate the androgen receptor in the prostate gland, we hypothesize that *P. mirifica* exhibits inhibitory effects on 5α-reductase. This, in turn, will result in reduced dihydrotestosterone (DHT) production and decreased proliferation of prostatic epithelial and stromal cells, leading to mitigation of benign prostatic hyperplasia (BPH) development in the testosterone-induced BPH rat model. Therefore, the objective of the current study is to evaluate the inhibitory effect of 5α-reductase by *P. mirifica* and its histomorphological effects in a rat model of testosterone-induced BPH.

MATERIALS AND METHODS

Extraction of *P. mirifica* tuberous root

The dried tuberous root of *P. mirifica* (Anhui Bozhou Qiaocheng, Guangzhao, China) was grounded into powder form with the grinder (Polymix, PX-MFC 90D, Kinematica, Switzerland). HPLC grade water (Merck, Germany) (250ml) was used to extract 50g of powdered *P. mirifica* which was incubated in a shaker water bath at 37°C for 12 hours. The solution was filtered through filter paper (Whatman, No.1) and dried in the freeze drier (Labconco, Kansas). Additionally, using the Soxhlet apparatus, the powdered *P. mirifica* was extracted with chloroform and methanol, following the polarity gradient at a ratio 1:10 (v/v). The solvent from all filtered extracts was eliminated under reduced pressure in a rotary evaporator (Buchi Rotavapor R-114, Switzerland).

5α-reductase Enzyme Inhibition Studies

5α-reductase inhibitory activity assay was carried out to investigate the inhibition potential of the *P. mirifica* tuberous root extract against prostatic hyperplasia spectrophotometrically. According to the method described earlier [25-27], an enzyme inhibition assay was conducted. Rat ventral prostate was dissected and weighed. Prostate tissue (200mg) was homogenised in 10 mL of medium (20 mM sodium phosphate, pH 6.5, containing 0.32 M sucrose and 1 mM EDTA), and was centrifuged at 4°C for 15 minutes at 716 x g (4000 rpm). Bradford Method for protein estimation was used to ascertain the enzyme content in the supernatant.

The NADPH standard curve and reaction mixtures were prepared according to the method described previously (25). The following reaction mixtures were used in the assay: blank (with NADPH and enzyme), negative control (with NADPH, enzyme and testosterone), positive control (with NADPH, enzyme, testosterone and finasteride) and *P. mirifica* extract samples (with NADPH, enzyme and testosterone). The reaction mixtures were spectrophotometrically determined at 340 nm every 5 minutes for 30 minutes. The absorbance was measured, and NADPH concentration was calculated using the NADPH standard curve. The calculation of the NADPH residue in the reaction mixture indicated the activity of 5α-reductase inhibition [26]. The 5α-reductase inhibition was calculated according to the formula below:

$$\text{Percentage inhibition} = \frac{\text{NADPH sample - NADPH blank}}{\text{ADPH sample}} \text{ X } 100$$

Animals Experimental Design

Male Sprague Dawley rats that were 12-weeks old and weighed 200g to 250g were kept in polypropylene cages in Laboratory Animal House, Faculty of

Medicine, Universiti Malaya. This study fulfils the ethical code sanctioned by the animal ethics committee of the Faculty of Medicine, University Malaya (Ethic No: No: ISB/30/05/2012/SSM(R)). The room temperature (25 + 2°C) with 12-hour light and dark cycles were maintained. Animal handling was conducted following the Institutional Animal Ethics Committee of University Malaya. The rats were given an adlibitum supply of water and a non-phytoestrogen pellet diet (Altromin, Lage, Germany). The rats were divided into 6 experimental groups at random after 7 days of acclimation. The groups are as follows: Group 1: Sham group (received vehicle); Group 2: BPH-induced control group; Group 3: FN group (finasteride 1mg/kg); Group 4: APM10 group (10 mg/kg *P. mirifica* aqueous extract); Group 5: APM100 (100 mg/kg *P. mirifica* aqueous extract); Group 6: APM1000 (1000 mg/kg *P. mirifica* aqueous extract). For 30 days, all groups but the sham group received subcutaneous injections of 3mg/kg testosterone propionate diluted in corn oil to induce prostate hyperplasia. The rats were administered vehicle Tween-20 (0.2% v/v, p.o) or finasteride (1 mg/kg, p.o) or *P. mirifica* aqueous extract (10, 100 or 1000 mg/kg, p.o) simultaneously. At the end of the study, the rats were anaesthetised, blood was collected intracardiac and the prostate was immediately dissected out and weighed.

Determination of Prostate Index

Prostates were weighed after being dissected, and prostatic index (PI), which was determined by dividing each rat's prostatic weight by body weight, was calculated as follows [40]:

Prostate index (PI) = (Prostate weight (g)) / (Bodyweight at day 30 (g))

Prostate enlargement inhibition by the experimental groups was determined as follows:

Percentage inhibition = 100 - (% Increase in prostatic weight)

The percentage increase in prostatic weight was calculated as follows:

Percentage increase in prostatic weight = ((PI treated group - PI Sham group)/ (PI BPH group - PI Sham group) X 100

Serum Dihydrotestosterone Assay

DHT levels in individual rats were measured using a DHT ELISA kit (Cusabio Biotech Co.Ltd) following the instruction in the kit.

Histomorphological Evaluation of Prostate Tissue

To evaluate the histopathological status of the prostate gland tissue by haematoxylin and eosin staining, a histomorphological examination of the prostate gland of testosterone-induced prostate hyperplasia was conducted. A score-chart protocol that had previously developed [28,29] was used to record and scored each characteristic that could be observed under the microscope (Table I). The histoscores were expressed in arbitrary units and were obtained from a thorough examination of 3 different sections, and 3 different levels (upper, middle and lower levels of tissue sections in the paraffin block), for each animal. The acinar morphology was considered in this approach, including crowding, intraluminal velocities, loss of basal nuclear polarity, and hyperplastic nodules, which were graded based on their severity and distribution pattern. The following are definitions of the pathological condition of the acinar structure: 1) Villamentous or villous projections are bordered by epithelial cells and indicate homogenous epithelial infolding into the lumen, showing a fine connective tissue core together with its longitudinal axis. 2) Papillary projections are enlarged epithelial infolding with a variety of ramifications that create a pattern resembling cauliflower. 3) Cribriform structure, which resembles a "glands in glands" pattern, results by the fusion of contralateral papillary projections. 4) The hyperplastic nodule is a multi-layered conglomerate with a localized increase of nuclei number. In piling up, the hyperplastic nodule protrudes into the acinar lumen and does not cross the basal membrane. In budding, the epithelial nodule bursts outside the basal membrane toward the connective tissue stroma. The hyperplastic nodules may appear as isolated or multiple foci in one or both lobes of the ventral prostate.

Statistical Analysis

Statistical analysis was analysed by the ANOVA test followed by Duncan's post hoc test. All results were expressed as the mean ± standard error of the mean (SEM). $p<0.05$was statistically significant.

RESULTS

5α-reductase Inhibitory Activity of *P. mirifica* Extracts

Figure 1a shows the residual concentration of NADPH significantly increased with time in a reaction mixture containing finasteride (positive control). The concentration of residual NADPH was 19.06 + 0.11 µg/ml at 0 minutes and significantly increased to 40.69 + 0.04 µg/ml at 30 minutes. The percentage inhibition of 5α-reductase activity was 54.91% at minute 30 (Figure 1b). When compared to finasteride, the aqueous extract of *P. mirifica* showed to be the most effective inhibitor of the 5α-reductase enzyme ($p<0.05$). Aqueous extract increased the NADPH concentration from 18.62 + 0.08 µg/ml at 0 minutes to 25.16 + 0.18 µg/ml at 30 minutes (Figure 1a). The percentage inhibition of 5α-reductase activity by water extract was 0.18% at minute 0 and significantly increased to 27.07% at minute 30 (Figure 1b). The methanol extract of *P. mirifica* tuberous root showed an increase of NADPH residual concentration from 18.33 + 0.06 µg/ml at 0 minutes

Table I : Cumulative chart score of histopathological findings (units) in rat ventral prostate

No.	Characteristics
	Low power magnification on prostate section
1	Luminal shape: regular (1), villous (3), papillary (4), cribriform (5)
2	Acinar shape: tubular (1), branched (3), irregular (5)
3	Interacinar space: large or moderate (1), back-to-back glands (5)
4	Stroma: fine (1), abundant (3), fibrosis/severe stroma hyperplasia (5)
	High power magnification on prostate section
1	Epithelial shape: flattened (1), cuboidal (1), cylindrical (3), hexagonal (5)
2	Number of layers: mono (1); oligo (3); pluri (5) • If .1, add: focal (3), diffuse (5)
3	Alignment: polar (1), apolar (3) • If there is piling up of epithelial cells add 3 • If there is budding out of epithelial cells into stroma add 5 • If periacinar clusters of epithelial cells are found add 3 • If isolated clusters of epithelial cells are found outside acini add 5
4	Lesion distribution (for apolar or budding out cells, no lesion 0): unilobar; isolated (2), multiple (6). bilobar; isolated (4), multiple (8)
5	• Nuclear shape: round, regular (1); irregular (5)
6	Nuclear size: small (2), large (2), small and large in the same acinus (4)
7	Mitoses per field: absent (0); isolated, 1–2 (2); abundant, 3–5 (5); excessive, >5 (10)
8	Basement membrane: intact (1); interrupted (5) thin (1); thick (5)

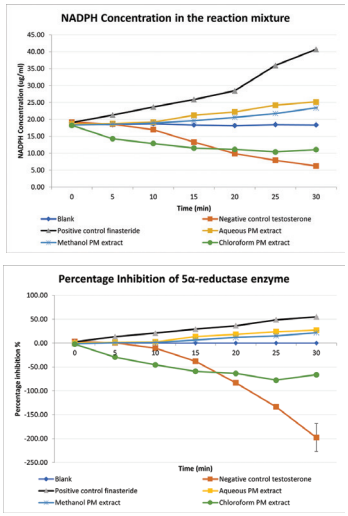

Figure 1 : Effects of *P. mirifica* extracts on the a) NADPH residual concentration, b) percentage inhibition of the 5α-reductase enzyme. The values are shown in mean ± SD (n=3). The values are shown in mean ± SD (n=3).

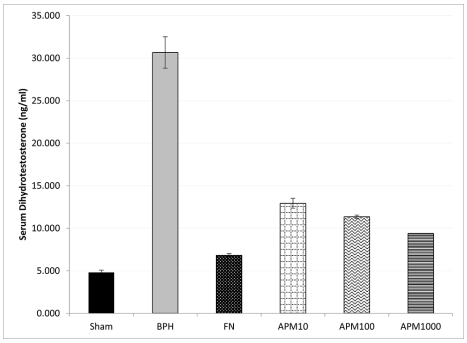

Figure 2 : Effect of orally administered *P. mirifica* aqueous extract on the serum DHT level. The histogram bars with different lowercase letters (a,b,c) are significantly different at $p<0.05$ (ANOVA, followed by Dunnet's multiple comparison tests). a= $p<0.05$ compared to sham control. b= $p<0.05$ compared to BPH group. c= $p<0.05$ compared to FN group. FN: Finsteride-treated group, APM10: 10mg/kg *P. mirifica* aqueous extract group, APM100: 100mg/kg *P. mirifica* aqueous extract group, APM1000: 1000mg/kg *P. mirifica* aqueous extract group.

Table II : Effect of aqueous extract of *P. mirifica* on prostate index and percentage inhibition.

Group treatment	Prostate Index ($x10^{-3}$)	% Inhibition
Sham	3.85 ± 0.09 ^{bc}	-
BPH	9.49 ± 0.27 ^{ac}	-
FN	4.92 ± 0.05 ^{ab}	81.11
APM10	7.16 ± 0.26 ^{abc}	41.37
APM100	6.52 ± 0.08 ^{abc}	52.58
APM1000	5.68 ± 0.09 ^{ab}	67.57

Data are mean ± SEM (n=6). The means with different lower-case letters (a, b, c) in the same column are significantly different at P<0.05 (ANOVA, followed by Dunnet's multiple comparison tests). a=P<0.05 compared to sham control. b= P<0.05 compared to BPH group. c=P<0. 05 compared to FN group. ns: not significant.

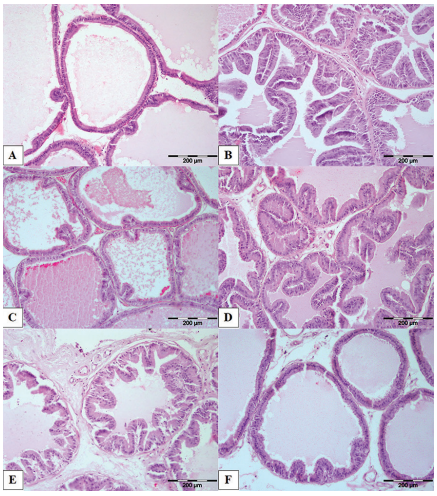

Figure 3 : Effect of P.mirifica water extracts on the prostate tissue of testosterone-induced prostatic hyperplasia rats. 100x magnification. H&E stains A) Sham group. B) BPH group. C) FN group. D) APM10 group. E) APM100 group. F) APM1000 group. s: stroma, il: intraluminal.

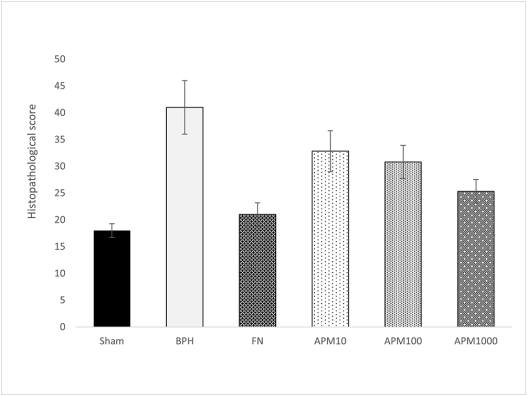

Figure 4 : Effect of orally administered *P. mirifica* water extract on the prostate histoscore. Data are mean + SEM (n=6). The means with different lower-case letters (a, b, c) in the same column are significantly different at p<0.05 (ANOVA, followed by Dunnet's multiple comparison tests). a= p<0.05 compared to sham control. b= p<0.05 compared to BPH group. c= p<0. 05 compared to FN group.

to 23.41 + 0.14 µg/ml at 30 minutes. The increment of NADPH concentration by time interval was significant. Similarly, the percentage inhibition also showed significant differences by time interval at p<0.05, where the percentage increased from -1.37% to 21.64%. The inhibitory activity of *P. mirifica* extracts was discovered in the following decreasing order: finasteride > aqueous > methanol > chloroform. The aqueous extract was chosen to study the treatment benefits of BPH in rats based on the findings.

Effects of *Pueraria mirifica* aqueous extract on the prostatic index

The evaluations of the therapeutic effects of *P. mirifica* aqueous extract on BPH in testosterone-induced prostatic hyperplasia are shown in Table II. As anticipated, testosterone propionate subcutaneous injection markedly higher prostatic weight and prostate index in BPH group in comparison to the sham group. When compared to the BPH group, the treatment received by APM10, APM100, APM1000 and FN groups significantly reduced the prostatic index (p<0.05).

Effects of *Pueraria mirifica* aqueous extract on the serum DHT levels

Displayed in Figure 2 are the effects of an aqueous extract of *P. mirifica* on serum dihydrotestosterone (DHT) level of testosterone-induced prostatic hyperplasia. BPH induction significantly increased DHT concentrations in the serum of BPH group (30.66 ± 33.30 ng/mL, p < 0.01) compared to the sham group (4.81 ± 32.09 ng/mL). The FN group, on the other hand, had a significantly lower serum DHT level (6.83 ± 29.88 ng/mL) than BPH group. Likewise, the DHT levels in the APM10 (12.93 ± 52.53 ng/

ml), APM100 (11.35 ± 40.79 ng/ml) and APM1000 (9.39 ± 40.79 ng/ml) groups were also significantly lower than BPH group.

Effects of *Pueraria mirifica* aqueous extract on Histomorphology of Prostate in Testosterone-Induced BPH Rats

Figure 3 shows the impact of *P. mirifica* aqueous extract treatment on the histological characteristics of the prostates from all groups of testosterone-induced BPH rats. The sham group displayed normal histological features of the prostate gland with an average histoscore corresponding to 18.0 + 1.29 (Figure 4). The acinar gland tubules were variable in diameter. The luminal shape was regular with prostatic secretion in the lumen observed. A single layer of cuboidal epithelium bordered the walls of the acini tubules. The nuclei were small, round and regular. The stroma matrixes were fine with the presence of blood vessels and moderate inter acinar spaces (Figure 3a).

The photomicrograph of the prostate tissue in BPH rats revealed a substantial alteration in the histomorphology of the tissue (Figure 3b), showing stromal and epithelial proliferation leading to glandular hyperplasia. The acinar gland tubules had an irregular acinar form and numerous villous extensions into the lumen, giving them a broader appearance. In contrast to the sham group, the tubule walls were lined with tall, cylindrical columnar cells that had epithelial pilling, resulting in a thicker epithelial layer. Each tubule has formed a significant involution projection into the lumen, which significantly lowers the volume of the lumen and partially obliterates it. The cribriform acini were arranged back-to-back with stromal and intraluminal papillary projections. Oedema, clogged blood arteries, and inflammatory cells were visible in the sparse interstitial stroma. These results were related to a higher histoscore value of 41.10 + 4.99, which, at 0.05, differed substantially from the sham and FN groups.

The prostate histomorphology in the FN group showed an improved prostate histoarchitecture when compared to BPH groups (Figure 3c). The scales of pathological changes were scored with histoscore corresponding to 23.00 ± 2.21. The acinar gland tubules were variable in diameter with regular luminal shapes. The luminal lining with cuboidal to tall columnar epithelial cells with minor hyperplastic alterations and epithelial piling in the FN group. The nuclei were round to oval, regular in shape, and basally situated. Involutions into the lumen were also reduced in number. The stroma distribution was normal, with prostatic acini that were less packed and projected less prominently.

Histological changes in the prostate of BPH-induced rats treated with *P. mirifica* aqueous extracts exhibited moderate glandular hyperplasia (Figure 3d-3f). An increasing dose of *P. mirifica* aqueous extract improved prostate histoarchitecture. The prostate of the APM10 group showed an irregular and massive enlargement of prostatic acini with intraluminal secretion (Figure 3d). The epithelium lining was still thicker with tall cuboidal epithelium cells. The involution projection into the lumen was frequently observed. The regular tubular prostatic acini were observed. The histoscore for rats in the APM100 group was 32.83 ± 3.10. The diameter and morphology of the prostatic acini varied, with villous and papillary luminal observed (Figure 3e). The cuboidal and cylindrical epithelial cells lining the lumen were intermingled with slight intraluminal villous projections. While in the APM1000 group (Figure 3f), prostatic tissues showed an improved histomorphology compared to the BPH group with a histoscore of 30.33 ± 2.20. Intraluminal secretions appeared and the morphology of the tubules was improved. Lumens of the tubules are regular to villous with a few small involution projections. The tubule walls were observed lined with cuboidal and cylindrical epithelium, with small epithelial piling-up formations observed in the group.

DISCUSSION

P. mirifica has a long history of use in ethnomedicine to address various ailments, including eye cataracts, memory enhancement, increased energy and vitality, and improving blood circulation and longevity, cosmetics and particularly related to the ageing process. In contemporary times, *P. mirifica* is available in various forms, such as tablets, extracts, creams, sprays, and powdered formulations. This versatility allows it to be incorporated into different medicinal preparations or combined with other herbs, as individual conditions may require varying applications and dosages [17-20,30]. Notably, with its 17 isoflavonoid compounds, *P. mirifica* is known to contain a high level of phytoestrogen [18,30], which earlier research has suggested may have beneficial effects in preventing cancer and benign prostate hyperplasia (BPH) [31-33]. The goal of the current study is to use in vivo models to examine the histomorphology preservation effect of *P. mirifica* from the tuberous root on BPH.

In BPH, the principal enzyme involved is 5α-reductase, which converts testosterone into the more potent DHT. It utilizes NADPH, as a co-enzyme, to convert testosterone into DHT, that ten times more potent than testosterone in triggering BPH [25,34-36]. This testosterone metabolite has ten times the ability of testosterone in triggering BPH disorder. By conducting an in vitro investigation, the extracts were screened for their ability to inhibit the 5α-reductase enzyme.

A 5-reductase inhibitor prevented the conversion of testosterone to DHT, and as the inhibition increased, more coenzyme NADPH accumulated. This occurred because NADPH molecules were freed from binding sites of 5α-reductase, and this implicated the 5α-reductase activity [25, 26]. In this study, we found out that aqueous extract was the strongest 5α-reductase inhibitor, as it increased the concentration of NADPH with time. Thus, only an aqueous extract of *P. mirifica* was taken up for *in vivo* experiments.

Testosterone-induced BPH rats were utilised widely as an experimental animal model for screening potential anti-BPH medications [32,35-42]. BPH in animal models is characterised by an increase in prostate weight and size, prostatic index and DHT levels. It also causes pathological changes, such as the proliferation of prostatic epithelium and stromal cells, enlargement of the glandular cavity, and infiltration of inflammatory cells, leading to the alteration in prostate histological features [37, 38]. In this current study, treatment of testosterone-induced BPH in rats with *P. mirifica* aqueous extract over 30 days period considerably mitigates the BPH progression through a decline in the prostatic index, DHT level and the severity of histological characteristics. The effects of medications used to treat prostatic hyperplasia have been conducted in the past with regard to the effects of testosterone and DHT on rats' prostatic development [43]. The prostatic index was used as a marker to demonstrate it [15, 37, 38].

A metabolite of testosterone called dihydrotestosterone (DHT) is more effective than testosterone in inducing BPH. The activity of 5α-reductase, for which NADPH serves as a coenzyme, is crucial in this conversion [34]. To control prostate growth, DHT binds to androgen receptors in the prostate. Due to this, increased androgen receptor expression may contribute to the aetiology of BPH in ageing men [44]. The androgen receptor expression in the prostate tissue of the BPH-induced rats was significantly reduced in the groups that received *P. mirifica* aqueous extract, as previously described [24]. This current study showed that the aqueous extract of *P. mirifica* decreases the DHT level by possibly inhibiting the activity of the enzyme of 5α-reductase. This provides an alleviation effect on BPH by aqueous extract of *P. mirifica*.

Moreover, previous research has demonstrated that testosterone-induced prostate weight gain in rats accompanied by histological changes suggestive of BPH and that treatments that prevented the prostate weight increase also reduced the severity of BPH on histological scores [41, 45]. The prostatic histoarchitecture has improved as seen by the histological findings, particularly in the cuboidal epithelial cells, intracellular lumen, tubular latency, and form, which further supports the possibility of using *P. mirifica* for the management of BPH. The glandular lumen was utterly obliterated in the prostate tissue of BPH-induced rats due to the significant proliferation of stromal and epithelial cells. The treatment with finasteride/ *P. mirifica* aqueous extract exhibited marked changes in the prostatic tissue histoarchitecture, where re-duction in epithelial involutions and stromal hyperplasia were obvious. The improvement in histoarchitecture of the prostate tissue is reflected in the inhibition of 5α-reductase activity. The current study agreed with previous studies that reported use of alternative therapies in BPH management to modulate hyperplasia by inhibiting the 5α-reductase activity [36, 40]. *P. mirifica* aqueous extract inhibit BPH progression in simultaneous induction with the extract treatment exhibited considerable effective prophylaxis.

Plants with a high content of isoflavonoids has been reported to alleviate BPH such as Uvaria rufa, Urtica dioica, Ganoderma lucidum and Benincasa hispida have been shown to alleviate BPH symptoms in experimental animals by reducing the DHT level via inhibiting the 5α-reductase [36, 39, 46]. The findings of the current study further support this notion, with the aqueous extract of *P. mirifica* being a potent 5α-reductase inhibitor, as evidenced by lower serum DHT levels with its administration. Treatment with aqueous extract of *P. mirifica* resulted in improved prostatic histological features, including reduced prostatic index, epithelial involutions, and stromal hyperplasia. Overall, the current study suggests that PM's aqueous extract has potential therapeutic benefits in mitigating BPH progression through its inhibitory effects on 5α-reductase and modulation of prostatic histological features. However, more research is needed to validate these effects in human BPH management.

The aetiology of BPH in humans is complex, and no other species exhibits the same level of complexity. To yet, animal models of BPH investigated do not appear to adequately mirror the stromal and epithelial alterations associated with BPH in people [47]. Therefore, animal models have limited utility in the investigation of BPH occurrences. Due to the pathogenesis of BPH being multifactorial and depending on a functional androgenic signal involving several components, BPH induced by testosterone or dihydrotestosterone does not reproduce all findings of BPH in humans [11]. It's essential to note that animal models of BPH may not fully mirror the complexity of the disease in humans, as BPH in humans involves prostatic oestrogens and α-adrenergic receptors, which are not entirely replicated in animal models Therefore, the limitations of the animal model used in this study should be considered when interpreting

the results in the context of human outcomes. Nevertheless, the impact of testosterone and dihydrotestosterone on prostatic development in rodents have previously been described and used to evaluate the impact of ethnomedicine used for prostatic hyperplasia treatment.

CONCLUSION

The aqueous extract of *P. mirifica* has demonstrated potent anti-BPH effects by inhibiting the activity of the 5α-reductase enzyme, which converts testosterone to DHT. This inhibition leads to a reduction in prostate gland enlargement and the reversal of testosterone-induced histological changes in experimental rats. The findings suggest that *P. mirifica* aqueous extract could be potentially used as an alternative treatment for managing BPH. Further investigation is warranted to fully explore its potential. It is essential to study the individual phytochemicals present in *P. mirifica* to identify the specific bioactive or phytochemical; and mechanism of action of each compound and determine if there is any synergy among them.

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REFERENCES

1. Briganti A, Capitanio U, Suardi N, Gallina A, Salonia A, Bianchi M, et al. Benign prostatic hyperplasia and its aetiologies. Eur Urol Suppl. 2009;8(13):865-71. doi: 10.1016/j.eursup.2009.11.002.
2. Pais P. Potency of a novel saw palmetto ethanol extract, SPET-085, for inhibition of 5alpha-reductase II. Adv Ther. 2010;27(8):555-63. doi: 10.1007/s12325-010-0041-6.
3. Roehrborn CG, Barkin J, Siami P, Tubaro A, Wilson TH, Morrill BB, et al. Clinical outcomes after combined therapy with dutasteride plus tamsulosin or either monotherapy in men with benign prostatic hyperplasia (BPH) by baseline characteristics: 4-year results from the randomized, double-blind Combination of Avodart and Tamsulosin (CombAT) trial. BJU Int. 2011;107(6):946-54. doi: 10.1111/j.1464-410X.2011.10124.x.
4. Jiwrajka M, Yaxley W, Ranasinghe S, Perera M, Roberts MJ, Yaxley J. Drugs for benign prostatic hypertrophy. Aust Prescr. 2018;41(5):150-53. doi: 10.18773/austprescr.2018.045.
5. Wang W, Guo Y, Zhang D, Tian Y, Zhang X. The prevalence of benign prostatic hyperplasia in mainland China: Evidence from epidemiological surveys. Sci Rep. 2015;5:13546. doi: 10.1038/srep13546.
6. McVary KT. BPH: epidemiology and comorbidities. Am J Manag Care. 2006;12(5 Suppl):S122-8. Available from: https://www.ajmc.com/view/apr06-2288ps122-s128
7. Nicholson TM, Ricke WA. Androgens and estrogens in benign prostatic hyperplasia: past, present and future. Differentiation. 2011;82(4-5):184-99. doi: 10.1016/j.diff.2011.04.006.
8. Nickel JC. Inflammation and benign prostatic hyperplasia. Urol Clin North Am. 2008;35(1):109-15. doi: 10.1016/j.ucl.2007.09.012.
9. Untergasser G, Madersbacher S, Berger P. Benign prostatic hyperplasia: Age-related tissue-remodeling. Exp Gerontol. 2005;40(3):121-8. doi: 10.1016/j.exger.2004.12.008.
10. Randall VA. Role of 5 alpha-reductase in health and disease. Baillieres Clin Endocrinol Metab. 1994;8(2):405-31. doi: 10.1016/s0950-351x(05)80259-9.
11. Carson C, Rittmaster R. The role of dihydrotestosterone in benign prostatic hyperplasia. Urology. 2003;61(4 Suppl 1):2-7. doi: 10.1016/s0090-4295(03)00045-1.
12. Roberts RO, Jacobson DJ, Rhodes T, Klee GG, Leiber MM, Jacobsen SJ. Serum sex hormones and measures of benign prostatic hyperplasia. Prostate. 2004;61(2):124-31. doi: 10.1002/pros.20080.
13. Jun JEJ, Kinkade A, Tung ACH, Tejani AM. 5α-reductase inhibitors for treatment of benign prostatic hyperplasia: A systematic review and meta-analysis. Can J Hosp Pharm. 2017;70(2):113-9. doi: 10.4212/cjhp.v70i2.1643.
14. Gravas S, Oelke M. Current status of 5alpha-reductase inhibitors in the management of lower urinary tract symptoms and BPH. World J Urol. 2010;28(1):9-15. doi: 10.1007/s00345-009-0493-y.
15. McVary KT, Roehrborn CG, Avins AL, Barry MJ, Bruskewitz RC, Donnell RF, et al. Update on AUA guideline on the management of benign prostatic hyperplasia. J Urol. 2011;185(5):1793-803. doi: 10.1016/j.juro.2011.01.074.
16. Kim EH, Brockman JA, Andriole GL. The use of 5-alpha reductase inhibitors in the treatment of benign prostatic hyperplasia. Asian Journal of Urology. 2018 Jan;5(1):28-32. doi: 10.1016/j.ajur.2017.11.005.
17. Bodner CC, Hymowitz T. Ethnobotany of Pueraria species. In: Keung WM, editor. London: Taylor & Francis; 2002. p. 29-58.
18. Cherdshewasart W, Sutjit W. Correlation of antioxidant activity and major isoflavonoid contents of the phytoestrogen-rich *Pueraria mirifica* and Pueraria lobata tubers. Phytomedicine. 2008;15(1-

2):38-43. doi: 10.1016/j.phymed.2007.07.058.
19. Suntara A. The Remedy pamphlet of Kwao Krua tuber of Luang Anusarnsuntarakromkarnphiset. Chiang Mai, Thailand: Chiang Mai Upatipongsa Press; 1931.
20. Wanadorn W. A reputed rejuvenator. Journal of the Siam Society. Nat Hist Suppl. 1933;9:145-147.
21. Trisomboon H, Malaivijitnond S, Watanabe G, Taya K. Estrogenic effects of *Pueraria mirifica* on the menstrual cycle and hormone-related ovarian functions in cyclic female cynomolgus monkeys. Journal of Pharmacological Sciences. 2004;94(1):51-9. doi: 10.1254/jphs.94.51.
22. Urasopon N, Hamada Y, Asaoka K, Cherdshewasart W, Malaivijitnond S. *Pueraria mirifica*, a phytoestrogen-rich herb, prevents bone loss in orchidectomized rats. Maturitas. 2007;56(3):322-31. doi: 10.1016/j.maturitas.2006.09.007.
23. Cherdshewasart W, Panriansaen R, Picha P. Pretreatment with phytoestrogen-rich plant decreases breast tumor incidence and exhibits lower profile of mammary ERalpha and ERbeta. Maturitas. 2007;58(2):174-81. doi: 10.1016/j.maturitas.2007.08.001.
24. Mohamad J, Masrudin SS, Alias Z, Muhamad NA. The effects of *Pueraria mirifica* extract, diadzein and genistein in testosterone-induced prostate hyperplasia in male Sprague Dawley rats. Mol Biol Rep. 2019;46(2):1855-1871. doi: 10.1007/s11033-019-04638-5.
25. Nahata A, Dixit VK. Evaluation of 5α-reductase inhibitory activity of certain herbs useful as antiandrogens. Andrologia. 2014;46(6):592-601. doi: 10.1111/and.12115.
26. Sun ZY, Zheng WJ, Feng J, Tu ZH. A convenient and rapid method to study enzymatic kinetics of steroid 5α-reductase inhibitors. Indian J Pharmacol. 1998;30:257-262. Available: https://www.ijp-online.com/article.asp?issn=0253-7613;year=1998;volume=30;issue=4;spage=257;epage=262;aulast=sun;type=0
27. Sharifan A, Hajhoseini A, Bakhtiari M. Clinical trial and in vitro study investigating topical application of Zataia multiflora Boiss. and Matricaria chamomilla extracts for androgenetic alopecia. Cogent Med. 2017;4(1):1421405. doi.org/10.1080/2331205X.2017.1421405
28. Scolnik MD, Servadio C, Abramovici A. Comparative study of experimentally induced benign and atypical hyperplasia in the ventral prostate of different rat strains. J Androl. 1994;15(4):287-97. https://doi.org/10.1002/j.1939-4640.1994.tb00451.x
29. Golomb E, Kruglikova A, Dvir D, Parnes N, Abramovici A. Induction of atypical prostatic hyperplasia in rats by sympathomimetic stimulation. Prostate. 1998;34(3):214-21. doi: 10.1002/(sici)1097-0045(19980215)34:3<214::aid-pros9>3.0.co;2-h.
30. Malaivijitnond S. Medical applications of phytoestrogens from the Thai herb *Pueraria mirifica*. Front Med. 2012;6(1):8-21. doi: 10.1007/s11684-012-0184-8
31. Geller J, Sionit L, Partido C, Li L, Tan X, Younkin T, et al. Genistein inhibits the growth of human-patient BPH and prostate cancer in histoculture. Prostate. 1998;34(2):75-9. doi: 10.1002/(sici)1097-0045(19980201)34:2<75::aid-pros1>3.0.co;2-i.
32. Huang YF, Feng Y, Pan LJ, Xia XY. Preventive effect of daidzein on testosterone-induced prostatic hyperplasia in rats. Zhonghua Nan Ke Xue. 2008;14(8):713-8.
33. Hsu A, Bray TM, Helfrich WG, Doerge DR, Ho E. Differential effects of whole soy extract and soy isoflavones on apoptosis in prostate cancer cells. Exp Biol Med (Maywood). 2010;235(1):90-7. doi: 10.1258/ebm.2009.009128.
34. Bartsch G, Rittmaster RS, Klocker H. Dihydrotestosterone and the concept of 5α-reductase inhibition in human benign prostatic hyperplasia. World J Urol. 2002;19(6):413-25. doi: 10.1007/s00345-002-0248-5.
35. Dhanotia R, Chauhan NS, Saraf DK, Dixit VK. Effect of Citrullus colocynthis Schrad fruits on testosterone-induced alopecia. Nat Prod Res. 2011;25(15):1432-43. doi: 10.1080/14786410802632820.
36. Nandecha C, Nahata A, Dixit VK. Effect of Benincasa hispida fruits on testosterone-induced prostatic hypertrophy in albino rats. Curr Ther Res Clin Exp. 2010;71(5):331-43. doi: 10.1016/j.curtheres.2010.10.006.
37. Wang R, Kobayashi Y, Lin Y, Rauwald HW, Fang L, Qiao H, Kuchta K. A phytosterol enriched refined extract of Brassica campestris L. pollen significantly improves benign prostatic hyperplasia (BPH) in a rat model as compared to the classical TCM pollen preparation Qianlie Kang Pule'an Tablets. Phytomedicine. 2015;22(1):145-52. doi: 10.1016/j.phymed.2014.10.001.
38. Yang X, Yuan L, Xiong C, Yin C, Ruan J. Abacopteris penangiana exerts testosterone-induced benign prostatic hyperplasia protective effect through regulating inflammatory responses, reducing oxidative stress and anti-proliferative. J Ethnopharmacol. 2014;157:105-13. doi: 10.1016/j.jep.2014.09.025.
39. Nahata A, Dixit VK. Ganoderma lucidum is an inhibitor of testosterone-induced prostatic hyperplasia in rats. Andrologia. 2012;44 Suppl 1:160-74. doi: 10.1111/j.1439-0272.2010.1155.x.
40. Veeresh Babu SV, Veeresh B, Patil AA, Warke YB. Lauric acid and myristic acid prevent testosterone induced prostatic hyperplasia in rats. Eur J Pharmacol. 2010;626(2-3):262-5. doi: 10.1016/j.ejphar.2009.09.037.
41. de Lourdes Arruzazabala M, Molina V, M6s R, Carbajal D, Marrero D, Gonz6lez V, Rodrнguez E. Effects of coconut oil on testosterone-induced

prostatic hyperplasia in Sprague-Dawley rats. J Pharm Pharmacol. 2007;59(7):995-9. doi: 10.1211/jpp.59.7.0012.
42. Gossell-Williams M, Davis A, O'Connor N. Inhibition of testosterone-induced hyperplasia of the prostate of sprague-dawley rats by pumpkin seed oil. J Med Food. 2006;9(2):284-6. doi: 10.1089/jmf.2006.9.284.
43. Nahata A, Dixit VK. Sphaeranthus indicus attenuates testosterone induced prostatic hypertrophy in albino rats. Phytother Res. 2011;25(12):1839-48. doi: 10.1002/ptr.3497.
44. Izumi K, Mizokami A, Lin WJ, Lai KP, Chang C. Androgen receptor roles in the development of benign prostate hyperplasia. Am J Pathol. 2013;182(6):1942-9. doi: 10.1016/j.ajpath.2013.02.028.
45. Elberry AA, Mufti S, Al-Maghrabi J, Abdel Sattar E, Ghareib SA, Mosli HA, Gabr SA. Immunomodulatory effect of red onion (Allium cepa Linn) scale extract on experimentally induced atypical prostatic hyperplasia in Wistar rats. Mediators Inflamm. 2014;2014:640746. doi: 10.1155/2014/640746.
46. Gьlзin I, Kьfrevioglu OI, Oktay M, Bьyьkokuroglu ME. Antioxidant, antimicrobial, antiulcer and analgesic activities of nettle (Urtica dioica L.). J Ethnopharmacol. 2004;90(2-3):205-15. doi: 10.1016/j.jep.2003.09.028.
47. Mahapokai W, Van Sluijs FJ, Schalken JA. Models for studying benign prostatic hyperplasia. Prostate Cancer Prostatic Dis. 2000;3(1):28-33. doi: 10.1038/sj.pcan.4500391.