Pharmacological investigation of an Ayurvedic formulation on testosterone propionate-induced benign prostatic hyperplasia rats

Soni Hardik¹, Mehta Hardik², Jani Deepi², Patel Ghanashyam¹

¹Vasu Research Centre, Vasu Healthcare Pvt. Ltd., Makarpura; ²Department of Pharmacology, Babaria Institute of Pharmacy, Varnama: Vadodara, Gujarat, India

INTRODUCTION

The prostate gland undergoes many changes during the course of man’s life. At birth, the prostate is about the size of a pea. It grows slightly until puberty and then it begins to enlarge rapidly, attaining normal adult size and shape [1]. Excessive cell proliferation can increase prostate size and weight. Benign prostatic hyperplasia (BPH) is a urological disorder caused by the noncancerous enlargement of the prostate as men age. As the prostate enlarges, it can constrict the urethra, inducing various symptoms including a weak urinary stream, incomplete bladder emptying, nocturia, dysuria and bladder outlet obstruction [2, 3]. These symptoms associated with BPH are known as lower urinary tract symptoms (LUTS) [4].

BPH is a common and progressive clinical disease of aging men, which may be associated with enlargement of the prostate, bothersome LUTS and bladder outlet obstruction (BOO). In the large scale multinational survey of the aging male, 34% of men in the USA, 29% of European men and 18% of men in Asian countries aged between 50-80 years reported moderate to severe LUTS [5]. Currently, the two main medications used for treatment of BPH are α₁-adrenergic receptor antagonists and 5α-reductase inhibitors [6]. The α₁-adrenergic receptor antagonists, including doxazosin, terazosin and tamsulosin, are the initial drugs for treating BPH, and they alleviate LUTS by relaxation of smooth muscle in the prostate and the neck of the bladder. On the other hand, the 5α-reductase inhibitors inhibit the development of BPH via a reduction in dihydrotestosterone (DHT) production [7]. However, the use of these drugs is limited because of their side effects, including decreased libido, ejaculatory or erectile dysfunction and nasal congestion [8, 9].

In this situation, medicinal plants may provide an alternative of new drugs. Indian system of medicine is a rich collection of knowledge on traditional medicine which narrates many plants having anti-inflammatory, diuretic, antioxidant and 5α-reductase activity [10]. The herbo-mineral formulation used in this study is such an Ayurvedic proprietary which contains extracts of Curcuma longa (Haridra) hizome [18], Punarnavadi (Lajjalu) Panchang [16], Mimosa pudica (Varun) bark [11-13], Tinospora Cordifolia (Haridra) rhizome [18], Emblica officinalis (Amalaki) fruit [19], Tinospora

Abstract

Objective: This study aimed to investigate the potential effect of an Ayurvedic herbo-mineral formulation in a rat model of testosterone propionate induced benign prostatic hyperplasia (BPH).

Methods: Male Wistar rats weighing 180-250 g were used. The selected animals were divided into six groups where each group consisted of six animals. Experimentally BPH was developed by subcutaneous administration of testosterone propionate (3 mg/kg) dissolved in corn oil for 28 days. The tested herbo-mineral formulation was administered orally for 28 consecutive days at 90 mg/kg, once or twice daily in different groups. On 29th day, Blood samples were collected to evaluate different parameters like blood urea nitrogen (BUN), serum creatinine, serum total protein, serum total cholesterol, serum albumin, serum globulin and serum A/G ratio. Prostate glands were dissected out for different prostatic parameters and histopathological examination.

Results: The herbo-mineral formulation showed significant effect on urine output and different prostatic parameters. Significant changes in urine volume, prostate weight, prostate weight index, prostate length and prostate width were observed. In addition, positive effects on different biochemical parameters like BUN, serum creatinine, serum total protein, etc. were recorded. The used formulation, at both dose levels, also showed significant reversal in histology of prostate glands when compared with disease control group. On the other hand, no side effects were observed according to the administered herbo-mineral formulation.

Conclusion: The findings of this study concludes that Herbo-mineral formulation has promising effect on testosterone propionate-induced BPH in male wistar rats.

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cordifolia (Guduchi) stem [20], Tribulus terrestris (Gokshur) fruit [21], powders of Chandraprabha Vati [21], and Shuddha Shilajit [21]. It has already been proven as standardized Ayurvedic herbo-mineral formulation [22]. It is manufactured and marketed by Vasu Healthcare Pvt. Ltd. (Vadodara, Gujarat, India) under the name of Ural-BPH capsule. Ingredients of the herbo-mineral formulation are well reported in Ayurvedic texts and scientific research publications for anti-inflammatory, diuretic, antioxidant and 5α-reductase activity. However, no such evidence was found which proves efficacy of their combination.

In the present study, an attempt was made to investigate effect of the abovementioned Ayurvedic herbo-mineral formulation on testosterone propionate-induced BPH in male Wistar rats.

MATERIALS AND METHODS

Preparation of test drug and dosage

The herbo-mineral formulation (Ural-BPH) was received from its manufacturer. Powder of the formulation was triturated with 1% carboxymethyl cellulose (CMC) solution to make suspension. Dose of the test drug was fixed by extrapolating the human dose to laboratory animals, based on body surface area ration as per the table of Paget and Barnes [23].

Experimental animals

Male Wistar rats weighing 180-250 g were used. Animals were housed in standard polypropylene cages (three rats/cage) and maintained under controlled room temperature (22 ± 2°C) and humidity (55 ± 5%) with 12:12 h light:dark cycle. All the rats were provided with commercially available rat pellets diet and water ad libitum, prior to the dietary manipulation. The guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) of the Indian Government were followed. Prior permission was taken from the institutional animal ethics committee (IAEC Protocol No. M.Ph. Sem-IV/12-13/05) for conducting the study.

Experimental design

The selected animals were divided into six groups where each group consisted of six animals:

- Group I (NC): served as normal control and received 1% CMC (p.o.) as vehicle + corn oil (s.c.)
- Group II (NC-UBPH1): served as normal control administered with herbo-mineral formulation once daily (90 mg/kg, once daily, p.o.) + corn oil (s.c.)
- Group III (NC-UBPH2): served as normal control administered with herbo-mineral formulation twice daily (90 mg/kg, twice daily, p.o.) + corn oil (s.c.)
- Group IV (DC): served as disease control and received testosterone propionate in corn oil (3 mg/kg, once daily, s.c.)
- Group V (TD-UBPH1): Served as test drug treated group and received herbo-mineral formulation once daily (90 mg/kg, p.o.) + testosterone propionate in corn oil (3 mg/kg, s.c.)
- Group VI (TD-UBPH2): Served as test drug treated group and received herbo-mineral formulation twice daily (90 mg/kg, p.o.) + testosterone propionate in corn oil (3 mg/kg, once daily, s.c.)

Testosterone propionate-induced BPH model

Experimentally, BPH was developed by s.c. administration of testosterone propionate (3 mg/kg) dissolved in corn oil for 28 days [7]. The test drug was administered orally for 28 consecutive days. Animals of groups II and III received test drug once daily and twice daily, respectively. Group-II and III were designed to evaluate a potential effect of the herbo-mineral formulation on healthy rats. Group V and VI were treated with the herbo-mineral formulation once and twice daily, respectively, along with subcutaneous administration of testosterone propionate. On 28th day, after administration of test drug, animals from different groups were kept in metabolic cages. After 3 h urine volume was measured. On 29th day, the rats were anesthetized and sacrificed. Blood samples were collected to evaluate different parameters like blood urea nitrogen (BUN) [24], serum creatinine [25], serum total protein [26], serum total cholesterol [27], serum albumin [28], serum globulin [28] and serum albumin/globulin (A/G) ratio. Immediately, prostate glands were dissected out and weighed. Length and width were also measured with the help of vernier caliper. Then prostates were preserved in 10% formalin solution for histopathological examination.

Prostate weight index

Prostate weight (PW) index was calculated by dividing prostate weight of each animal by its body weight and multiplied with 100 [16].

Histopathology of prostate

Under anesthesia, prostate gland from animals of each group was removed after sacrificing. They were preserved in bottle containing 10% formalin solution and immediately processed by paraffin technique. Section of approximately 5 μm thickness was cut and stained by hematoxylin and eosin (H&E). Sections were examined under microscope to evaluate structural changes.

Statistical analysis

Analysis was done with the help of GraphPad Prism (version 5) software. Results were expressed as mean ± standard error of the mean. Different groups were compared with analysis of variance (ANOVA) followed by post hoc Bonferroni’s test. P < 0.05 was considered as statistically significant.
RESULTS

Effect on urine volume

Urine volume of DC group was reduced significantly (P < 0.001) as compared to NC. No significant change was observed due to treatment of the herbo-mineral formulation in NC-UBPH1 and NC-UBPH2. On the other hand, TD-UBPH1 and TD-UBPH2 showed significant increase (P < 0.001) in urine volume as compared to DC group (Fig.1).

Effect on prostatic parameters

The DC group animals were treated with testosterone injection, it showed significant decreases (P < 0.001) in PW, PW index, prostate length and prostate width compared to NC group. Treatment with herbo-mineral formulation (TD-UBPH1 and TD-UBPH2 groups) presented significant increases in PW, PW index, prostate length and prostate width when compared with DC group. No change was observed due to treatment with the herbo-mineral formulation in NC-UBPH1 and NC-UBPH2 groups. (Table 1)

Effect on serum biochemical parameters

The level of BUN was significantly (P < 0.001) elevated in DC group when compared with NC group. Significant reversal (P < 0.001) was observed in TD-UBPH1 and TD-UBPH2 groups in comparison to DC group. Administration of herbo-mineral formulation did not affect the normal level of BUN as shown in groups NC-UBPH1 and NC-UBPH2. (Table 2)

The levels of serum creatinine, total protein and cholesterol were significantly increased (P < 0.001) in DC group with respect to NC group. The herbo-mineral formulation showed significant reduction (P < 0.001) in all three parameters at both dose levels when compared with DC group. (Table 2)

The levels of serum albumin and globulin significantly increased (P < 0.01 and 0.001, respectively) in DC group when compared with NC. TD-UBPH1 group did not show reduction in the elevated level of serum albumin, but TD-UBPH2 group caused significant reduction (P < 0.01) of the same in comparison to DC group. Serum globulin level was significantly reduced (P < 0.001) in TD-UBPH1 and TD-UBPH2 groups compared to DC group. Serum A/G ratio was also altered at significant level (P < 0.01) (Table 2).

Histopathological findings

Histology of prostate gland in NC group showed normal histological features. Acini of variable diameter were observed normal in this group. The tubules of variable diameter and irregular lumen with single layer of epithelium were observed. The lumens were filled with prostatic secretions and the matrix of connective tissue was normal (Fig.2a). Treatment of herbo-mineral formulation in normal rats did not show any histological changes of prostate gland; they resembled characters of NC group (Figs.2bc). DC group showed mild to moderate disruption of cyto-architecture structure of the gland. The walls of tubules were thickened and almost every tubule developed large involutions projecting into the lumen, reducing the volume of the lumen compared with the NC group. Overall histology of DC group revealed characteristics of prostatic tissue hyperplasia (Fig.2d). TD-UBPH1 and TD-UBPH2 groups showed marked reduction in thickness of epithelium wall of tubules. There was better cyto-architectural arrangement comparable with normal prostatic tissues in TD-UBPH1 and TD-UBPH2 groups as compared to DC animals (Figs.2ef).

Table 1. Effect of herbo-mineral formulation on prostatic parameters (mean ± SEM, n = 6)

<table>
<thead>
<tr>
<th>Groups</th>
<th>Prostate weight (g)</th>
<th>Prostate weight index</th>
<th>Prostate length (cm)</th>
<th>Prostate width (cm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>0.61 ± 0.04</td>
<td>0.24 ± 0.02</td>
<td>1.42 ± 0.19</td>
<td>1.41 ± 0.15</td>
</tr>
<tr>
<td>NC-UBPH1</td>
<td>0.59 ± 0.03</td>
<td>0.24 ± 0.01</td>
<td>1.4 ± 0.11</td>
<td>1.48 ± 0.11</td>
</tr>
<tr>
<td>NC-UBPH2</td>
<td>0.64 ± 0.04</td>
<td>0.26 ± 0.02</td>
<td>1.52 ± 0.13</td>
<td>1.51 ± 0.13</td>
</tr>
<tr>
<td>DC</td>
<td>2.1 ± 0.07**##</td>
<td>0.84 ± 0.03**##</td>
<td>2.58 ± 0.19**##</td>
<td>2.56 ± 0.18**##</td>
</tr>
<tr>
<td>TD-UBPH1</td>
<td>0.96 ± 0.06***##</td>
<td>0.38 ± 0.02***##</td>
<td>1.67 ± 0.11**</td>
<td>1.85 ± 0.08**</td>
</tr>
<tr>
<td>TD-UBPH2</td>
<td>0.84 ± 0.06***##</td>
<td>0.33 ± 0.02***##</td>
<td>1.66 ± 0.09***##</td>
<td>1.67 ± 0.08***##</td>
</tr>
</tbody>
</table>

##P < 0.01, ###P < 0.001 compared to normal control (NC); **P < 0.01, ***P < 0.001 compared to disease control (DC) group.

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Table 2. Effect of herbo-mineral formulation on serum biochemical parameters (mean ± SEM, n = 6)

<table>
<thead>
<tr>
<th>Groups</th>
<th>BUN (mg/dl)</th>
<th>Creatinine (mg/dl)</th>
<th>Total protein (g/dl)</th>
<th>Total cholesterol (mg/dl)</th>
<th>Albumin (g/dl)</th>
<th>Globulin (g/dl)</th>
<th>A/G ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>NC</td>
<td>40.81±1.3</td>
<td>0.67±0.03</td>
<td>5.11±0.58</td>
<td>56.52±4.06</td>
<td>3.89±0.41</td>
<td>1.6±0.56</td>
<td>2.43±0.57</td>
</tr>
<tr>
<td>NC-UBPH1</td>
<td>40.67±2.22</td>
<td>0.68±0.04</td>
<td>5.23±0.48</td>
<td>58.31±4.09</td>
<td>3.67±0.35</td>
<td>1.78±0.42</td>
<td>2.06±0.69</td>
</tr>
<tr>
<td>NC-UBPH2</td>
<td>41.9±1.85</td>
<td>0.66±0.04</td>
<td>5.16±0.68</td>
<td>64.97±4.17</td>
<td>3.86±0.39</td>
<td>1.76±0.56</td>
<td>2.19±0.32</td>
</tr>
<tr>
<td>DC</td>
<td>163±3.13###</td>
<td>1.51±0.09###</td>
<td>9.61±0.65###</td>
<td>189±11.61###</td>
<td>5.79±0.35###</td>
<td>3.78±0.64###</td>
<td>1.53±0.31###</td>
</tr>
<tr>
<td>TD-UBPH1</td>
<td>53.22±1.91***</td>
<td>0.87±0.04***</td>
<td>5.79±0.51***</td>
<td>64.96±5.49***</td>
<td>4.43±0.34</td>
<td>1.37±0.36***</td>
<td>3.23±1.04***</td>
</tr>
<tr>
<td>TD-UBPH2</td>
<td>45.57±1.97***</td>
<td>0.72±0.04***</td>
<td>4.88±0.63***</td>
<td>50.07±6.12***</td>
<td>3.75±0.22**</td>
<td>1.41±0.51***</td>
<td>2.65±0.93***</td>
</tr>
</tbody>
</table>

**P < 0.01, ***P < 0.001 compared to normal control (NC); 'P < 0.01, ***P < 0.001 compared to disease control (DC) group.

DISCUSSION

Benign prostatic hyperplasia (BPH) is one of the most common diseases among aged men. It produces lower urinary tract symptoms which reduce the quality of daily life [2]. Safe and effective natural interventions that reduce the symptoms and reverse or halt the progression of BPH have been the subject of considerable research interest. The present study was undertaken to investigate safety as well as efficacy of an Ayurvedic herbo-mineral formulation in testosterone propionate-induced BPH in male Wistar rats. Treatment of herbo-mineral formulation was also provided to normal rats in order to evaluate safety profile in healthy subjects.

Cell proliferation in the prostate gland can obstruct urine flow by constricting the urethra which affects the evacuation of urine [11]. Urine output was significantly increased after treatment with the herbo-mineral formulation when compared with DC group. This result indicates the positive effect of the used herbo-mineral formulation on urine flow (Fig. 1).

The prostate gland is an androgen dependent organ. In the body, testosterone is converted into DHT in the...
prostate stroma under the action of the 5α-reductase and exerts its effects through the specific receptor on the nuclear membrane of the prostate stromal cells. Testosterone plays important role in development of BPH which reflects by increase in prostate weight and size [29]. The DC rats, treated with s.c. injection of testosterone exhibited enlargement of prostate in significant manner. Treatment with the herbo-mineral formulation showed resulted in significant reduction in PW, PW index, prostate length and width when compared with DC group. No marked changes were observed in prostatic parameters in normal rats treated with herbo-mineral formulation. This indicates that the test drug has no adverse effect on prostatic parameters of healthy rats (Table 1).

Clinically BPH is reflected by LUTS, urinary tract infections (UTI) or acute urinary retention (AUR) due to urethral obstruction. Diabetes or hypertension appears to be the most likely cause of elevated serum creatinine measurements in men with BPH [30]. Administration of herbo-mineral formulation at both dose levels showed significant reduction in serum creatinine and BUN when compared to DC group. The level of serum creatinine and BUN were not affected with treatment of herbo-mineral formulation in healthy rats (Table 2).

Chronic inflammation has been reported to be associated with the pathogenesis of BPH and LUTS. It might be attributed to the over-expression of the pro-inflammatory cytokines including interleukin (IL)-6, IL-8 and tumor necrosis factor (TNF)-α. It is reported that serum total protein level is significantly associated with inflammation of prostate in BPH patients [7, 31]. Serum total protein content was significantly increased in DC group in comparison to NC group. Treatment with herbo-mineral formulation showed significant reduction in elevated level of serum total protein (Table 2).

Nandeesha et al [32] found that total cholesterol and low density lipoprotein (LDL)-cholesterol were significantly higher and high density lipoprotein (HDL)-cholesterol was significantly lower in BPH cases compared to control. They reported that insulin had a significant regression with cholesterol, triglycerides, LDL cholesterol and VLDL cholesterol. Insulin is claimed to be involved in pathogenesis of BPH through its action on sympathetic nerve activity, sex hormones and insulin-like growth factor (IGF) axis. They suggested that dyslipidemia in BPH occurs due to insulin resistance and insulin has some role in promotion of prostate growth. Similarly, significant elevation was found in DC group when compared to NC group in our study. Elevated level of serum cholesterol was significantly reduced by treatment with the herbo-mineral formulation at both dose levels (Table 2). Data indicates that the herbo-mineral formulation might be useful to treat risk factors such as diabetes mellitus, hypertension, obesity and dyslipidemia in the patient with BPH.

Elevated level of serum total protein in association with concentration of serum albumin and globulin are also affected in BPH patients [33]. DC group showed significant elevation in level of serum albumin and globulin when compared with NC group. Treatment with the herbo-mineral formulation at both dose levels showed significant reduction in globulin level. TD-UBPH2 group showed significant reduction in serum albumin level while TD-UBPH1 showed reduction but not at significant level (Table 2).

Histopathology of prostate glands revealed that treatment with the herbo-mineral formulation in this study did not produce any cyto-architectural changes on healthy male wistar rats. Subcutaneous induction of testosterone showed marked characteristic changes of hyperplasia in histology of prostate glands which was significantly reversed by herbo-mineral treatment at both dose levels (Fig.2).

On the basis of study data it can be concluded that the used Ayurvedic herbo-mineral formulation has promising effect on testosterone propionate-induced BPH in male Wistar rats. It has been observed that the test drug did not show any side effect on the healthy rats. This study data reveals that the tested herbo-mineral formulation is safe and effective in BPH.

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COMPETING INTERESTS

The author(s) have no competing interests for finance, publication of this research, patents and royalties through this collaborative research. All authors were equally involved in discussed research work. There is no financial conflict with the subject matter discussed in the manuscript.

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