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Scientific Evaluation of A Unani Pharmacopoeia-Based Formulation on BPH in Animal Model

Mohammad Shujauddin, Shamshad Alam, Sumbul Rehman, Murad Ahmad

Abstract: ***BPH (Benign prostatic hyperplasia) is a common problem of the prostate gland that affects elderly men with age and major cause of lower urinary tract symptoms (LUTS) like urgency of urination, frequency of micturition, dysuria, nocturia, dribbling, and burning of urine. If left untreated, the condition can lead to severe urinary retention, recurrent UTI, and, in severe cases, renal failure, etc. Alpha-blockers and 5-alpha reductase inhibitors (5-ARIs) are two main classes of drugs that are used in conventional therapy to treat BPH. These phytochemical therapies are extremely effective, but standard control methods have unfavorable side effects too. Conversely, evidence, though limited, has shown that alternative medicines are safer and have the potential to improve LUTS and quality of life, in addition to improving sexual dysfunction in patients with BPH. The purpose of the study is to assess the efficacy of 50% hydroalcoholic UPBF extract on BPH in TP-induced rats, and the effects of the drug were compared between the treated and untreated groups. The experimental design for anti-BPH activity was carried out following the Bhavin model. 48 Wistar rats were divided into 6 groups, with 8 rats in each group. Group 1, plane control (untreated rats), group 2, negative control (treated with TP 5 mg/kg), group 3, negative control (treated with TP+ finasteride 1 mg/kg), and groups 4,5 and 6 served as tests controls (A, B, and C) treated with TP+ UPBF extract 100 mg/kg, 150 mg/kg, and 200 mg/kg as low, medium, and high doses, respectively. BPH was induced in rats by administration of testosterone propionate (TP) in arachis oil, and treatment was done orally with a standard drug (finasteride) and a test drug (UPBF) once a day for 28 consecutive days. Before and after initiating the study, each rat was weighed and documented, and after 28 days, the collection of blood samples and the prostate gland was done under light anesthesia. The parameters of the study were accessed, like prostate weight, prostate-to-body weight ratio, histopathological investigation of the prostate, percentage of inhibition, percentage of recovery, and determination of ST and STP levels. Data from the results were expressed as the mean ± standard error of the mean (SEM). Evaluation between group results was carried out with the one-way ANOVA test followed by Tukey's test, and the significant value of p was <0.05. Both histopathological and biochemical parameter results showed significant improvements in rats treated with finasteride and UPBF extract at a dose of 200 mg/kg (p<0.001).***

In addition, ST and STP levels showed a significant reduction in comparison with the BPH group. It can be concluded from the findings of the results in both biochemicals and histopathological parameters that the test drug (UPBF) possesses significant effects against the TP-induced BPH in rats. The findings suggested that the test drug can be used independently in the management of BPH as well as for lower urinary tract symptoms.

Keywords: ***Waram-e-Gudda-i-Madhi, Insidad-e-Majra-i-Mathana, UFBF on BPH, Tribulus terrestris in the Unani system, benign prostatic hyperplasia, lower urinary tract symptoms (LUTS).***

I. INTRODUCTION

Benign prostatic hyperplasia (BPH) is a chronic andrological disorder with a high prevalence among men. Its prevalence is approximately 60% in people aged 50 to 60, and it increases to up to 70% in men in the sixth decade of life. BPH, the nonmalignant growth of prostate glands, is associated with urinary obstruction in men[1]. This can affect the quality of life, and patients may suffer from complicated problems such as burning micturition, retention of urine, urgency and frequency of micturition, UTI, and bladder stones[2]. Many factors and etiologies are involved that can lead to this disease, such as an imbalance in sexual hormones, especially androgens and their metabolites, aging, and a dietary lifestyle. Aerobic exercise, which can lower insulin, insulin-like growth factor-1, estradiol, or testosterone levels in the body, is one lifestyle factor that experts have discovered to be significant for BPH[3]. Moreover, they verified the relationship between BPH and high blood pressure, obesity, and metabolic diseases such as diabetes[4]. Although androgens are not directly linked to BPH, an excess of androgen hormones can be observed in this condition.[5]. Androgen is converted to estrogen and dihydrotestosterone by the enzyme's aromatase and 5a-reductase, respectively. The activation of these enzymes increases with age. Due to decreased testosterone levels, an increase in its metabolites, and the role of estrogen in prostate cell growth, the risk of BPH increases [6]. The prostate glands are anatomically made of capsules and stromal tissue, which is highly concentrated with androgen receptors, while the bladder has a few alpha receptors[7]. During drug therapy, these receptors and enzymes are selectively targeted[8].

1.1. Concept of BPH in the Unani system of medicine: Unani medical philosophy is based on the traditional theory of four humours (*Akhlat*), which holds each person in their bodies in a certain balance, namely: phlegm (*Balgham*), blood (*Dam*), yellow bile (*Safra*), and black bile (*Sauda*)[9]. Any disturbance in their equilibrium will result in distemper (*Su-i-mizaj*) and, consequently, sickness.

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*Correspondence Author (s)
Dr. Mohammad Shujauddin*, Research Scholar, Department of Ilmul Advia, Ajmal Khan Tibbia College, Faculty of Unani Medicine, AMU, Aligarh (UP), India. E-mail: ms.suza@rediffmail.com, ORCID ID: 0009-0003-3384-3282
Shamshad Alam, Assistant Professor, Department of Ilmul Advia, Ajmal Khan Tibbia College, Faculty of Unani Medicine, AMU, Aligarh (UP), India.
Sumbul Rehman, Assistant Professor, Department of Ilmul Advia, Ajmal Khan Tibbia College, Faculty of Unani Medicine, AMU, Aligarh (UP), India.
Murad Ahmad, Assistant professor, Department of Pathology, Jawahar Lal Nehru Medical College, AMU, Aligarh (UP), India.

Blood predominates, resulting in a sanguine temperament, and bile, resulting in biliousness. Phlegum, resulting in a phlegmatic temperament, and black bile, resulting in a melancholic temperament. Temperaments with a sanguine or bilious nature are hot, whereas those with a phlegmatic or melancholic nature are cold[10]. The fundamental ideas behind any treatment of disease in the Unani system are to eradicate the causes (*Azala-e-Sabab*), restore humoral equilibrium (*Tadeel-e-Akhlat*), and normalize organs and tissues (*Tadeel-e-Aza*)[10]. In the classical literature of the Unani books, the word BPH can be found under the terms *Sal'a Ghudda-i-Madhi* (prostatic tumor) or *Waram-e-Ghudda-i-Madhi* (prostatitis), *Waram-e-Unuq-al-Mathana* (bladder neck swelling), and *Insidad-e-Majra-i-Mathana* (bladder outlet obstruction)[10]. Avicenna, an Arabic physician, and philosopher (980–1037 CE), claimed that this swelling could be either hot (*Waram-e-Harr*) or hard (*Waram-e-Sulb*). Along with the obstructive urinary symptoms, hot swelling, also known as inflammation (*Iltihab*), causes the symptoms of hot distemper (*Su'-i-mizaj-e-Harr*), such as burning micturition (*Sozish-e-Bol*), increased thirst, and fever. Contrarily, hard swelling (*Waram-e-Sulb*), which frequently appears after inflammation or as a result of wear and tear, prevents both the urine and feces from leaving the body, causing dysuria (*Usr-e-Bawl*), anuria (*Ihtibas-e-Bawl*), dribbling (*Taqtir-e-Bawl*), and constipation (*Qabz*)[10]. Allama Jurjani (Arabic physician, 1041–1136 CE) has categorized the swelling at the bladder neck into hot swelling (*Waram-e-Harr*) and cold swelling (*Waram-e-Barid*). According to him, hot swelling is due to the humours of hot temperaments, which are blood or yellow bile, while cold swelling is due to the humours of cold temperaments, which are phlegm or black bile[11]. Additionally, hormones are divided into two categories: phlegm and white humor[12]. The most widely known etiology of BPH in the current era is a disturbance with the testosterone-to-DHT ratio or simply a hormonal reason, which strongly supports our humoural theory that hormonal disequilibrium is truly a humoural disequilibrium[13]. In the Unani perspective, the disequilibrium of phlegm in the body is the primary cause of prostatic hypertrophy. The most significant signs of phlegmatic disorders are abnormal white-colored urine (*Bawl-e-Abyaz*), increased frequency of micturition or polyurea (*Kasrat-e-Bawl*), urgency, and incontinence[14]. All the above statements support the correlation between swelling, prostatic hyperplasia, or prostatitis, and LUST-like symptoms. Traditional medicines for the management of BPH in Unani literature, the herbs that have been documented to be used as nephroprotective agents may be useful in prostate disease as well. The seeds of *Ajwain* (*Trachyspermum ammi*) have been used as a digestive, appetizer, carminative, diuretic, and emmenagogue[15]. The seeds of *kharpazah* (*Cucumis melo*) are used as demulcent, diuretic, lithotriptic, and detergent and are also used in burning micturition and oliguria[16]. The seeds of *khayar* (*Cucumis sativus*) are used fully in thirst, strangury, painful micturition, oliguria, and renal and vesicle calculi[17]. The roots of Taraxacum officinale are effectively used in chronic renal disorders[18]. *Banadiq-ul-buzoor* is a polyherbal formulation that is widely used in LUTS, and recently it has been investigated for BPH[19]. Some crude herbs taken in formulation form for the current study are (a) *Baadiyaan* (*Foeniculum vulgare* Mill., from the Umbelliferae family). Its major components are volatile oil, fenchone, methyl chavicol, flavonoid, etc. The seeds are widely used as an anti-inflammatory, anti-cancer, diuretic, anti-carcinogenic, etc[20]. (b) *Giloe* (*Tinospora cardifolia* Miers., from the Menispermaceae family). Diterpenoid lactones, glycosides, steroids, sesquiterpenoids, phenolics, and aliphatic compounds are its major components[21]. Due to their drug properties, it is used as an anti-inflammatory and anti-cancer[22]. (c) *Kasni* (*Cichorium intybus* Linn., from the Asteraceae family). The root and leaves are used in Unani medicine, its valuable components are sesquiterpene lactones (sonchusides A and C), cytokinin, crepidiase B, cichoriolide A, etc[23]. Due to these components, the drug is used as an anti-inflammatory, anti-tumor, and anti-fungal[24]. (d) *Khar-e-Khasak Khurd* (*Tribulus terrestris* Linn., from the Zygophyllaceae family). Its bioactive components are saponins, alkaloids, steroids, flavonoids, tannins, vitamins, glutamic acid, and aspartic acid[25]. So, the drug is used as a diuretic, anti-inflammatory, nephroprotective, and anti-tumor[26]. (e) *Kundur* (*Boswellia serrata* Roxb., from the Burseraceae family). Its exudation and oleo gum resin are used as medicine[27]. Terpenoids and boswellic acid are major components[28]. Anti-tumor and anti-carcinogenic[29], anti-inflammatory[30], and anti-fungal studies have been done[31]. (f) *Mako* (*Solanum nigrum* Linn., from the Solanaceae family). Its major active components are glycoalkaloids, glycoproteins, and polysaccharides. It also contains polyphenolic compounds[32]. Various studies have been done on cardio-protective activity[33], anti-tumor activity, anti-cancerous activity[34], and anti-HCV activity[35]. (g) *Qurtum* (*Carthamus tinctorius* Linn., from the Compositae family). Isolated chemical ingredients are carthamin, flavonoids, alkaloids, lignans, carboxylic acids, steroids, quinochalcone, and C-glycosides[36-37]. The drug is investigated for anti-inflammatory, antipyretic, and analgesic effects[38], an antidiabetic effect[39], and an antitumor effect[40].

II. MATERIAL AND METHODS

2.1. Collection of Drug Samples: The sample of test drugs was procured from Dawakhana Tibbiya College, A.M.U. Aligarh, U.P., and identified properly by a plant taxonomist at the pharmacognosy department of A.K.T.C., AMU, Aligarh. All ingredients have been submitted with voucher numbers to the Ibne Baitar Advia Museum, Department of Ilmul Advia, AMU, Aligarh, for record and future reference.

2.2. Preparation of the test drug for extraction: All drugs were cleaned and dried under shade, grounded into coarse powder, and passed through sieve no. 80 to establish uniformity in particle size. Fractions of 100 g of the dry powder were packed in filter paper thimbles and extracted by using a Soxhlet extractor. The temperature was maintained between 70 and 80°C for 2–3 hours. Thereafter, it was concentrated until the entire ethanol solvent was evaporated in the water bath at 45°C. After that, it was preserved in a vacuum desiccator over silica gel and used to conduct the study.

2.3. Animals: 48 male Wistar rats weighing 150–200 g were procured from the Central Animal Facility, AIIMS, New Delhi, and they were housed in propylene cages under standard laboratory conditions (half-day light/dark cycle at 22 ± 3°C). During the experimental period, they were fed a balanced commercial pellet diet and water ad libitum. After 7 days of acclimatization, they were randomly divided into experimental groups. All experimental procedures were followed according to CPCSEA guidelines. The study was reviewed and approved by the Institutional Animal Ethics Committee, AKTC, AMU, Aligarh.

2.4 Experimental design and doses: The study design was followed by the Bhavin model (2013)[41]. 48 Wistar rats were divided into six groups, each group containing eight rats. Group 1 (plane control) was the reference group without BPH induction, and Group 2 (disease control) was treated with just 5 mg/kg of TP (S.C.), in arachis oil, group 3 serves as the standard control, treated with 5 mg/kg of TP injection and 1 mg/kg of finasteride by gavage, and groups 4, 5, and 6 (test-A, B, and C control) treated with 5 mg/kg of TP+100 mg/kg, 150 mg/kg, and 200 mg/kg of UPBF extract as low, medium, and high doses by gavage once a day for consecutive 28 days.

2.5. Body weight: The body weight of each animal was documented weekly throughout the study period, and at the end of the study, they were weighed before being sacrificed.

2.6 Prostate Weight: After the 28th day, the rats had no access to food or drinking water for 12 hours. They were scarified by light ether anesthesia, and the prostate was removed and weighed immediately.

2.7 Prostate weight to body weight ratio. It was calculated as follows. (Bhavin *et., al.* 2013)

$$\textbf{Prostate index} = \frac{\textbf{Prostate weight}}{\textbf{Body weight}}$$

2.8. Percentage of Inhibition. It was calculated as follows (Veeresh Babu *et al.*,2010) (Table 2.5)

$$\textbf{100- \{[}\frac{\textbf{Prostate weight of treated group} - \textbf{P.W. of plane control}}{\textbf{the Prostate weight of disease control} - \textbf{P.W. of plane control}}\textbf{]}\times\textbf{100}$$

2.9. Percentage of recovery: The 100% increase in prostate weight induced by testosterone was to be considered alone and used as a standard reading for the comparison of test groups as follows. **Percent of recovery of the test sample = [A - B]**, where A was considered an increased percentage of prostate weight induced by testosterone injection and B was considered an increased percentage of prostate weight in the test sample. (testosterone +finasteride or UPBF extract.

2.10. Serum testosterone and protein level: After collection of the blood sample, it was placed in test tubes, labeled, left at room temperature for 2-3 hours to clot, and centrifuged for 5 minutes at 2000 rpm. The separated serum was tested for serum testosterone and serum total protein level by using standard diagnostic kits (ELISA-enzyme-linked immunosorbent assay kit).

2.11. Histopathological investigation: The entire prostate was isolated from the body and weighed immediately and preserved in formalin (10%) before processing. The prostate was dehydrated with 100% ethanol first before being engrafted with paraffin wax. A 4-5 µm-thick section was then cut and stained with eosin and hematoxylin. A photomicroscope (under 40x) was used to examine the slides.

2.12. Statistical Analysis: Data from the results were expressed as the mean ± standard error of the mean (SEM). Evaluation between group results was carried out with the one-way ANOVA test followed by Tukey's test, and the statistically significant value of p was <0.05. Statistical analysis was performed with GraphPad Software Inc., USA.

III. RESULTS

3.1. Prostate weight: A significant increment ($p<0.001$) in prostate weight was found in the TP (5 mg/kg) treated group when compared with the plane control group, and a specific reduction in increased prostate weight was also found in the finasteride (1 mg/kg) and UPBF extract treated groups (low, medium, and high dose) when compared with the testosterone-treated group. These findings were statistically significant ($p<0.001$). (Table 1, Fig.1)

3.2. Prostate weight to body weight ratio: The groups treated with TP (5 mg/kg) were found to significantly increase the prostate-to-body weight ratio in comparison with the plane control ($p<0.001$), and the finasteride-treated group and test-A group were found to be similar to the TP-treated group. The finding was statistically not specific ($p<0.05$), and groups B and C treated with UPBF extract were found to significantly decrease the prostate-to-body weight ratio when compared with the TP-treated group. (Table- 1)

3.3. Percentage of recovery in P.W: >7% recovery in PW was found in the positive control and test-C groups when compared with the BPH group. (Table 1, Fig.1)

3.4. Serum testosterone: The group treated with TP (5 mg/kg) was found to have significantly elevated ST levels ($p<0.001$) in comparison with the plane control, and the group treated with finasteride and test-B and C (treated with UPBF extract) displayed a meaningful relationship between the standard drug and UPBF extract when compared with the disease group ($p<0.001$) and ($p<0.001$), respectively. The group treated with UPBF extract at a low dose (100 mg/kg) showed a dose-dependent effect on ST level when compared with the finasteride group. (Table 2, Fig. 2)

3.5. Serum total protein: A significant elevation of STP level was found in the TP (5 mg/kg) treated group when compared with the plane control group ($p<0.001$). The group treated with finasteride and test-B and C (treated with UPBF extract) in STP levels was found to decrease in comparison with the disease group ($p<0.001$), ($p<0.01$), and ($p<0.01$), respectively.

The group test-A was treated with a low dose (100 mg/kg), but nothing responded at the minimum dose, so no significant difference was observed between the disease control and the UPBF extract with a dose of 100 mg/kg ($p<0.05$) (Table 2, Fig. 2).

3.6. Histopathology examines: According to H&E Figure 5, the BPH control group indicated an obvious disruption in prostatic tissue, shown as complex architectures, increased prostate gland-to-stromal ratio, inflammatory cell hyperplasia, hypertrophy of the epithelium, and progression of stromal proliferation. A few glands show epitheliosis. The focal areas are visible in prostatic intraepithelial neoplasm grade II. These results are significant when compared with the plane control group. A meaningful reduction is noticed in test control-C (UPBF extract at a dose of 200 mg/kg) on the progression of stromal proliferation, inflammatory cell hyperplasia, and epithelial cell hypertrophy. In comparison with the BPH group, it was found that the maximum dose of UPBF extract (150 mg/kg–200 mg/kg) is fully effective in the prevention of inflammatory cell hyperplasia, epithelial cell hypertrophy, and the progression of stromal proliferation. In this test, a clear benefit of finasteride is recorded in the prevention of inflammatory cell hyperplasia, epithelial cell hypertrophy, and the progression of stromal proliferation. Overall, the histopathological section shows similarity to the positive control group in covering prostate tissue injury. (Fig-3.1-3.6)

Table 1: Effects of UPBF extract on BW and Prostate Enlargement in Testosterone-Treated Rats

Groups (n=6)	Initial body weight (g)	Final body weight	P.W. (g)	% of inhibition	PW/BW ratio (g/g×1000)	% of inhibition	% of recovery
Plan. Cont.	151.5±2.74	267.25±7.96	0.742±0.039 $b^{3\#}$ c^3 d^3 e^3 f^3	----------	6.410±0.116 b^3 c^3 d^3 e^1 f^2	----------	----------
N. Cont.	155.75±2.15	235.25±8.53	1.51±0.055 a^3 c^3 d^1 e^2 f^0	----------	18.704±0.189 a^3 c^0 d^0 e^1 f^0	----------	----------
P. Cont.	157.5±2.79	261.25±12.45	1.487±0.117 a^3 b^0 d^0 e^1 f^0	97%	11.277±0.486 a^3 b^3 d0 e^1 f^1	39.58%	7.42%
Test – A	156.25±3.161	238.75±7.425	1.462±0.082 a^3 b^0 c^0 e^3 f^2	93%	17.721±0.487 a^3 b^3 c^0 e^3 f^3	92%	0.98%
Test – B	156.5±4.08	258.125±5.97	1.17±0.030 a^3 b^0 c^0 d^0 f^0	55.72%	14.793±0.244 a^3 b^0 c^0 e^0 f^3	68%	4.41%
Test – C	157.75±0.08	268.75±3.981	1.262±0.041 a^3 b^3 c^0 d^0 e^3	67.70%	11.369±0.142 a^3 b^0 c^0 e^0	40.33%	7.35%

(PW: prostate weight, BW: body weight, TP: testosterone propionate, UPBF: Unani pharmacopoeia-based formulation, Pln. Cont.: plane control (untreated group), N. Cont.: negative control (treated with TP 5 mg/kg), P. Cont.: positive control (treated with TP+ finasteride 1 mg/kg), Test-A (treated with TP+ UPBF extract 100 mg/kg), Test-B (treated with TP+ UPBF extract 150 mg/kg), Test-A (treated with TP+ UPBF extract 200 mg/kg); the values are expressed as mean ± S.E.M. statistical analysis is done by one-way ANOVA followed by Tukey's multiple comparison test. (a=against plane control (0=p>0.05), b=against negative control (1=p<0.05), c=against positive control (2=p<0.01), d=against test-A (3=p<0.001), e=against test-B, f=against test-C)

Table 2: Effects of 50% HAE of the test drug on ST and STP levels in BPH-induced rats

S. NO.	GROUPS (N=6)	S.T. (µG/ML)		S.T.P. (G/DL)	
1.	**Plane control**	1.867±0.021	b^3 c^3 d^3 e^3 f^3	2.456±0.099	b^3 c^3 d^3 e^1 f^0
2.	**Negative control**	12.832±0.586	a^3 c^3 d^3 e^3 f^3	3.406±0.189	a^3 c^0 d^2 e^3 f^3
3.	**Positive control**	7.287±0.288	a^3 b^0 d^0 e^0 f^0	2.3±0.125	a^3 b^0 d^0 e^3 f^3
4.	**Test control-A**	10.621±0.349	a^3 b^0 c^3 e^3 f^3	3.237±0.161	a^3 b^2 c^1 e^3 f^3
5.	**Test control -B**	7.288±0.166	a^3 b^0 c^3 d^0 f^1	2.743±0.114	a^3 b^0 c^0 d^0 f^1
6.	**Test control -C**	7.281±0.325	a^3 b^0 c^3 d^0 e^1	2.54±0.126	a^3 b^0 c^0 d^0 e^0

(HAE: hydroalcoholic extract, ST: serum testosterone, STP: serum total protein, BPH: benign prostatic hyperplasia. Group-1 (untreated animal), group-2 (treated with TP 5mg/kg), group-3 (treated with TP+ finasteride 1mg/kg), group-4 (treated with TP+ UPBF extract 100mg/kg), group-5 (treated with TP+ UPBF extract 150mg/kg), group-6 (treated with TP+ UPBF extract 200mg/kg), the values are expressed as mean ± S.E.M. statistical analysis is done by one-way ANOVA followed by Tukey's multiple comparison test). (a = against plane control (0 = p>0.05), b = against negative control (1 = p<0.05), c = against positive control (2 = p<0.01), d = against test-A (3 = p<0.001), e = against test-B, f = against test-C).

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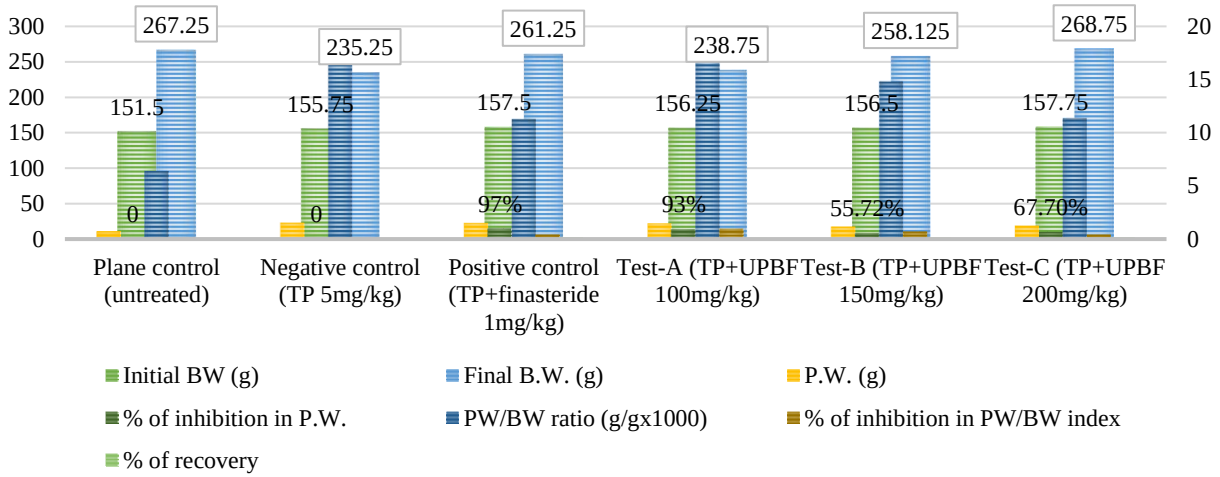

Fig. 1: Effect of UPBF extract on prostate enlargement and PW/BW index in TP-induced rats The findings are expressed by a column chart between groups. Statistical analysis was performed with GraphPad Software Inc., USA.

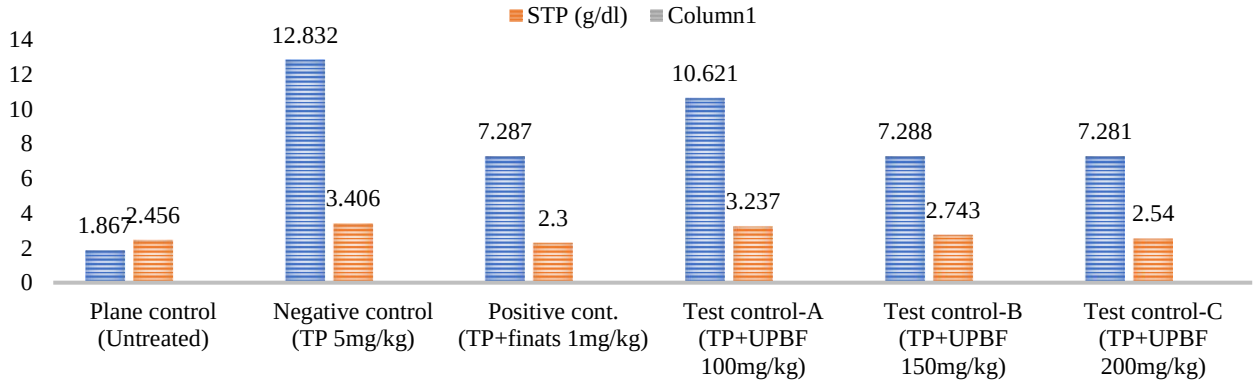

Fig-2: At the end of the study, ST (serum testosterone) and STP (serum total protein) levels were shown by a chart between groups: plane control (arachis oil), negative control (TP 5 mg/kg), positive control (TP+ finasteride 1 mg/kg), test-A, B, and C (TP+UPBF extract at doses of 100 mg/kg, 150 mg/kg, and 200 mg/kg). Statistical analysis was performed with GraphPad Software Inc., USA).

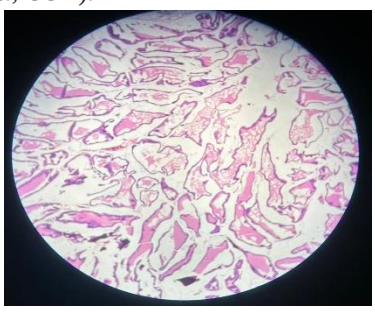

Fig:-3.1 (Plane control)

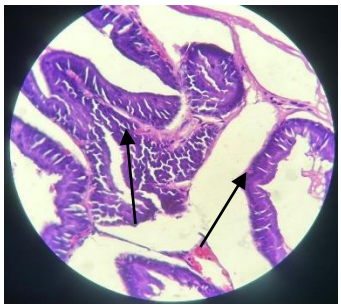

Fig:-3.2 (Negative control)

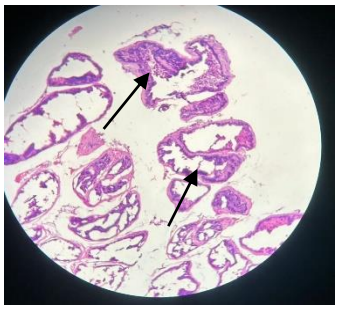

Fig:-3.3 (Positive control

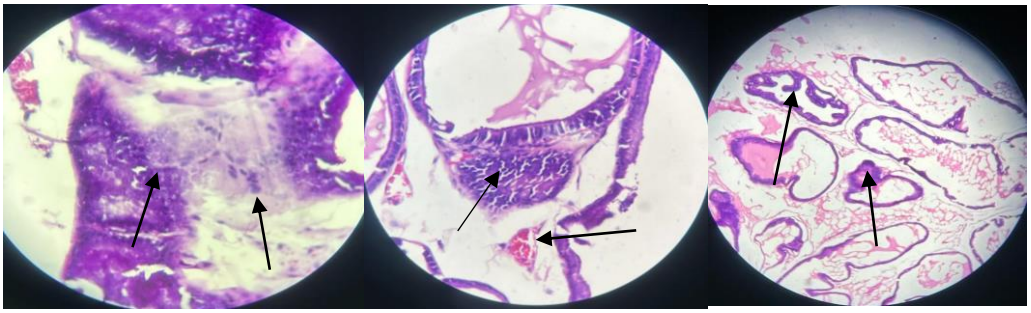

Fig:- 3.4 (Test control- A) *Fig:- 3.5 (Test control- B)* *Fig:- 3.6 (Test control- C)*

Photomicrograph of the prostate ventral lobe (H&E 10^{x}). The arrows represent morphological changes such as stromal proliferation, abnormal acinar fold, and hypertrophy, area PIN grade-II. Plane control (untreated animal), negative control (treated with TP 5mg/kg), positive control (treated with TP+ finasteride 1mg/kg), test control-A (treated with TP+ UPBF extract 100mg/kg), test control-B (treated with TP+ UPBF extract 150mg/kg), test control-C (treated with TP+ UPBF extract 200mg/kg.

IV. DISCUSSION

Unani medicine has a promising position to tackle the *Warm-e-Ghudda-i-Madhi* (BPH) and associated ailments, as this system of medicine shows peculiar characteristics to prevent the disease and strengthen the organs for their normal function. The Unani System of Medicine is based on four classical humoral theories, as mentioned earlier[38-39]. A unique balance of these humors determines the temperament of each organ and the whole body as well. The disturbance of their balance (equilibrium) leads to distemperament (*Sue-Mizaj*), which ultimately causes disease. The symptoms developed from obstruction due to the cold swelling (*Warm-e-Barid*), like the symptoms of BPH. So, the fundamental principles of treatment in Unani medicine to eliminate the disease are the elimination of the cause (*Izala-e-Sabab*), balance in humor (*Tadeele Akhlat*), and restoring the normal function of the organ (*Tadeel-e-Aza*). According to Western theory, BPH is a chronic andrological disorder in which the frequent inflammation of prostatic epithelial cells or stromal cells is an important factor that leads to hyperplasia of the prostate gland, which subsequently results in LUTS. BPH is related to inflammation, cell proliferation, and oxidative stress. The antioxidants are free radical scavengers that inhibit cell damage and related diseases. In this study, the administration of testosterone induces oxidative stress in prostate tissue, and this was observed in the BPH group. On the other hand, the test drugs are rich in anti-oxidant properties like flavonoids and phenol components. Due to the high number of flavonoids, phenolic compounds, and antioxidant capacity of the extract, this could be a possible mechanism for dealing with BPH. One more significant finding to emerge from this study is that UPBF extract can reduce BPH complications by inhibiting cell division and having an anti-inflammatory effect. The purpose of this study is to assess the efficacy of UPBF extract against anti-proliferative activity in rats, and numerous studies have been done on BPH using herbal drugs[75]. Therefore, we have made our best efforts to select the drugs among those that have been frequently prescribed for various renal and urinary disorders. Based on various pharmacological studies, the test drug was taken in combination for the study. In this study, biomarker examination showed a significant reduction in ST, STP level, and prostatic index ($p<0.001$) in comparison with the BPH group. Furthermore, no significant correlation was found between the high dose (200 mg/kg) of UPBF extract and standard medicine (finasteride). This finding ($p<0.001$) suggests that the efficacy of UPBF extract at a dose of 200 mg/kg was similar to finasteride in the treatment of BPH. If a complete reduction of prostate weight is found, that indicates the curative effect of the test drug, but if a partial or mild reduction is found, it means the protective effect of the test drug. The weight of the prostate gland in BPH-induced rats was found to be significantly ($p<0.001$) increased in comparison with the control group, which indicates the hormonal effects on the prostate gland. The standard medicine-treated group was found to be similar to the medium- to high-dose-treated group with UPBF extract (150 mg/kg–200 mg/kg), which was the curative effect of the test drugs. These findings are statistically significant ($p<0.001$) in comparison with the disease group. While a partial reduction in prostate weight was observed in the low-dose treated group with UPBF extract (150 mg/kg), these findings are statistically not significant ($p<0.05$). In histopathological examination, the glands of the negative control group displayed a typical sign of hyperplasia. The focal areas visible with prostatic intraepithelial neoplasm grade II and the finasteride-treated group showed focal atrophy of glands, and the lining became flat. So, this is a clear benefit of the effects of standard drugs. In the low-dose-treated group with UPBF extract (100 mg/kg), partial atrophic changes were found, which indicates the action of the test drug against hormone-induced hyperplasia, while in the medium- to high-dose-treated group (150–200 mg/kg), the glands were completely atrophic, and it gave a better result than the low-dose-treated group. This proves that the extract produced increased activity in a dose-dependent manner.

V. CONCLUSION

Given the complete discussion, it can be concluded that all doses of test drugs have significant effects against testosterone-induced BPH in rats. However, the effect exerted by the high dose of the test drug was more marked and was about equal to that of the standard drug (finasteride). The test drug substantiated overall findings with histopathological studies as it reduced tubular projection into the lumen of glands and also improved the histoarchitecture of the prostate in a dose-dependent manner, so the test drug may be effective in the structural derangement of the prostate gland. The test drug is categorized as *Muqawwi* (tonic), *Muhafiz* (protective), and *Mofatteh* (deobstruent). Therefore, the test drug may be extensively used in various inflammatory conditions like prostitis, cystitis, nephritis, etc. Based on the safety study of the test drug, it can also be concluded that the test drug is quite safe and has a wide therapeutic index; therefore, it can be used safely even at high doses. The claim of Unani medicine that the ingredients of the test drug have an anti-inflammatory effect, which is quite beneficial in prostatic disease as well as in LUTS, is validated scientifically. Further, the test drug may be used in BPH and its associated symptoms like dysuria, urine retention, dribbling, and incontinence of urine independently or as an adjuvant with other drugs clinically to overcome the burden of Western medicine and to avoid their adverse effects.

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Availability of Data and Material/ Data Access Statement	All materials, including rats, testosterone propionate, finasteride, testosterone, and protein ELISA kits, and hydro-alcohol for extraction of the test sample, were provided by Ajmal Khan, Tibbiya College, A.M.U., Aligarh, U.P. India.
Authors Contributions	All authors have equal participation in this article.

REFERENCES

1. Roehrborn, C. G. Benign prostatic hyperplasia: An overview. Rev Urology, **vol. 7**, Sup. 9, S3-S14. (2007). https://doi.org/10.1016/S0084-4071(08)70077-0
2. Iscaife, A., Anjos, A. D., Neto, C. B., Nahas, C. *et al*. The role of bladder diverticula in the prevalence of acute urinary retention in patients with BPH who are candidates for surgery. Int Braz J. Urology Jul-Aug; **vol. 44**, Suppl 4, pp. 765-70, (2018). https://doi.org/10.1590/s1677-5538.ibju.2017.0605
3. Barnard, R. J., Aronson, W. J. Benign Prostatic Hyperplasia: Does Lifestyle Play a Role? The Physician and Sports Medicine, **vol. 37**, Sup. 4, pp. 141–46, (2009). https://doi.org/10.3810/psm.2009.12.1752
4. DiBello, J. R., Ioannou, C., Rees, J., *et al*. Prevalence of metabolic syndrome and its components among men with and without clinical benign prostatic hyperplasia: a large, cross-sectional, UK epidemiological study. BJU International, **vol. 117,** no. 5, pp. 801–8, (2016). https://doi.org/10.1111/bju.13334
5. Vickman, R. E., Franco, O. E. *et al*. The role of the androgen receptor in prostate development and benign prostatic hyperplasia. A review. Asian J. Urology. Jul; **vol. 7,** no. 3, pp. 191-202, (2020). https://doi.org/10.1016/j.ajur.2019.10.003
6. Krieg, M., Nass, R., Tunn, S. Effect of aging on endogenous levels of 5 alpha-dihydrotestosterone, testosterone, estradiol, and estrone in the epithelium and stroma of normal and hyperplastic human prostate. J Clin Endocrinology Metab. Aug; **vol. 77**, no. 2, pp. 375-81, (1993). https://doi.org/10.1210/jcem.77.2.7688377
7. Le, V., He, Y., Aldahl, J., Hooker, E., Yu, EJ., Olson, A., *et al*. Loss of androgen signaling in mesenchymal sonic hedgehog-responsive cells diminishes prostate development, growth, and regeneration. PLoS Genet. Jan 13; **vol. 16**, no. 1, pp. 1-22, (2020). https://doi.org/10.1371/journal.pgen.1008588
8. Kato, M., Sasaki, T., Inoue, T. Current experimental human tissue-derived models for prostate cancer research. Int. J. Urol. Feb; **vol. 28**, no. 2, pp. 150-62, (2021). https://doi.org/10.1111/iju.14441
9. Ibn Zuhr, A. M. *Amraz Kuliya Wa Mathana*. (Edit. Ahmad, B.) *Kitab-Al-Taiseer-Fil-Madawat-Al Tadbir*. New Delhi: Central Council for Research in Unani Medicine, pp. 164-166, (1986).
10. Kantori, G. H. *Amraz-e-Juz'ia* in *Kitab at-Qanun fit-Tibb* (Edit. & transl. Sina, S. R.). New Delhi: Ejaz Publishing House, New Delhi, pp. 1018-30, (2010).
11. Jurjani, A. H. *Amraz Mathana* in *Zakhıra Khwarzam Shahi* (edit. & Transl. Khan, H. H.) New Delhi: Idara Kitabul Shifa, pp. 525-31, (2010).
12. Razi, A. B. Z. *Amraz Minal Qarnel al- Qadam*. In *Kitabul-Hawı-fil-Tibb* (Edit. and transl. CCRUM) New Delhi: Central Council for Research in Unani Medicine, pp. 148-78, (2002)
13. Lee, C. L., Kuo, H. C. Pathophysiology of benign prostate enlargement and lower urinary tract symptoms: current concepts. Ci Ji Yi Xue Za Zhi. Apr-Jun, **vol. 29**, no. 2, pp 79-83, (2017). https://doi.org/10.4103/tcmj.tcmj_20_17
14. Khan, M. A. Amraz Mathana in *Aks-ır-i-Azam* (Edit. Kabiruddin, M.) New Delhi: Ejaz Publishing House, pp. 728-34, (2010).
15. Mohammad, M., Zarshenas, M. M., Moein, M., Samani, S. M., Petramfar, P. An Overview of Ajwain (Trachyspermum ammi) Pharmacological Effects. Modern and Traditional Journal of Natural Remedies, **vol. 14,** no. 1, pp. 99–105, (2014).
16. Vishwakarma, V. K., Gupta, J. K., Upadhyay, P. K. PHARMACOLOGICAL IMPORTANCE OF CUCUMIS MELO L: AN OVERVIEW. Asian Journal of Pharmaceutical and Clinical Research. Mar, **vol. 10**, no. 3, pp. 8–12, (2017). https://doi.org/10.22159/ajpcr.2017.v10i3.13849
17. Pethakar, S. R., Hurkadale, P.J., Hiremath, R.D. Evaluation of Anti-urolithiasis Potentials of Hydro-Alcoholic Extract of Cucumis sativus L. Indian Journal of Pharmaceutical Education and Research, **vol. 51,** no. 10-12, pp. 607-14, (2017). https://doi.org/10.5530/ijper.51.4s.89
18. Hu, C., Kitts, D. D. Dandelion (Taraxacum officinale) flower extract suppresses both reactive oxygen species and nitric oxide and prevents lipid oxidation in vitro. Phytomedicine. Aug, **vol. 12,** no. 8, pp. 588-97, (2005). https://doi.org/10.1016/j.phymed.2003.12.012
19. Ahmad, K., Najar, F. A., Faisal, M., Ansari, T. A. Efficacy of Banadiq-ul-Buzoor in Improving the Quality of Life of Patients of Benign Prostatic Hypertrophy: An Open Labelled Observational Clinical Study. Research & Reviews: Journal of Surgery. **vol. 6**, no. 3, pp. 7–11, (2017).
20. Choi, E. M., Hwang, J. K. Anti-inflammatory, analgesic and antioxidant activities of the fruit of Foeniculum vulgare. Fitoterapia. Sep; **vol. 75**, no. 6, pp. 557-65, (2004). https://doi.org/10.1016/j.fitote.2004.05.005
21. Khan, M., Haque, M. S., Chowdhury, M. S. I. Medicinal use of the unique plant Tinospora cordifolia: evidence from the traditional medicine and recent research. Asian Journal of Medical and Biological Research, **vol. 2**, no. 4, pp.508–512, (2017). https://doi.org/10.3329/ajmbr.v2i4.30989
22. Sharma, P., Parmar, J., Verma, P., Sharma, P., Goyal, P. K. Anti-tumor activity of Phyllanthus niruri (a medicinal plant) on chemical-induced skin carcinogenesis in mice. Asian Pac J Cancer Prev, **vol. 10**, no. 6, pp. 1089-94, (2009).
23. Mattioli, R., Francioso, A., Mosca, L., and Silva, P. Anthocyanins: A comprehensive review of their chemical properties and health effects on cardiovascular and neurodegenerative diseases. Molecules, **vol. 25**, no.17, Aug 21, (2020) https://doi.org/10.3390/molecules25173809

24. Cavin, C., Delannoy, M., Touche, A., Courtois, D., and Schilter, B. Inhibition of the expression and activity of cyclooxygenase2 by chicory extract: Biochem Biophys Res Commun, **vol. 327,** no. 3, pp. 742-749, (2005). https://doi.org/10.1016/j.bbrc.2004.12.061
25. Nadkarni, K. M. *Indian Materia Medica*. Popular Prakashan, Mumbai, **vol. I** no. 3, pp. 1229–1232, (1984).
26. Jabbar, A., Nazir, A., Ansari, N.I., Javad, F., Mahmood, K. *et al.* Effects of Tribulus terrestris on urine output and electrolytes in rabbits. Professional Med J, **vol. 19**, no. 6, pp. 843–847, (2012). https://doi.org/10.29309/TPMJ/2012.19.06.2464
27. Karnick, C. R. *Pharmacology of Ayurvedic Medicinal Plants*. Sri Satguru Publications, Delhi, December 31, pp. 17, (1996).
28. Duke, J. A. *Handbook of Medicinal Herbs*. CRC Press, New York, Edition 2, pp. 113-114, (2002). https://doi.org/10.1201/9781420040463
29. Huang, M. T., Badmaev, V., Ding, Y., Liu, Y., Xie, JG., Ho, CT. *et al*. Anti-tumor and anti-carcinogenic activities of triterpenoid, beta-boswellic acid. Biofactors, vol. 13, no. 1-4, pp. 225-30, (2000). https://doi.org/10.1002/biof.5520130135
30. Siddiqui, M. Z. Boswellia serrata, a potential anti-inflammatory agent: an overview. Indian J Pharm Sci. (2011) May, **vol. 73**, no. 3, pp. 255-61, (2011).
31. Garg, S. C., Siddiqui, N. Antifungal activity of some essential oil isolates. Pharmazie, Jun; **vol. 47**, no. 6, pp. 467-8, (1992).
32. Sikdar, M., Dutta, U. Traditional phytotherapy among the Nath people of Assam. Ethno-Med, **vol. 2**, pp. 39-45, (2008). https://doi.org/10.1080/09735070.2008.11886313
33. Lai, Y. J., Tai, C. J., Wang, C. W., Choong, C. Y., Lee, B. H. Anti-Cancer Activity of Solanum nigrum (AESN) through Suppression of Mitochondrial Function and Epithelial-Mesenchymal Transition (EMT) in Breast Cancer Cells. Molecules. Apr; **vol. 21**, no. 5, pp. 553, (2016). https://doi.org/10.3390/molecules21050553
34. Gabrani, R., Jain, R., Sharma, A., Sarethy, I. P., Dang, S., Gupta, S. Antiproliferative Effect of Solanum nigrum on Human Leukemic Cell Lines. Indian J. Pharm Sci. Sep; **vol. 74**, no. 5, pp. 451-3, (2012). https://doi.org/10.4103/0250-474X.108421
35. Javed, T., Ashfaq, U. A., Riaz, S., Rehman, S., Riazuddin, S. In-vitro antiviral activity of Solanum nigrum against Hepatitis C Virus. Virol J. Jan, **vol. 8**, no. 26, (2011). PMID: 21247464 https://doi.org/10.1186/1743-422X-8-26
36. Hattori, M., Huang, X. L., Che, Q. M., Kawata, Y. et al. 6-hydroxy kaempferol and its glycosides from Carthamus tinctorius petals. Phytochemistry, **vol. 31**, pp. 4001-4004, (1992). https://doi.org/10.1016/S0031-9422(00)97572-1
37. Chang, H. T., Han, H. X., and Tu, P. F. Chemical constituents and pharmacological effects of safflower". Xiandai Yaowu Yu Linchuang, **vol. 14**, pp. 201-203, (1999).
38. Tien, Y.C., Lin, J. Y., Lai, C. H., Kuo, C. H. *et al*. Carthamus tinctorius L. prevents LPS-induced TNFalpha signaling activation and cell apoptosis through JNK1/2-NFkappaB pathway inhibition in H9c2 cardiomyoblast cells. J Ethnopharmacology. (2010) Aug 9; **vol. 130**, no. 3, pp. 505–13, (2010). https://doi.org/10.1016/j.jep.2010.05.038
39. Qazi, N., Khan, R. A., Rizwani, G. H., Feroz, Z. Effect of Carthamus tinctorius (Safflower) on fasting blood glucose and insulin levels in alloxan-induced diabetic rabbits. Pak J Pharm Sci. Mar; **vol. 27**, no. 2, pp. 377-80, (2014).
40. Arpornsuwan, T., Petvises, S., Thim-am, A., Boondech, A., and Roytrakul, S. Effects of Carthamus tinctorius L. solvent extracts on the anti-proliferation of human colon cancer (SW 620 cell line) via apoptosis and the growth promotion of lymphocytes. Songklanakarin J Sci Technol, pp. 45–51, (2012).
41. Shin, I. S., Lee, M. Y., Ha, H. K. *et al*. Inhibitory effect of Yukmijihwang-tang, a traditional herbal formula against testosterone-induced benign prostatic hyperplasia in rats. BMC Complement Altern Med. **Vol. 12**, no. 48, (2012). https://doi.org/10.1186/1472-6882-12-48
42. Keskar, M., & Maktedar, Dr. D. (2019). Enhancing Classifier Accuracy in Ayurvedic Medicinal Plants using WO-DNN. In International Journal of Engineering and Advanced Technology (Vol. 9, Issue 1, pp. 6705–6714). https://doi.org/10.35940/ijeat.a2001.109119
43. Medicinal Plants Segmentation using Thresholding and Edge based Techniques. (2019). In International Journal of Innovative Technology and Exploring Engineering (Vol. 8, Issue 6S4, pp. 71–76). https://doi.org/10.35940/ijitee.f1014.0486s419

AUTHOR PROFILE

Dr. Mohammad Shujauddin (MD), Department of Ilmul Advia, AKTC, AMU, Aligarh, UP, 202002, India. Mohammad Shujauddin is a dedicated and enthusiastic researcher with three years of experience in the field of Unani pharmacognosy and the standardization of single drugs with compound formulations. He completed his Bachelor's degree in Unani from Aligarh Muslim University in 2018 and has since been actively involved in conducting research and contributing to scientific publications. Mohammad Shujauddin pursued his Master's degree in Unani from Aligarh Muslim University in 2022.