Original Article

PROPHYLACTIC EFFECT OF CITRULLUS LANATUS & CURCURBITA PEPO SEEDS EXTRACT VERSUS DUTASTERIDE ON HORMONAL PARAMETERS IN BENIGN PROSTATIC HYPERPLASIA RATS

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ABSTRACT

Background: Benign Prostatic Hyperplasia (BPH) is the most common benign neoplasm and is a possible cause of urinary obstruction in elderly males.The current study evaluated the Prophylactic effect of Citrullus lanatus and Curcurbita Pepo seeds extract versus Dutasteride on hormonal parameters in BPH rats.

Material and Methods: Over the 29-day research period, 42 healthy adult male albino rats were divided into 6 groups of 7 animals, except group 1, each received daily testosterone 3mg/kg s.c and medications as per designated groups for initial 14 days for BPH induction & from 15-29 days for maintenance. Group 1 was normal healthy control & 2 was disease control. Other groups were group 3 (3mg/kg Testosterone s.c + 0.5mg/kg dutasteride p.o), group 4 (3mg/kg Testosterone + 2g/kg Methanolic Extract of Citrullus Lanatus Seeds, MECLS p.o), group 5 (3mg/kg Testosterone s.c + 5g/kg Petroleum Ether Extract of Curcurbuta Pepo Seeds, PECPS p.o) & group 6 (3mg/kg Testosterone s.c + 1g/kg MECLS p. o+2.5g/kg PECPS p.o). Their blood samples were taken on d 0, 14 and 29 days for testosterone and PSA estimation by ELISA technique.

Results: Prophylactic combination of 1g/kg Methanolic Extract of Citrullus Lanatus Seeds & 2.5g/kg Petroleum Ether Extract of Curcurbuta Pepo Seeds produced significant improvement in serum PSA & testosterone equivalent to Dutasteride. The individual herbs 2g/kg MECLS & 5 g/kg PECPS produced gradual similar improvement over a prolonged duration (29 days) without any side effects.

Conclusions: This suggests MECLS+PECPS to be equivalently effective & safe as Dutasteride in the prophylaxis of the BPH murine model.

Key Words: Benign prostatic hyperplasia, urine output, Dutasteride, serum PSA and testosterone.

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INTRODUCTION

Benign Prostatic Enlargement (BPE) is a likely diagnosis to describe the large prostate gland size. In contrast, BPH is the diagnosis relating to an increased number of smooth muscles and epithelial cells located in the transition zone of the gland.[1]

As the prostate enlarges, the gland squeezes the urethra. The bladder wall becomes thicker. Eventually, the bladder may weaken and lose the ability to empty, leaving some

urine in the bladder. The narrowing of the urethra and urinary retention—the inability to empty the bladder—cause many problems associated with benign prostatic hyperplasia.[2]
The incidence of BPH is increasing with age and affects 80 percent of men above their eighties.[4]
The etiology and pathophysiology of BPH are poorly understood. However, the testicular androgen i-e Dihydrotestosterone (DHT) is produced from testosterone in body tissue which is more effective in prostate development. Whereas DHT could be harmful in adulthood as it would be responsible for increased prostate growth.[5]
Alpha blockers and 5 alpha reductase inhibitors are the two medical treatment options. Administration of a 5 alpha reductase inhibitor (Dutasteride and Finasteride) causes prolonged inhibition of DHT. The patients are more prone to drug-related problems (DRPs), e.g. alpha blockers cause dizziness and orthostatic hypotension and 5 alpha reductase inhibitors responsible for sexual dysfunction and gynecomastia.[6]
Citrullus lanatus is a floral vine with flowers of both genders and many lengthy stems. The fruit is oval to round, mostly upto 20cm in size and not found in bunches.[7] The flattened seeds are smooth,varying in colour from yellow to brown or black. The seed oil constitutes glycosides of linoleic, oleic, palmitic and stearic acids as noticeable by its high fat content.[8] Curcurbita pepo is known as pepitas, paitha kaddu (urdu) or pumpkin with flat, dark green seeds.[9] The pumpkin seeds are good source of proteins, phytosterols, carbohydrates, essential fatty acids i-e oleic, linoleic, palmitic and stearic acids and minerals including selenium, zinc, calcium, copper, iron, manganese, phosphorous, magnesium and potassium & tocopherols and carotenoids (lutein and beta carotene).[10]
Scientific data exists on their therapeutic benefits in BPH. However, no comparative or prophylactic data as well as of their combined usage could be found, which lays the basis of our study.

The objectives of this study are formation of a rat model of benign prostatic hyperplasia by testosterone, assortment of the rats into different groups for prophylactic treatment with Citrallus lanatus, Curcurbita pepo seeds extract and Dutasteride– alone and in different combinations & Evaluation of biochemical parameters i.e, serum testosterone and PSA in different experimental groups at baseline, on day 14 after the BPH induction and after the completion of treatment on day 29.

MATERIAL AND METHODS

The study was carried out in an experimental research laboratory at Postgraduate Medical Institute (PGMI), Lahore, completed in 12 months after approval of the synopsis. Biochemical parameters were done at Biochemistry laboratories at Shaikh Zayed Hospital Lahore, respectively.
42 healthy albino male rats of 180-200 grams were purchased from Veterinary and Animal Sciences University, Lahore. Rats were housed and maintained in standard polypropylene cages at the PGMI Animal House at a controlled temperature (25±10c), humidity of 60-70% and appropriate lighting conditions.
Healthy control was included in group 1 and disease control in group 2. Other groups were group 3 (3mg/kg s.c Testosterone +0.5mg/kg p.o dutasteride), group 4 (3mg/kg s.c Testosterone + 2g/kg Methanolic Extract of Citrullus Lannatus Seeds p.o), group 5 (3mg/kg s.c Testosterone +5g/kg Petroleum Ether extract of Curcurbita Pepo Seeds p.o) & group 6 (3mg/kg s.c Testosterone + 1g/kg Methanolic Extract of Citrullus Lannatus Seeds p.o+ 2.5g/kg Petroleum Ether extract of Curcurbita Pepo Seeds p.o).
Healthy adult albino rats (male) of age 2 months having weight = 140-194 grams were

included and diseased rats were excluded from this study.

Simple random sampling was done by using the lottery method in this experimental design study. A sample size of 7 rats was estimated for each of the 6 groups by using 95% confidence level and 93% power with an expected mean testosterone level of 45,43,42,42 ng/ml (61) for healthy control, positive control, Citrullus lanatus seed extract and Curcurbuita pepo seed extract respectively. The sample size was calculated using power and precision 3.0 software with a 0.82 effect size with an Error SD of 1.5.

Injection of Testosterone propionate (Tesvot) 250mg/ml & tablets of Dutasteride (Galaxosmith) 0.5 mg were purchased from Clinix Pharmacy.

Biochemical kits for Specific mouse testosterone and PSA ELISA Kits for rats were purchased from Spinreact Company (Spain) & Bio Check, Inc. company, respectively.

Seeds of Citrullus lanatus and Curcurbita pepo were purchased from Hamdard Dawakhana, Lahore. The Methanolic Extract of Catullus Lanatus seed (MECLS) and Petroleum Ether Extract of Curcurbita Pepo seed (PECPS) were made in PCSIR, Lahore.

324g of Citrullus lanatus seeds were weighed and powdered. Percolation of the powdered sample was done in 800ml of Methanol for 48 hours, and the filtrate was concentrated using a rotary evaporator, diluted with corn oil, producing a 100mg/ml stock solution. The desired concentrations were given by insulin syringe.

Two hundred grams of Curcurbita Pepo ground seeds were defatted with petroleum ether in a Soxhlet-type extractor and then digested with 1.25% (v/v) H2SO4 and 1.25% (v/v) NaOH. The residues were heated at 1300°C for 2 hours, cooled in a desiccator and weighed.[12] The data was entered and analyzed using SPSS 23.0. Mean ± SEM was given for quantitative variables, i-e, serum testosterone and serum PSA.

As the data distribution was not normal for serum PSA and testosterone, nonparametric tests, including Wilcoxon Rank & Mann Witheny tests, were applied & deteriorated the differences among all groups & within pairs of individual groups. A p-value of < 0.05 was considered statistically significant.

RESULTS

At the end of the study, compared to the healthy control group, the DC group showed a 440% (p value <0.001) increase in testosterone level because of exogenous testosterone administration.

Within groups 2, 3, 5 & 6, a significant and gradual rise of serum testosterone levels of 195%, 38%, 112% and 29% was seen from baseline till the end of the study. Group 4 showed a gradual decline over the same period in serum testosterone level (9.3%). In comparison to the healthy control group, oral prophylactic treatment received by group 6(MECLS+PECPS), 5(PECPS), 4(MECLS) and 3(Dutasteride) showed a very highly significant decrease in serum testosterone levels ranging from 225, 160, 225 and 300% respectively.

Compared with the normal control group, the disease control exhibited elevated serum PSA levels of upto 50% & within itself 75% by the end of the study. The treatment groups (3-6) receiving oral prophylactic treatment for the whole study period showed 25% (p value <0.001) decrease in PSA levels in each vs DC group. A similar pattern was seen as compared to the healthy control group. However, within groups, from baseline to the point of BPH induction at day 14, there was a significant rise in serum PSA in groups & 6 of 9% & 66% respectively, followed by a decline of 18% in group 5 with no change in group 6 till the end of the study.

Table-1: Table presenting serum testosterone levels for 6 study groups

Serum testosterone	N	Base line (Mean± S.D)	Day 14 (Mean± S.D)	Day 28 (Mean± S.D)
Group 1	7	0.43±0.02	0.41±0.01	0.43±0.07
Group 2	7	0.73±0.37	2.21±0.49	2.16±0.57
Group 3	7	1.29±0.27	1.61±1.07	1.79±0.70
Group 4	7	1.50±0.29	1.31±0.38	1.06±0.73
Group 5	7	1.50±0.29	1.04±0.46	1.93±0.73
Group 6	7	1.86±0.85	1.33±0.36	1.31±0.38
Total	42	1.12±0.62	1.32±0.76	1.45±0.81
P Value (Anova)		<0.001	<0.001	<0.005

Table-2: Table presenting mean serum PSA level for six study groups (Serum PSA of animals on day 0,14,29)

Serum PSA	N	Base line (Mean± S.D)	Day 14 (Mean± S.D)	Day 28 (Mean± S.D)
Group 1	7	0.007±0.002	0.008±0.001	0.007±0.002
Group 2	7	0.008±0.001	0.012±0.002	0.014±0.002
Group 3	7	0.013±0.002	0.010±0.003	0.012±0.007
Group 4	7	0.012±0.002	0.010±0.004	0.009±0.001
Group 5	7	0.011±0.002	0.012±0.004	0.009±0.001
Group 6	7	0.009±0.003	0.015±0.002	0.009±0.001
Total	42	0.010±0.003	0.011±0.003	0.010±0.004
p-value (ANOVA)		<0.001	0.001	0.005

DISCUSSION

BPH is the most common noncancerous disease of the prostate in elderly men. It is described by excessive growth of the prostate and urinary symptoms i.e., frequency, urgency, nocturia, and dysuria, which badly affects the quality of life of an individual.

The development of BPH depends upon the presence of testicular androgens in growing age, which is dependent upon testosterone conversion into more potent Dihydrotestosterone (DHT).[13]

Alpha blockers and 5 alpha reductase inhibitors are the two medical treatment options available, of which alpha blockers are

less commonly used. Dutasteride and Finasteride are 5 alpha reductase inhibitors that cause quick and constant DHT inhibition and are the major current treatment for BPH.[14,15] Due to allopathic medication's side effects, the research trends have been inclined towards natural products. The plants studied for BPH treatment include *Saw palmetto*, African plum tree (*Pygeum africanum*) and rye pollen (*Secale cerale).*[16] Research has been conducted on the anti-prostatic hyperplastic activity of Citrullus lanatus and pumpkin seed, showing their therapeutic potential based upon the presence of certain antioxidant and fatty compounds.[17] However, there is a gap in knowledge to determine whether their anti BPH activity is more beneficial prophylactically, both singly and in combination, versus our standard drug i-e Dutasteride.

In our murine study, forty-two healthy male albino adult rats were segregated into six groups of seven animals. Except for healthy control, all groups received daily testosterone 3mg/kg s.c and medications as per group designation for initial 14 days for BPH induction & from 15-28 days for maintenance. Biochemical investigations (serum testosterone, PSA) were measured at baseline, day 14 and day 28.

The marked decline in testosterone levels was due to the overwhelming diminution of DHT due to each group's alpha reductase inhibiting activity not being counteracted by exogenous testosterone.[18.19] In comparison to the healthy control group, oral prophylactic treatment received by group 6 (MECLS+PECPS),5(PECPS),4(MECLS) & 3 (Dutasteride) showed very highly significant decreases in serum testosterone levels, respectively. Our results are not matchable to other studies where alpha reductase inhibition by different doses of MECLS[11] and Finasteride was the cause of the rise in serum testosterone levels. At the same time, we used a single dose of MECLS. Another research on the effect of different components of crushed pumpkin seeds but not extract on serum testosterone, prostate size, prostate binding proteins and histopathology of testis was evaluated, which was again different from our research.[12]

Compared with a normal control group, the disease control showed increased serum PSA levels at the end of the study. This was due to an increased concentration of DHT secondary to the unopposed administration of exogenous testosterone, resulting in increased secretion of PSA by prostatic epithelial cells.[20] However, within groups, from baseline to the point of BPH induction at day 14, there was a significant rise in serum PSA in groups 5 & 6 followed by a decline in group 5 with no change in group 6 till the end of the study. The reason for the initial rise by group 5 (PECPS) was not a direct effect but rather an indirect one through exogenous testosterone. However, the latter decline in serum PSA was due to 3 & 5-omega fatty acids, which have antioxidant & anti-inflammatory activity & beta-sitosterol, which binds to the prostate and reduces inflammation & secretions of the prostate.[21]

In contrast, the Dutasteride treated group 3 showed a 23% decrease in serum PSA at day 14 with almost no significant change at day 29, showing 5 alpha reductase inhibiting activity.[21]

Group 4 was the most different, which showed a consistent decline of up to 25% in serum PSA by day 29 due to polyphenolic compounds present in MECLS, resulting in prostatic cell death and reduced PSA secretion.[22] Therefore, MECLS & PECPS could be highly beneficial in treating prostatitis and prostatic cancer in addition to BPH.

No comparable study could be found on the combination effect of MECLS & PECPS on serum PSA. Earlier research was on the effect of watermelon seeds on sperm count and serum PSA[11] and that of a combination of saw

palmetto and oil of pumpkin seed on serum PSA, the volume of the prostate and maximal flow rate of urine, which were not comparable.[23]

CONCLUSION

Prophylactic combination treatment with 1g/kg Methanolic Extract of Citrullus Lanatus Seeds (MECLS) & 2.5g/kg Petroleum Ether extract of Curcurbita Pepo Seeds (PECPS) decreased serum PSA levels and testosterone levels equivalent to Dutasteride, the drug of choice for BPH.

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Conflict of interest: None

AUTHOR'S CONTRIBUTION

BFA: Conceptualization of project
SSA: Drafting, Revision
MIP: Writing of manuscript
TZ: Data collection
SJ: Literature search
MM: Statistical analysis

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