Preventive Effect of Citrullus lanatus and Curcurbita pepo Seeds Extract against Dutasteride in Testosterone-Induced BPH Rats: A Histopathological Evaluation

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Abstract

Objective: To investigate the protective effects of extracts from Watermelon (Citrullus Lanatus) and Pumpkin (Cucurbita pepo) seeds against the histopathological changes caused by dutasteride in hyperplastic rats.

Material and Methods: The research was conducted in an experimental laboratory at the Post Graduate Medical Institute (PGMI), Lahore, and completed within 12 months (2018–2019) following authorization of the research proposal. Histopathological analysis of the prostate was carried out at the histopathology department of Post Graduate Medical Institute (PGMI), Lahore. Forty-two healthy adult male albino rats were divided into six groups (seven rats per group). All groups, except the control group, received daily subcutaneous testosterone injections (3 mg/kg) for 14 days to induce benign prostatic hyperplasia (BPH), followed by maintenance therapy from days 15 to 29. The groups included: 1) normal healthy control, 2) disease control (DC), 3) testosterone +0.5 mg/kg dutasteride, 4) testosterone + 2 g/kg methanolic extract of watermelon seeds (MECLS), 5) testosterone +5 g/kg petroleum ether extract of pumpkin seeds (PECPS), and 6) testosterone +1 g/kg MECLS +2.5 g/kg PECPS. At the end of the study, animals were sacrificed, and their prostates were weighed and examined histopathologically.

Results: The preventive combination of 1 g/kg of methanolic extract of Watermelon seeds (MECLS) and 2.5 g/kg of petroleum ether extract of Pumpkin seeds (PECPS) restored prostate histopathology to levels similar to those achieved with dutasteride.

Conclusion: These findings suggest that the synergistic effects of MECLS and PECPS could offer an alternative or complementary approach for managing prostate health

Keywords: Benign prostatic hyperplasia, Dutasteride, Prophylactic, Histopathology

How to cite: Aslam BF, Alam SS, Sultan F, Naz N, Sadia, Maqsood S. Preventive Effect of Citrullus lanatus and Curcurbita pepo Seeds Extract against Dutasteride in Testosterone-Induced BPH Rats: A Histopathological Evaluation. Esculapio - JSIMS 2025;21(01): 9-14

DOI: https://doi.org/10.51273/esc25.2513212

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14-09-2024
03-02-2025
11-03-2025

Introduction

Benign Prostatic Hyperplasia (BPH) is characterized by the abnormal enlargement of stromal and epithelial cells in the prostatic transition zone. This enlargement leads to lower urinary tract symptoms (LUTS), such as excessive urination, urgency, difficulty starting urination, nocturia, painful urination, and a feeling of incomplete bladder emptying. A definitive diagnosis is made through histological analysis.^{1,2} The prevlence of BPH increases with age, impacting approximately 50 percent of men between the ages of 51 and 60, and around 80 percent of those in their eighties.³ As the prostate size increases, it exerts pressure on the urethra resulting in narrowing of the urinary passage and urinary retention. These complications are mainly responsible for the issues linked to benign prostatic hyperplasia. Furthermore, BPH significantly impacts a patient's quality of life.⁴

The exact mechanism of BPH is unclear. However, the progression of BPH depends upon testicular androgens during prostate development and growing age.⁵ Within body tissues, testosterone is converted into dihydrotestosterone (DHT), a more potent androgen that plays a pivotal role in prostate development.⁶

The medical management of BPH primarily involves two treatment options: alpha-blockers and 5-alpha reductase inhibitors. Among these, 5-alpha reductase inhibitors, such as Dutasteride, are widely used as they provide rapid and sustained suppression of DHT levels,⁷ making them a key therapeutic choice for BPH. However, patients may encounter drug-related problems (DRPs) with these treatments. Alphablockers are linked to side effects such as dizziness and orthostatic hypotension,⁸ whereas 5-alpha reductase inhibitors can lead to sexual dysfunction and gynecomastia.⁹

The scientific name of watermelon has Greek origins, with "citrus" meaning fruit, and Latin "lanatus" meaning woolly, reflecting the fruit's texture.¹⁰ The seeds are flattened and smooth, displaying a range of colors from yellow to brown or black. In a 100 g serving of dried, shell-less seeds, the composition includes: 5.1% water, and 2340 kJ (557 kcal) of energy derived from nutrients such as 28.3% protein, 47.4% fat, and 15.3% carbohydrates. The mineral content includes 0.05% calcium, 0.75% phosphorus, and 0.007% iron. Additionally, the vitamin content consists of 0.00019% thiamine, 0.00015% riboflavin, 0.0035% niacin, and 0.000058% folate.¹¹

Cucurbita pepo, widely recognized as pumpkin, yields flat, dark green seeds. Pumpkin seeds are valued for high proportions of proteins, essential amino acids, fatty acids¹² and microelements.¹³

The reason for choosing Dutasteride, a standard medication known for its side effects, for our study was that it is frequently prescribed for the treatment of BPH but not for its prophylaxis. Consequently, our research aimed to explore safe and effective phytoalternatives for the prevention of BPH.

Material and Methods

The research was conducted in an experimental laboratory at the Post Graduate Medical Institute (PGMI), Lahore, and completed within 12 months (2018–2019) following authorization of the research proposal. Histopathological analysis of the prostate was carried out at the histopathology department of Post Graduate Medical Institute (PGMI), Lahore. After the taking approval from Ethical Committee Ref No. F.1-24/M.Edn/881/2016 Dated: 20-08-2026. A total of 42 healthy male albino rats, weighing between 180 and 200 grams, were obtained from the University of Veterinary and Animal Sciences, Lahore. The rats were kept in standard polypropylene cages at the PGMI Animal Facility, where environmental conditions were carefully maintained: a temperature of $25\pm10^{\circ}$ C, humidity at 60–70%, and suitable lighting.

The rats were divided into six groups: Group 1 served as the healthy control.

Group 2 was designated as the disease control.

Group 3 received 3 mg/kg subcutaneous (s.c.) testosterone and 0.5 mg/kg oral (p.o.) dutasteride.

Group 4 was treated with 3 mg/kg s.c. testosterone and 2 g/kg p.o. methanolic extract of watermelon seeds.

Group 5 was administered 3 mg/kg s.c. testosterone and 5 g/kg p.o. petroleum ether extract of pumpkin seeds.

Group 6 received 3 mg/kg s.c. testosterone combined with 1 g/kg p.o. methanolic extract of watermelon seeds and 2.5 g/kg p.o. petroleum ether extract of pumpkin seeds.

Testosterone propionate injection (Tesvot, 250 mg/ml) and dutasteride tablets (0.5 mg, Galaxosmith) were procured from Clinix Pharmacy. Watermelon and pumpkin seeds were sourced from Hamdard Pharmaceuticals in Lahore. The methanolic and petroleum ether extracts of Citrullus lanatus and Cucurbita pepo seeds, respectively, were prepared at PCSIR in Lahore.

To prepare MECLS, 324 grams of watermelon seeds were ground into a fine powder and soaked in 800 ml of methanol for 48 hours. The mixture was filtered, concentrated using a rotary evaporator, and diluted with corn oil to create a stock solution (100 mg/ml). The solution was administered to rats using an insulin syringe.¹⁴ For PECPS, 200 grams of Cucurbita pepo seeds were defatted using a Soxhlet-type extractor with petroleum ether. The defatted seeds were then treated with 1.25% sulfuric acid and 1.25% sodium hydroxide, heated at 1300°C for 2 hours, allowed to cool in a desiccator, and then weighed.¹⁵

At the end of the study, the rats were sacrificed. A midline incision was made in the lower abdomen, and the dorsal and ventral prostate tissues were identified, excised, and preserved in labeled containers with 10% formalin solution for histopathological analysis. These specimens were submitted to the histopathology department of PGMI, where laboratory numbers were assigned. The tissues were processed into paraffin blocks according to international standards, using an automatic tissue processor for dehydration (ethyl alcohol), clearing (xylene), and paraffin infiltration. Paraffin blocks were cut into slides with a thickness of 3–5 microns and stained with hematoxylin and eosin for microscopic examination.

The histopathological evaluation included:

Epithelial cell count, acini and tubule structures, and prostatic secretions.

Stroma composition, including smooth muscles, connective tissue, fibroblasts, blood vessels, and lymphatics.

Due to the absence of a Benign Prostatic Hyperplasia (BPH) grading system, the Chi-square test was not applicable for statistical analysis.

Results

Prostate Histology:



Figure-1: (Control) A.Tubules are surrounded by epithelial cells i-e outer lower cuboidal and inner tall columnar mucin producing cells.B.Prostatic

secretions are obvious. C. Fibro muscular stroma between tubules and acini is thin & condensed. (Fig.1)



Figure-2: (Testosterone induced group) shows A. All tubules are dilated and distorted. Their epithelial cells are flattened. (Epithelial hyperplasia). B. Prostatic secretions are increased C. Stromal tissue is abundant, blood vessels are dilated (stromal hyperplasia). (Fig.2)



Figure-3 Group-3: (Dutasteride + testosterone group) shows A.Epithelium is less distorted and flattened. No involution or projections seen.B. Prostatic secretions are reduced.C. Fibromuscular stroma is decreased



Figure-4 Group-4: (*Testosterone* + *MECLS Group*) *A. Size of glands and epithelial size are decreased,*

Esculapio - Volume 21, Issue 01, January - March 2025 - www.esculapio.pk - 11

epithelium restore its normal state. B. Prostatic secretions are also reduced in glands

C. Fibromuscular stroma is less likely to be decreased.



Figure-5 Group-5: (Testosterone + PECPS) A. Glandular epithelial distortion is decreased B. Prostatic secretion is not markedly decreased. C. Fibromuscular stroma is decreased more than MECLS group.



Fig-6 Group-6: (Testosterone + MECLS+PECPS) A. Tuboalveolar glands are not dilated and their epithelium consists of outer cuboidal and inner columnar cells B.Prostatic secretions are reduced. C. Fibromuscular stroma is thin and condensed between tubules.

Discussion

Benign prostatic hyperplasia (BPH) is a prevalent health issue among aging men, characterized by abnormal prostate enlargement16. This condition often leads to urinary symptoms such as increased frequency, urgency, nocturia, and dysuria, all of which can greatly affect an individual's quality of life.¹⁷ Given the adverse effects associated with conventional allopathic treatments, research has increasingly focused on exploring natural alternatives. Several plants have been studied for their potential in managing BPH, including saw palmetto, African plum tree and rye pollen.¹⁸ Studies have also examined the anti-prostatic hyperplastic effects of Citrullus lanatus (watermelon) and pumpkin seeds, attributing their therapeutic potential to specific antioxidant and fatty acid compounds.^{19,20} However, it remains unclear whether these natural remedies are more effective when used preventively, either alone or in combination, compared to the standard pharmaceutical treatment with Dutasteride. Histopathology revealed normal prostatic glands lined by outer cuboidal cells and inner mucin secreting columnar cells, surrounded by thin and condensed stroma filled with prostatic secretions (corpora amylacea) in normal healthy controlgroup.

In comparison continuous exogenous testosterone administration in DC group resulted in 42% tubuloalveolar glands enlargement, dilatation and folded projections. Stromal size and prostatic secretions were also increased. All these changes were due to testosterone conversion to DHT which causes cellular multiplication and proliferation by interacting with androgen receptors enhancing proliferation while inhibiting apoptosis.²¹

Dutasteride treatment given in Group 3 due to its alpha reductase inhibiting activity culminated in DHT reduction and 28% less epithelial distortion with no involutions and projections.²² Prostatic secretions and fibromuscular stroma were reduced as well.

One of our research plants, MECLS produced 42% less glandular size and prostatic concretions. In addition, there was an increase in size of the fibromuscular stroma (connective tissue) layer. These changes were due to polyphenolic content of MECLS having antioxidant properties responsible for prostatic involution and cell death.²³

Our other plant of interest PECPS produced 29% less glandular epithelial distortion when administered in group 5. Prostatic secretions and fibromuscular stroma were however not reduced significantly. Another Research showed fatty acids and zinc present in Curcurbita pepo seeds interrupt prostate cell multiplication by testosterone and DHT & the β -sitosterol content causes mitotic arrest in PC-3 cells.²⁴⁻

²⁶ Finally no further improvement was noted in group 6 where both MECLS+PECPS were administered and 42% of animals showed tubulo alveolar glands epithelial normalization with outer cuboidal ,and inner columnar cells filled with prostatic secretions interspersed with thin & condensed fibromuscular stroma. Thus at the structural level MECLS+PECPS donot lead to cumulative improvement in prostate histology.

Therefore, MECLS singly & MECLS+PECPS produced significant postate histology normalization in comparison to dutasteride. No comparable data could be found to support or refute our results.

Conclusion

Therefore, MECLS singly & MECLS+PECPS in combination produced significant postate histology normalization in comparison to Dutasteride.

Conflict of Interest	None
Funding Source	None

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Authors Contribution

BFA: Conceptualization of Project

FS: Data Collection

NN: Literature Search

SM: Statistical Analysis

SSA: Drafting, Revision

SM: Writing of Manuscript