Open Access

Original Article

Evaluation of the Role of Losartan in the Prevention of Benign Prostate Hyperplasia in Rats

Arooj Shahid, Akbar Waheed*, Shabana Ali, Kulsoom Farhat, Sikandar Mehmood, Mehwish Amin

Department of Pharmacology, Army Medical College/ National University of Medical Sciences (NUMS), Rawalpindi Pakistan, *Department of Pharmacology, Riphah International Medical College, Rawalpindi Pakistan

ABSTRACT

Objective: To evaluate the preventive role of Losartan in testosterone-induced Benign Prostate Hyperplasia in an adult rat model.
Study Design: Laboratory-based experimental study.
Place and Duration of Study: National Institute for Health, Islamabad Pakistan, for four weeks in Oct 2021.
Methodology: A total of ninety (n=90) rats were divided into three equal Groups, having thirty rats each. In group-A, no active intervention was carried out. In groups B and C, subcutaneous injections of testosterone were administered daily for 28 days to induce a Benign Prostate Hyperplasia model. In group-C, Losartan was administered daily. Animals were sacrificed after the study period of 4 weeks. The animal and prostatic weights were measured to calculate the prostatic index. Blood samples were taken to assess Prostate-Specific Antigen levels, and prostate samples were preserved for histo-pathological analysis.
Results: Mean prostatic weight, prostatic index and serum Prostate-Specific Antigen showed a statistically significant decrease in group-C, where Losartan was given, compared to the disease control group-B (p-value <0.001). Moreover, histo-pathological features like glandular and stromal hyperplasia and the formation of intra-luminal papillary folds were significantly reduced in the drug-treated group-C compared to group-B.
Conclusion: Losartan is an effective drug for the prevention of Benign Prostate Hyperplasia.

Keywords: Benign prostatic hyperplasia, Losartan, Prostatic index.

How to Cite This Article: *Shahid A, Waheed A, Ali S, Farhat K, Mehmood S, Amin S. Evaluation of the Role of Losartan in the Prevention of Benign Prostate Hyperplasia in Rats. Pak Armed Forces Med J 2024; 74(6): 1614-1618. DOI: https://doi.org/10.51253/pafmj.v74i6.10693*

This is an Open Access article distributed under the terms of the Creative Commons Attribution License (https://creativecommons.org/licenses/by-nc/4.0/), which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

INTRODUCTION

Benign prostatic hyperplasia (BPH) is one of the most prevalent disorders in the elderly male population. It is a cause of considerable morbidity but an uncommon cause of mortality.[1] It is characterized by benign overgrowth of prostatic tissue around the prostatic urethra, leading to constriction of the urethral opening and, ultimately, lower urinary tract symptoms (LUTS). This results in symptoms of increased frequency of urination, incomplete urination and a weak urinary stream.[2] Histologically, BPH is described as a non-malignant proliferation of the prostate epithelium and stroma within the transitional zone associated with lower urinary tract symptoms.[3]

The Renin-Angiotensin System (RAS) has been explored to have a prominent role in several inflammatory diseases, including BPH.[4] RAS has a prime function as a regulator of blood pressure. However, there is accumulating evidence that its signalling is also involved in the development of inflammation identified in various disease states. RAS plays a role in the pathogenesis of BPH via Angiotensin II (Ang II), a potent mitogen, a significant pro-inflammatory mediator, and an anti-apoptotic molecule. Ang II exerts its effects by directly activating Angiotensin-1 (AT 1) receptors that enhance pro-survival signaling. The stimulation of the AT 1 receptor leads to the activation of the Nuclear Factor kappa B (NF-κB) pathway, causing the development of BPH.[5]

Presently, BPH is being treated medically and surgically. Patients with mild to moderate symptoms are treated with 5-alpha reductase inhibitors and alpha blockers.[6] These drugs effectively improve the clinical symptoms of the disorder. However, these drugs neither have a role in the prevention of the onset of BPH nor do they hinder the progression of the disorder. In addition, no other drug has been found to have a definitive and effective role in preventing BPH.[7] Drugs acting on RAS include Angiotensin receptor blockers like Losartan. It acts on Angiotensin II receptors, ultimately decreasing the effects of Angiotensin II. It exerts prominent anti-inflammatory action in addition to its blood pressure-lowering properties[8] as anti-hypertensive drugs have to be taken daily, so they can be evaluated in the preventive

Correspondence: Dr Arooj Shahid, Department of Pharmacology, Army Medical College, Rawalpindi Pakistan
Received:01 Aug 2023; revision received: 13 Mar2024; accepted: 19 Mar 2024

role of different common disorders, like BPH. This will not only help decrease morbidity but also decrease the financial burden imposed on them by the involvement of a co-morbid condition.[9,10] Thus, we aim to evaluate the preventive effects of Losartan in testosterone-induced benign prostatic hyperplasia in an adult rat model. This might prove beneficial in the elderly male population with diagnosed BPH and hypertension.

METHODOLOGY

The laboratory-based experimental study was conducted at the Department of Pharmacology and Therapeutics and Department of Pathology, Army Medical College, Rawalpindi, in collaboration with the National Institute of Health, Islamabad Pakistan, for four weeks in Oct 2021. The Ethics Review Committee of the Center for Research in Experimental and Applied Medicine (CREAM), Army Medical College, approved the study vide ERC dated 16 Dec 2021.

Inclusion Criteria: Adult healthy male rats of the Sprague Dawley breed, 2 to 3 months of age, weighing about 250 to 300 grams, were included.

Exclusion Criteria: Female rats and rats with an apparent disease or deformity were excluded.

Ninety animals were procured from NIH and nurtured in its animal house during the study, which lasted 28 days. Biochemical and tissue analysis was conducted in the Pathology laboratory of Army Medical College, Rawalpindi. All the data collected was statistically analyzed in the Department of Pharmacology and Therapeutics, Army Medical College, Rawalpindi.

All animal experimental procedures were carried out in accordance with the NIH Islamabad guide for the care and use of laboratory animals. The rats were kept in wire-topped cages with six rats in each cage. After 1 week of acclimatization to the laboratory environment, rats were randomly divided into three experimental groups (n=30 each). The cages were labelled as A, B, and C according to their groups. The standard environmental conditions were maintained, including room temperature between 25±5˚C and humidity of 50±10%. Twelve hours a day and night cycle was provided to maintain the circadian rhythm of the rats. Free access to clean drinking water and standard rodent diet ad libitum was provided during the study duration.[11]

Testosterone propionate, manufactured by Bayer Pharmaceutical, was purchased from a local pharmacy. Losartan, manufactured by Werrick Pharmaceuticals, was also purchased from a local pharmacy. All the animals were euthanized at the end of the experiment. The intervention protocol is described in Table-I.

Seventy-two hours after the final testosterone injection, animals were weighed. They were euthanized by chloroform, and blood samples were collected by intra-cardiac puncture. Prostate tissues were dissected out and weighed to calculate the prostatic index. Prostate specimens were placed in 10% formalin for 24 hours. They were processed by dehydration, embedded in paraffin wax, and 5-micro-metre thick sections were taken with a microtome. The specimens were then mounted on glass slides and stained with hematoxylin and eosin to be assessed for histopathological changes. The following parameters were assessed: weight: each rat's prostate was removed and weighed, prostatic index: the prostatic index was calculated as the ratio of prostate weight to body weight (mg/g) for each rat.

Serum PSA level was measured quantitatively by rat PSA enzyme-linked immunosorbent assay (ELISA) kit. Standards and samples were prepared according to the manufacturer's protocol.

Analysis of histopathological changes was done qualitatively based on the presence of the following features seen under a microscope regarding 1) Glandular hyperplasia seen as glandular epithelial proliferation, 2) Stromal hyperplasia seen as smooth muscle proliferation, 3) Formation of intra-luminal papillary folds.

The collected data was analyzed using Statistical Package for the Social Sciences (SPSS) version 28. For the qualitative parameter of histopathological assessment, Chi-square was applied. Quantitative variables like prostatic weight, index, and serum PSA levels were expressed in mean±SD and ANOVA test was applied for an intergroup analysis. The *p*-value of ≤0.05 was considered statistically significant.

RESULTS

Healthy male rats of 2 to 3 months of age selected randomly with a baseline weight of 250 + 50 gm were observed in the study. The effect of Losartan was observed using different parameters, including prostatic weight, prostatic index, serum PSA level, and histopathology. At the end of the study, the rats were weighed. Blood samples were drawn for serum PSA, and prostates were dissected to check prostate weight and prostatic index.

The mean prostatic weight, prostatic index, and serum PSA were all increased in group-B compared to group-A. Group-C showed all these parameters to be significantly reduced. All of these quantitative parameters were expressed as mean±SD, as shown in Table-II.The three parameters among the three study groups were compared inter-group using the post-hoc Tukey Test, shown in Table-III.

Table–I: Groups of Experimental Rats and Interventions Carried Out in each Group (n=90)

Groups (n=30)	Subcutaneous Injection given daily for 4 Weeks	Oral Treatment given daily for 4 Weeks
Group A Negative Control	Nil	Nil
Group B Disease Control	3mg/kg/day testosterone[12]	Nil
Group C Losartan	3mg/kg/day testosterone	20mg/kg/day[13]

Table–II: Results of Quantitative Parameters in Study Groups (n=90)

Parameters (Mean+SD)	Group A (n=30)	Group B (n=30)	Group C (n=30)	*p*-value
Prostatic weight	0.41+0.07	0.96+0.05	0.48+0.04	<0.001*
Prostatic index	1.36+0.24	3.07+0.09	1.67+0.15	<0.001*
Serum PSA	0.18+0.02	0.29+0.03	0.19+0.02	<0.001*

**Statistically Significant (p<0.05)*

Table-III: Intergroup Comparison (Post Hoc Analysis) of the Study Groups (n=90)

Parameters	Group A vs B	Group B vs C	Group A vs C
Prostatic Weight	0.001*	0.001*	0.001*
Prostatic Index	0.001*	0.001*	0.001*
Serum PSA	0.001*	0.001*	0.004*

**Statistically Significant (p<0.05)*

Table–IV: Histopathological Grading of Samples in Study Groups (n=90)

Groups (n=90)	Histopathology				*p*-value
	Normal Architecture	Mild BPH	Moderate BPH	Severe BPH	
Group A (n=30)	30(100%)	0(0%)	0(0%)	0(0%)	<0.001*
Group B (n=30)	0(0%)	0(0%)	0(0%)	28(100%)	
Group C (n=30)	0(0%)	3(10%)	27(90%)	0(0%)	

Histopathologically, prostate samples were assessed based on three features: stromal hyperplasia, glandular hyperplasia and papillary folds. All of these features showed severe BPH; the presence of two features meant moderate BPH; the presence of just one feature determined mild BPH, while the absence of all features was considered normal architecture (Figure).

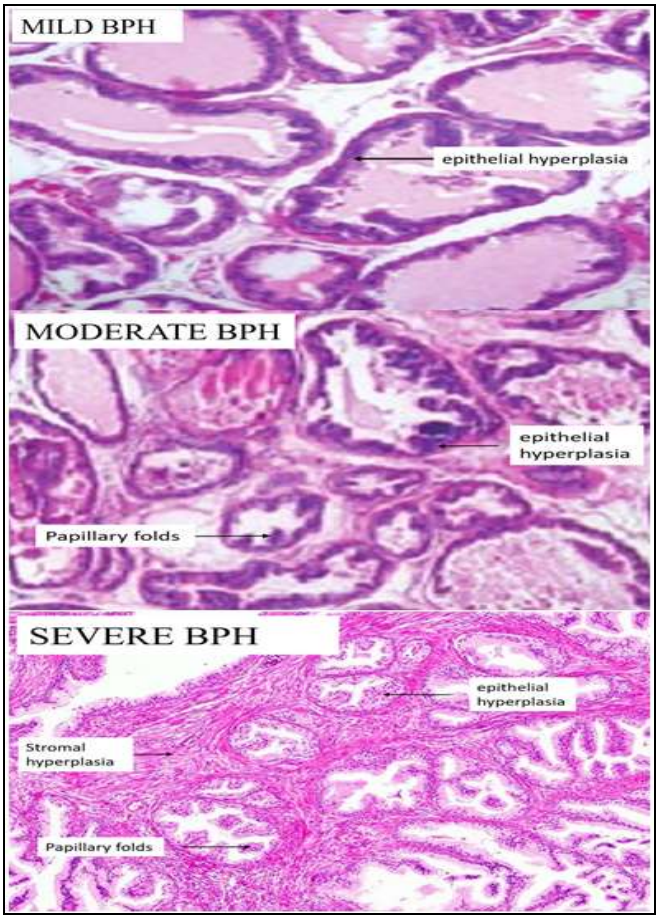

Figure: Photomicrographs showing severity of BPH based on presence of Histopathological Features

DISCUSSION

As BPH is a condition of elderly males, it is frequently accompanied by several other diseases or conditions related to the geriatric population. The most common among these include hypertension and diabetes.[12,13] The FDA has approved several drugs for the treatment of symptoms of BPH, including finasteride, a 5-alpha-reductase inhibitor. These drugs improve the overall quality of life of the patients by improving the signs and symptoms. However, to the best of our knowledge, a drug has yet to be approved by the FDA for its prevention.[14]

Our study was designed to explore new therapeutic potentials of drugs commonly used in the treatment of hypertension for their role in the prevention of BPH. This study was unique in the sense

that only those anti-hypertensive drugs that sequentially inhibit different steps involved in the synthesis and action of Ang II. This study not only evaluates and compares the role of this drug in the prevention of BPH but also consolidates the concept of the involvement of the RAS pathway in the development of BPH.

Rats were selected for the study because, as mammals, their physiological and genetic characteristics are extensively comparable to humans. The model for BPH was created using testosterone propionate injected subcutaneously daily for 30 days. A similar model has been created in several studies using different doses of daily subcutaneous injection of testosterone. It is one of the extensively used models created for BPH for research purposes because of its appreciative resemblance to clinically relevant BPH.[15] BPH was assessed using a number of physical, biochemical, and histopathological markers. The BPH model created in rats resembles the human condition in gross evaluation, microscopy, and biochemical markers.[16]

Prostatic weight was one of the first parameters to be assessed after dissection. It was observed that the mean prostatic weight in group-B rats, in which the BPH model was created by injecting testosterone, was much higher than in group-A, which was the negative control group. Similarly, the mean prostatic weight in group-B was prominently higher than that in group-C, in which drug intervention was done. This result was consistent with studies that had been conducted previously. Mostafa *et al*., found that the increase in weight of the prostate in the testosterone-injected group was 118% compared to the control group. The decrease in prostatic weight was 39% with rats treated with Captopril for one month.[4] The prostatic index was calculated as a ratio of prostatic weight in mg to body weight in gm. In the present study, the mean value of prostatic index seen in group-A surged to a mean value of almost double in group-B after daily injections of testosterone. However, the mean prostatic index significantly decreased in group-C when treated with RAS inhibitors (p-value <0.001). Ishola *et al*., conducted a study by creating a model of testosterone-induced BPH in male Sprague Dawley rats to determine the inhibitory effects of Ponciri Fructus. This study induced BPH by daily administration of testosterone propionate in corn oil. At the end of the study, it was concluded that ponciri fructis significantly inhibited the development of BPH, as measured by the relative decrease in prostatic index compared to the control group. These findings were attributed to the antiproliferative and antioxidant effects of the experimental agent on the prostate.[17]

In the present study, Losartan-treated group-C showed a prominent fall in the PSA level compared to group-B. This is in correspondence with a study by Patel *et al*., in which thirty rats were divided into equal Groups comprising six rats each. The BPH model was created in a similar way as in our study. Two groups were treated with enalapril and Losartan each. Their study observed a significant decrease in PSA levels in both groups treated with enalapril and Losartan.[18]

Group-A's histopathological findings showed normal prostate gland histology in 100% of specimens. The specimen showed a marked histopathological difference in group-C compared to group-B. Similar results were obtained by Mostafa *et al*., who conducted a study on 40 male Sprague-Dawley rats, and the BPH model was created by subcutaneously injecting testosterone propionate (3mg/kg/day) for four weeks.[4]

CONCLUSION

From a holistic point of view, Losartan showed a preventive effect on the development of BPH, as assessed by all the parameters.

Conflict of Interest: None.

Funding Source: None.

Authors' Contribution

Following authors have made substantial contributions to the manuscript as under:

AS & AW: Conception, study design, drafting the manuscript, approval of the final version to be published.

SA & KF: Data acquisition, data analysis, data interpretation, critical review, approval of the final version to be published.

SM & MA: Conception, data acquisition, drafting the manuscript, approval of the final version to be published.

Authors agree to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

REFERENCES

1. Cannarella R, Condorelli RA, Barbagallo F, La Vignera S, Calogero AE. Endocrinology of the aging prostate: current concepts. Frontiers in Endocrinology 2021; 12: 554078. https://doi.org/10.3389/fendo.2021.554078
2. Lee SW, Chan EM, Lai YK. The global burden of lower urinary tract symptoms suggestive of benign prostatic hyperplasia: a systematic review and meta-analysis. Sci Rep 2017; 7(1): 6628. https://doi.org/10.1038/s41598-017-06628-8

3. Vafa A, Afzal SM, Barnwal P, Rashid S, Shahid A, Alpashree, et al. Protective role of diosmin against testosterone propionate-induced prostatic hyperplasia in Wistar rats: Plausible role of oxidative stress and inflammation. Human Exp Toxicol 2020; 39(9): 1133-1146. https://doi.org/10.1177/0960327119889655
4. Mostafa F, Mantawy EM, Azab SS, El-Demerdash E. The angiotensin converting enzyme inhibitor captopril attenuates testosterone-induced benign prostatic hyperplasia in rats; a mechanistic approach. Eur J Pharmacol 2019; 865: 172729. https://doi.org/10.1016/j.ejphar.2019.172729
5. Hunyady L, Catt KJ. Pleiotropic AT1 receptor signaling pathways mediating physiological and pathogenic actions of angiotensin II. Mol Endocrinol 2019; 20(5): 953-970. https://doi.org/10.1210/me.2004-0536
6. Lokeshwar SD, Harper BT, Webb E, Jordan A, Dykes TA, Neal Jr DE, et al. Epidemiology and treatment modalities for the management of benign prostatic hyperplasia. Translat Androl Urol 2019; 8(5): 529. https://doi.org/10.21037/tau.2019.10.01
7. Leung SKW, McNeill SA. Therapeutic Options for Benign Prostate Hyperplasia (BPH) and Prostatic Cancer. In: Schill WB, Comhaire F, Hargreave TB. (eds) Andrology for the Clinician. Springer, Berlin, Heidelberg; 2006. https://doi.org/10.1007/3-540-33713-X_91
8. Adaramoye OA, Oladipo TD, Akanni OO, Abiola OJ. Hexane fraction of Annona muricata (Sour sop) seed ameliorates testosterone-induced benign prostatic hyperplasia in rats. Biomed Pharmacother 2019; 111: 403-413. https://doi.org/10.1016/j.biopha.2018.12.038
9. Bjerre HL, Christensen JB, Buus NH, Simonsen U, Su J. The role of aliskiren in the management of hypertension and major cardiovascular outcomes: a systematic review and meta-analysis. J Human Hypertens 2019; 33(11): 795-806. https://doi.org/10.1038/s41371-018-0149-8
10. Ozhan O, Parlakpinar H, Acet A. Comparison of the effects of losartan, captopril, angiotensin II type 2 receptor agonist compound 21, and MAS receptor agonist AVE 0991 on myocardial ischemia–reperfusion necrosis in rats. Fundament Clin Pharmacol 2021; 35(4): 669-680. https://doi.org/10.1111/fcp.12599
11. Vásquez-Velásquez C, Gasco M, Fano-Sizgorich D, Gonzales GF. Inflammatory pathway employed by Red Maca to treat induced benign prostatic hyperplasia in rats. Andrologia 2020; 52(3): e13516. https://doi.org/10.1111/and.13516
12. de Assis AM, Moreira AM, Carnevale FC, Marcelino AS, de Oliveira Cerri LM, Antunes AA, et al. Effects of prostatic artery embolization on the dynamic component of benign prostate hyperplasia as assessed by ultrasound elastography: a pilot series. Cardiovasc Intervent Radiol 2019; 42(7): 1001-1007. https://doi.org/10.1007/s00270-019-02220-x
13. Golchin-Rad K, Mogheiseh A, Nazifi S, Khafi MS, Derakhshandeh N, Abbaszadeh-Hasiri M. Changes in the serum prostatic biomarkers during the treatment of benign prostatic hyperplasia with a 5alpha-reductase inhibitor: finasteride. Top Companion Animal Med 2020; 38: 100405. https://doi.org/10.1016/j.tcam.2020.100405
14. Zhang J, Zhang M, Tang J, Yin G, Long Z, He L, et al. Animal models of benign prostatic hyperplasia. Prostate Cancer Prostatic Dis 2021; 24(1): 49-57. https://doi.org/10.1038/s41391-020-00277-1
15. Jeon WY, Kim OS, Seo CS, Jin SE, Kim J, Shin HK, et al. Inhibitory effects of Ponciri Fructus on testosterone-induced benign prostatic hyperplasia in rats. BMC Complement Alter Med 2018; 17(1): 1. https://doi.org/10.1186/s12906-017-1877-y
16. Al-Trad B, Al-Zoubi M, Qar J, Al-Batayneh K, Hussien E, Muhaidat R, et al. Inhibitory effect of thymoquinone on testosterone-induced benign prostatic hyperplasia in Wistar rats. Phytother Res 2017; 31(12): 1910-1915. https://doi.org/10.1002/ptr.5936
17. Ishola IO, Anunobi CC, Tijani KH, Afolayan O, Udokwu VU. Potential of telmisartan in the treatment of benign prostatic hyperplasia. Fundament Clin Pharmacol 2017; 31(6): 643-651. https://doi.org/10.1111/fcp.12304
18. Patel SB, Patel V, Captan H. Effect of enalapril and losartan on testosterone induced benign prostatic hyperplasia in rats. Pharmacol Exp Therapeut 2013; 27(S1): 1170. https://doi.org/10.1096/fasebj.27.1_supplement.1170.4