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Evaluation and Comparison of the Preventive Role of Nebivolol and Alpha- Tocopherol in Testosterone-Induced Benign Prostatic Hyperplasia in Rats

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ABSTRACT

***Objective*:** To evaluate and compare the preventive role of Nebivolol and Alpha Tocopherol on testosterone-induced benign prostatic hyperplasia in rats.

***Study Design*:** Laboratory-based experimental study.

***Place and Duration of Study*:** Department of Pharmacology and Pathology, Army Medical College, Rawalpindi, in collaboration with National Institute of Health (NIH), Islamabad Pakistan, from Dec 2021 to Jun 2022.

***Methodology*:** One hundred fifty healthy male Sprague-Dawley rats were procured from the NIH, Islamabad. They were placed into five groups, each with 30 rats.

***Results*:** Out of a total 150 sample of five groups, the mean prostatic index in Group-A, Group-B, Group-C, Group-D, and Group-E were 1.49±0.09, 2.35±0.48, 1.87±0.11, 1.72±0.04 and 1.68±0.08 respectively (p-value<0.001). Mean Prostate Specific Antigen in Group-A, Group-B, Group-C, Group-D and Group-E was 0.19±0.02, 0.23±0.01, 0.18±0.02, 0.18±0.02 and 0.16±0.02 respectively (p-value<0.001). On gross examination, in Group-A, all (100%) prostate samples were normal-sized. In Group-B, all (100%) prostate samples were enlarged. In Group-C, 53.3% were mild, and 47.7% of samples were moderately enlarged. In Group-D, 73.3% of samples were mildly enlarged, and 26.7% were moderately enlarged. In Group E, 26.7% of samples were normal, and 73.3% were mildly enlarged (p-value<0.001).

***Conclusion*:** Prostatic index is markedly declined following treatment with the combination of Nebivolol and Alpha-Tocopherol. There is also a declining trend in Prostate Specific Antigen following treatment with a combination of Nebivolol and Alpha-Tocopherol.

Keywords: Alpha-tocopherol, Benign prostatic hyperplasia, Nebivolol.

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INTRODUCTION

Benign Prostatic Hyperplasia (BPH) is among the most common ailments in ageing males. The incidence of BPH is directly proportional to individuals' age. More than 50% of cases of BPH occur in the fifth decade of life and more than 80% in the eighth decade. Although an uncommon cause of mortality, it is associated with considerable morbidity, disability, and poor quality of life.[1,2]

The pathogenesis of BPH is multifactorial, including genetic and environmental factors. Over the past decade, a rise in the cases of metabolic syndrome, hypertension, ischemic heart disease, diabetes and obesity has led to an increasing trend in the incidence of BPH.[3] Inflammatory mediators, dietary factors, hormones, environmental factors and oxidative stress also influence the pathogenesis of BPH. Obesity, smoking, alcohol consumption and hereditary status also have a great impact on the development and progression of the disease. [4,5]

Pakistan is a developing country, and the financial repercussions substantially burden the individual and national economies.[6] A beneficial impact on the progression of BPH is noted with drugs imparting a reduction in oxidative stress. The role of beta blockers in slowing the progression of prostatic carcinoma has been proven by literature.[7,8] Nebivolol is a 3rd generation selective beta-adrenoceptor antagonist. It promotes nitric oxide-based vaso-dilatation by activating endothelial Nitric Oxide synthase-mediated beta 3-adrenoceptors.[9] Alpha-Tocopherol is a well-known anti-oxidant. An Alpha-Tocopherol Beta-Carotene Cancer Prevention (ATBC) study proved the beneficial role of Alpha-Tocopherol in the Carcinoma of Prostate.[10] This study will assess and compare the potential role of the above drugs with anti-oxidant properties in preventing BPH.

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METHODOLOGY

The laboratory-based experimental study was conducted at the Department of Pharmacology and Pathology, Army Medical College, Rawalpindi, in collaboration with the National Institute of Health (NIH), Islamabad Pakistan, from December 2021 to June 2022, after approval of the Ethical Review Board Committee (Dated; 17 Dec 2021).

Inclusion Criteria: Healthy adult Sprague-Dawley male rats aged 2 to 3 months and weight 200 to 300 grams, were included.

Exclusion Criteria: Rats with any obvious disease or deformity were excluded.

A total number of 150 healthy male Sprague-Dawley rats were procured from NIH, Islamabad. After one week of acclimatisation to the laboratory environment, rats were randomly distributed into five groups (n=30 each). Group-A (Negative Control Group), Group-B (Disease Control Group), Group-C (Nebivolol Group), Group-D (Alpha-Tocopherol Group) and Group-E (Nebivolol and Alpha-Tocopherol Group). The stan-dard environmental conditions and diet comprised roughly 69% carbs, 19.6% protein, 4.6% fat, and 4.8% fibre. BPH was induced by injecting testosterone propionate subcutaneously 3mg/kg/day to each rat from group A to E. Nebivolol was administered orally to rats of Group C and E for four weeks at a dose of 10mg per kg per day. Alpha Tocopherol was also administered orally to Group D and E rats for four weeks at 100mg per kg per day.

Seventy-two hours after administering the last injection on day 28, each rat was weighed. They were euthanised with the help of chloroform by inhalational route. Prostate specimens were dissected and weighed in order to count the prostatic index. Blood samples were gathered by doing intra-cardiac puncture. Serum prostate specific antigen (PSA) level was measured by rat PSA using an ELISA kit. Prostate specimens of all animals were excised immediately and washed with a cold saline solution. All samples were stored at 20 °C until processing. Formalin fixation and paraffin embedding (FFPE) technique was used to preserve the tissue morphology.

Statistical analysis was conducted using Statistical Package for the Social Sciences (SPSS) version 26.0. Quantitative variables were expressed as Mean±SD and qualitative variables were expressed as frequency and percentages. Chi-square test was applied to explore the inferential statistics. One-way analysis of variance (ANOVA) was applied to gauge the mean differences among the groups. The group differences were calculated using Post Hoc test (Tukey HSD). The *p*-value lower than or up to 0.05 was considered as significant.

RESULTS

Out of 150 total samples from five groups, the mean prostatic index in Group-A, Group-B, Group-C, Group-D, and Group-E were 1.49±0.09, 2.35±0.48, 1.87±0.11, 1.72±0.04 and 1.68±0.08 respectively (*p*-value <0.001). Mean PSA in Group-A, Group-B, Group-C, Group-D and Group-E was 0.19±0.02, 0.23±0.01, 0.18±0.02, 0.18±0.02 and 0.16±0.02, respectively (*p*-value <0.001) shown in Table-I.

Table-I: Comparison of Prostatic Index and Prostate Specific Antigen (n=150)

Parameters	Group-A (n=30)	Group-B (n=30)	Group-C (n=30)	Group-D (n=30)	Group-E (n=30)	p-value
	Mean±SD	Mean±SD	Mean±SD	Mean±SD	Mean±SD	
Prostatic Index	1.49±0.09	2.35±0.48	1.87±0.11	1.72±0.04	1.68±0.08	<0.001*
Prostate Specific Antigen	0.19±0.02	0.23±0.01	0.18±0.02	0.18±0.02	0.16±0.02	<0.001*

On gross examination, in Group-A, all (100%) prostate samples were normal-sized. In Group-B, all (100%) prostate samples were much enlarged with fluid. In Group-C, 53.3% of prostate samples were mildly enlarged, and 47.7% of prostate samples were moderately enlarged. In Group-D, 73.3% of prostate samples were mildly enlarged, and 26.7% of prostate samples were moderately enlarged. In Group-E, 26.7% of prostate samples were normal-sized, and 73.3% of prostate samples were mildly enlarged (*p*-value< 0.001) (Table-II).

On microscopic examination, in Group-A, all 100% prostate samples were normal. In Group-B, all 100% of prostate samples had severe hyperplasia. In Group-C, 53.3% of prostate samples had mild hyperplasia, and 47.7% of prostate samples had moderate hyperplasia. In Group-D, 73.3% of prostate samples had mild hyperplasia, and 26.7% of prostate samples had moderate hyperplasia. In Group-E, 26.7% of pros-

tate samples were normal, and 73.3% of prostate samples had mild hyperplasia (*p*-value <0.001) (Table-III)

Table-II: Comparison of Gross Features of Prostate in Five Groups (n=150)

Study Group	Gross features of prostate				Total n (%)	*p*-value
	Normal size n (%)	Mild enlarged n (%)	Moderate enlarged, n (%)	Much enlarged with fluid, n (%)		
Group-A	30(100.0%)	0	0	0	30(100.0%)	<0.001*
Group-B	0	0	0	30(100.0%)	30(100.0%)	
Group-C	0	16(53.3%)	14(46.7%)	0	30(100.0%)	
Group-D	0	22(73.3%)	08(26.7%)	0	30(100.0%)	
Group-E	08(26.7%)	22(73.3%)	0	0	30(100.0%)	

Table-III: Comparison of Microscopic Features of Prostate in Five Groups (n=150)

Study Groups	Microscopic features of Prostate				Total n (%)	*p*-value
	Normal n (%)	Mild hyperplasia n (%)	Moderate hyperplasia, n (%)	Severe hyperplasia, n (%)		
Group-A	30(100.0%)	0	0	0	30(100.0%)	<0.001
Group-B	0	0	0	30(100.0%)	30(100.0%)	
Group-C	0	16(53.3%)	14(46.7%)	00	30(100.0%)	
Group-D	0	22(73.3%)	8(26.7%)	0	30(100.0%)	
Group-E	8(26.7%)	22(73.3%)	0	0	30(100.0%)	

DISCUSSION

Benign prostatic hyperplasia is the leading cause of lower urinary tract symptoms in men. As the prostate is located around the male urethra, its enlargement can lead to physical obstruction of the urethra, which in turn hinders the normal flow of urine from the urethra. This commences the symptomatic appearance of BPH.[11] Several drugs are used, including 5-5-alpha reductase inhibitors finasteride and alpha blockers like Tamsulosin. However, to our knowledge, a drug has yet to be approved by the FDA for its prevention.[12]

Our study was designed to explore new therapeutic potentials of drugs commonly used in the treatment of hypertension and ischemic heart disease for their role in the prevention of BPH. Nebivolol promotes Nitric oxide-induced vasodilatation via eNOS and demonstrates anti-oxidant effects by decreasing superoxide production by inhibiting NADPH oxidase and blockade of eNOS uncoupling. Due to reduced oxidative stress, Nebivolol inhibits nuclear factor- kappa B activation, decreasing the production of multiple pro-inflammatory cytokines. Moreover, Nebivolol demonstrates robust anti-apoptotic properties through its Nitric Oxide governing effect. Such actions lay the foundations for investigating the influence of Nebivolol on the prevention of the development of BPH by reducing oxidative stress.

Rats of the Sprague-Dawley breed were used in our study. Rats were selected for the study because, being mammals, their physiological and genetic characteristics are extensively comparable to those of humans.[13,14] The prostatic growth in our study animals was induced by exogenously administered testosterone. This was consistent with similar studies that had been conducted previously. In a study by Constantinou *et al*., it has been observed that testosterone hormonal treatment prompts prostate growth in rats.[15] Sabur *et al*. also showed the induction of growth in the epithelial cell layer and stromal cell space in both the rat prostate lobes by testosterone.[16]

The duration of intervention in our study was similar to the work done by other scientists. However, it was longer than six weeks of a study conducted by Baha Al-Trad *et al*. [17] 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 more prominent than in Groups C, D, and E, where drug intervention was done. However, the most prominent effect was seen in Group E, in which both drugs were used. The prostatic index was determined 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 index value significantly decreased in the three groups treated with drugs. A comparison of values shows that the most significant

effect was seen in group E, treated with Nebivolol and Alpha-Tocopherol (*p*-value <0.001).

This result was consistent with previous studies. Grytli explored the correlation of β-blocker use in carcinomas of the prostate in a study carried out in Norway. In a meta-analysis by Lu and his colleagues, the use of beta-blockers in reducing mortality in prostate cancer patients was analysed. Results from the analysis proved a beneficial impact of beta-blocker use in reducing cancer-specific mortality due to carcinoma of the prostate (hazard ratio =0.85, 95% confidence interval =0.77–0.94).[18]

A study carried out by Grytli *et al*. analysed data from 3,561 patients. According to the analysis, patients who used beta-blockers experienced a 21% reduced risk of prostate cancer-specific mortality.[19] Our results about Alpha-Tocopherol are also consistent with previously conducted similar studies. Gossell-Williams *et al*. found that pumpkin seed oil (a source of vitamin E; Tocopherol) can limit testosterone-induced prostate hyperplasia in rats. [20]

In our study, the size of the prostate gland was observed for all groups and it was seen that the combined effect of Nebivolol and alpha-tocopherol showed a normal to mild enlargement of the prostate. Mild prostate enlargement was observed in the samples of individually administered groups, Nebivolol (Group C) and Alpha-Tocopherol (Group D).

In the present study, the biochemical marker of serum PSA was calculated at the conclusion of the study period, one month before dissection. It was observed that the serum PSA level of Group B was much higher than that of Group A. In addition, drug-treated groups showed a prominent fall in the level of PSA. However, the difference between drug-treated groups was insignificant, as observed in our study. Group A's histopathological findings showed normal prostate gland histology in 100% of specimens. Group B was given daily injections of subcutaneous testosterone. All of the prostate specimens of Group B showed the presence of all the characteristic features of BPH as seen on histopathology under a microscope. However, it was observed that in groups C, D, and E, in which active intervention was done using oral drugs, the specimens showed marked differences in histopathology compared to group B. The most prominent effect on histopathology was seen in group E, where both drugs were used.

This study observed that Alpha-Tocopherol and Nebivolol in combination have the most prominent role in preventing BPH, followed by Alpha-Tocopherol alone and then Nebivolol alone. This evidenced a prominent reduction in prostatic weight, index, and serum PSA levels. Hence, Alpha-Tocopherol and Nebivolol both have preventive roles in the development of BPH.

This study was unique because Nebivolol has never been previously evaluated for its role in preventing BPH. In addition, to the best of our knowledge, no comparison between Nebivolol and potent antioxidants has been conducted in BPH prevention. Hence, this study validates the research hypothesis that Nebivolol and Alpha-Tocopherol help prevent BPH.

CONCLUSION

The prostatic index was significantly dropped following treatment with a combination of Nebivolol and Alpha-Tocopherol compared to Nebivolol and Alpha-Tocopherol alone. Gross and microscopic features of the prostate were normal after treatment with a combination of Nebivolol and Alpha-Tocopherol as compared to Nebivolol and Alpha-Tocopherol alone. PSA was significantly dropped following treatment with a combination of Nebivolol and Alpha-Tocopherol and Nebivolol and Alpha-Tocopherol alone, but the difference was not statistically significant between the treatment groups.

Conflict of Interest: None.

Authors Contribution

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

MSM & AW: Data acquisition, data analysis, drafting the manuscript, critical review, approval of the final version to be published.

SA & AS: Study design, data interpretation, drafting the manuscript, critical review, approval of the final version to be published.

UN : 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.

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