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RESEARCH ARTICLE

Protective Effect of Naringin on Testosterone Induced Benign Prostatic Hyperplasia in Rats

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ABSTRACT

Major components of plants being flavonoids containing polyphenolic derivatives which posses antioxidant property have shown to improve uncontrolled growth of the prostate gland and urinary tract symptoms, which are associated with benign prostatic hyperplasia. Our study investigated whether Naringin prevents testosterone induced prostatic hyperplasia in rats by virtue of its antioxidant property. In vitro studies were carried out to assess the protective effect of prostate tumor cell lines. BPH was induced in experimental groups by intramuscular injection of Testosterone Enanthate on day 1, 7 and 14. Naringin was administered daily by oral gavage for a period of 21 days. On 22nd day, rats were sacrificed, prostate tissue weighed and histopathological studies were carried out. Prostate zinc, oxidative parameters were measured. Treatment with Naringin showed significant inhibition of prostate enlargement and restored the histoarchitecture when compared with positive control group. In conclusion, the present study showed that Naringin reduced the elevated levels of both prostate weight and prostate weight to body weight ratio, markers of testosterone induced prostatic hyperplasia in rats.

KEYWORDS

Benign prostatic hyperplasia, Testosterone, Antioxidant, Naringin.

INTRODUCTION

Non-malignant and uncontrolled enlargement of the prostate involving the proliferation of epithelium and fibromusclar tissue is benign prostatic hyperplasia.[1] It is a common condition in elderly men, with a prevalence of up to 85%.[2] Benign prostatic hyperplasia is characterized by lower urinary tract symptoms like urinary frequency, urgency, weak and intermittent stream, a sense of incomplete emptying, nocturia and can lead to complications including acute urinary retention, obstructive uropathy and urinary tract infection.[3,4] Although the etiology of benign prostatic hyperplasia is not completely elucidated.

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it involves hormonal changes in the aging man. The development and growth of prostate gland depends on androgen stimulation, mainly by Dihydrotestoterone (DHT), an active metabolite formed due to enzymatic conversion of testosterone by steroid 5α- reductase. Production and accumulation of DHT in the prostate increases with aging which results in encouraging cell growth and induction of hyperplasia[5,6]. BPH also involves augmented adrenergic tone in prostate smooth muscle, regulated through α1-adrenoceptors[7]. Conventional drugs like steroid 5 alpha-reductase inhibitors (finasteride and dutasteride), alpha-adrenoceptor antagonists (alfuzosin, doxazosin, tamsulosin, terazosin) are used to treat benign prostatic hyperplasia, but they possess various side effects like impotence,

decreased libido, ejaculation disorder, gynaecomastia, dizziness, upper respiratory tract infection, headache, fatigue, and additional responses were reported in post marketing investigation which includes rash, tachycardia, and chest pain[8]. Along with conventional therapy, some alternative therapies are also available to treat prostatic hyperplasia.

Narinign is a flavonoid containing polyphenolic derivative found in various citrus fruits and is a part of dietary supplement. Recent studies showed that naringenin suppresses proliferation and stimulates DNA repair following oxidative damage in LNCaP human prostate cancer cells[9], various herbal drugs like gallic acid[10], HMBA, lupeol[11], vitamin D[12] has proved to be effective in treating benign prostatic hyperplasia by virtue of their antioxidant property[13].

MATERIALS AND METHODS

Animals

Male wistar rats weighing 170-190g were procured from National Institute of Nutrition (NIN), Hyderabad. Animals were placed individually in clean, transparent polypropylene cages with free access to food and water with 12: 12 hr dark/light cycle was followed. They were acclimatized for a period of one week and divided into five experimental groups. All the experimental conditions and procedures were carried in accordance with the committee for the purpose of control and supervision of experiments (320/CPCSCEA dated 03-01-2001) on animals. This study was approved by the Institutional Animal Ethical committee, G. Pulla Reddy College of pharmacy, Hyderabad.

Chemicals

1. Naringin purchased from Sigma Aldrich.
2. Testosterone Enanthate was purchased from commercially available Testoviron Depot from Cadila healthcare limited, Goa
3. Finasteride – Gift sample from Dr. Reddy's laboratories, Hyderabad.
4. TBAR's, butanol, pyridine and other chemicals were purchased from sigma Aldrich.

Experimental Design

Male wistar rats were divided into five groups (n=8) and received the following treatment for 21days[7]. Test drugs were suspended in 5% CMC solution and administered orally, (Group 1) negative control received vehicle 5% CMC; (Group 2) Positive control, received testosterone 25mg/day intramuscular on day 1,7 and 14th to induce prostatic hyperplasia; (Group 3) Finasteride (5mg/kg, p.o); (Group 4 and 5) Narinign (40 and 80mg/kg, p.o) respectively. Change in body weights was observed from day1 and day21. On 22nd day all the animals were sacrificed by cervical dislocation. The prostate gland was isolated and weighed immediately. Finally prostatic tissue was used for biochemical estimations (MDA, GSH, SOD, and Nitrite), tissue zinc analysis and histopathological examination.

Body Weight

Animals were weighed before initiation and after completion of the experiment[14].

Prostate Weight

Animals were sacrificed by cervical dislocation and prostates were removed and weighed immediately.

Prostate Weight to Body Weight Ratio

Prostate weight to body weight ratio were calculated.

Percentage of Inhibition

a) Prostate weight

b) Prostate weight/body weight ratios.

Percentage of inhibition was calculated as follows:

100 − [(treated group−negative control)/ (positive control−negative control) × 100].

Estimation of Zinc Levels

Prostate tissue were isolated and separated for ventral and lateral lobes. These lobes were digested using nitric acid to perchloric acid (1:1)v/v% and made upto final volume with distilled water and assayed for atomic

absorption spectroscopy and the zinc content was measured in g/L[7].

Histopathological Investigation

The paired prostates were dissected and freed from fascia and fixed in Bouin's solution with haematoxylin and eosin stain and observed under light microscope (40×).

Statistical Analysis

Data were expressed as mean ± S.E.M. Statistical analysis is done by one-way ANOVA followed by Bonferroni's multiple comparison Test. P<0.0001 considered as significant.

RESULTS AND DISCUSSION

Body Weight Changes

There were no significant differences in body weights of rats before and after testosterone treatment among the groups (Table 1).

Evaluation of Prostate Enlargement

Prostate Weights

Administration of testosterone significantly increased prostate weight when compared with normal control. Treatment with Naringin (40mg/kg and 80mg/kg, p.o) dose dependently decreased prostate weights when compared to testosterone treated group and this effect was restored to the normal levels at higher dose. Similar effect was observed in Finasteride (5mg/kg p.o) treated group. There was no change prostate weight in normal animal treated with Naringin (80mg/kg) alone when compared to normal control Percentage inhibition for Finasteride, Naringin 40mg/kg and 80mg/kg, were found to be 92%, 60.0%, and 78.6% respectively (Table 1).

Prostate Weights to Body Weights Ratio

Induction of BPH significantly increased prostatic index in testosterone treated group when compared with normal control. Treatment with Naringin (40mg/kg and 80mg/kg, p.o) and Finasteride (5mg/kg, p.o) significantly decreased testosterone induced prostatic index when compared with positive control group. Percentage inhibition of PI was found to be 93%, 73.7%, and 89% for Finasteride, Naringin 40mg/kg and 80mg/kg respectively (Table 1).

Prostate Zinc Levels

Prostate zinc in normal tissue was found to be 0.43±0.04mg/L, in disease conditions it was increased to 3.43±0.15mg/L. Treating with standard, berberine low and high doses brought down to 0.67±0.02, 1.18±0.06 & 0.83±0.06 respect.

Histopathology of Prostate

The tissue were tightly packed, epithelium was cuboidal and regular in size (Fig.1A). In positive control group, there was disruption in the histoarchitecture of the prostate tissue.

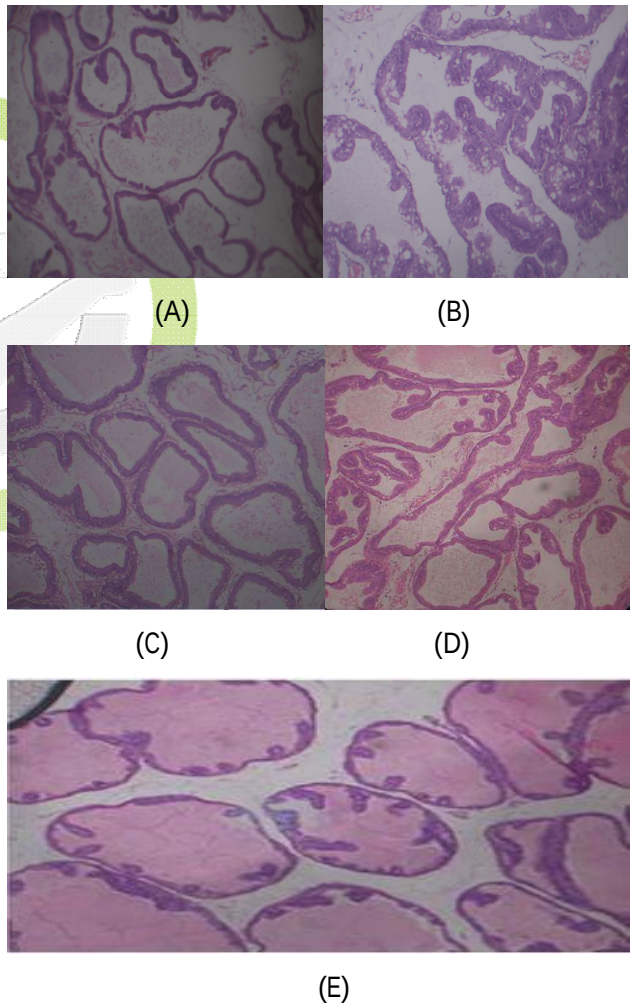

Figure 1: Effects of Naringin on Histoarchitecture of Prostate. A: Negative control; B: Positive Control; C: Finasteride treated group; D: TE+Naringin (40mg/kg) E: TE+Naringin (80mg/kg);

Table 1: Effect of Naringin on Body weights and prostatic enlargement in testosterone treated rats

S. No.	Treatment	Body (gm) weights		PW (mg)	% inhibition	PW/BW ratio	% inhibition
		Initial	Final			(×10−3)	
I	Vehicle	198 ±2.1	205 ± 15.1	440 ±6.4	--	2.14	--
II	Testosterone (25mg/day)	208 ±6.5	212.5 ± 7.3	844±60.7	--	3.99	--
III	Finasteride (5mg/kg)	222 ±3.6	224.6±19.3	528±57.7	77.6[a]	2.44[a]	84
IV	Testosterone + Naringin (40mg/kg)	203 ±5.0	201±11.9	552±80.7	65[b]	3.13[b]	60
V	Testosterone + Naringin (80mg/kg)	203 ±1.6	204±22.6	479±95.8	76[b]	2.78[b]	71.4

PW: prostate weight, BW: body weight, Group I: negative control, Group II: positive control, Group III: Finasteride (5 mg/kg), Group IV: Naringin (40mg/kg), Group V: Naringin (80mg/kg). Values are expressed as mean ±S.E.M. Statistical analysis is done by one-way ANOVA followed by Bonferroni's multiple comparison test. [a]P<0.01 when compared with normal control.; [b]P<0.001 when compared with positive control.
* a & b indicates level of significance when compared with normal and positive control groups.

The amount of connective tissue was well marked and inward projections of epithelial cells were observed (Fig.1B). Disease induced animals when treated with Naringin (40mg/kg and 80mg/kg)showed reduction of epithelial cell layers and stromal cell spaces of the prostate compared to the positive control group, which similar to that observed in the Finasteride treated group (Fig.1C). No stromal appearance was found in Naringin (80mg/kg) treated group (Fig.1E). Mild glandular hyperplasia of prostatic cells was found in the Naringin (40mg/kg) treated group (Fig.1D). These findings indicate marked reduction in histoarchitecture disruption of prostate in Naringin, when compared with positive control group. Previous studies suggest that Naringin is capable of inhibiting, retarding or reversing the carcinogenic process including the prostate cancer development. Naringin showed the effect of apoptotic cell death of prostate cancer cells viz., androgen-independent (PC-3) and androgen-dependent (LNCaP) cell lines *in-vitro*[15,16]. In the present study, treatment with Naringin for 21 days significantly inhibited the development of testosterone induced prostatic hyperplasia, which was evidenced by reduction in elevated prostate weight, prostate weight to body weight ratio and prostate tissue zinc levels. The effect of treatment on prostate weight gain was evident and that should be related to body weight changes; therefore the prostate weight to body weight ratio has been used as the main marker for the treatment. The treatment did not significantly affect body weight, but the effect was on prostate weight to

body weight ratio. Also histopathological studies revealed that treatment with Naringin (80mg/kg) and treatment with Finasteride showed reversal of the histoarchitecture when compared with positive control group. The histological findings have shown the recovery in the prostatic histoarchitecture particularly in the cuboidal epithelial cells, intracellular lumen and shape which further support to make Naringin a strong candidate for the management of prostatic hyperplasia. However, the protective effect of Naringin needs to be tested on testosterone induced prostatic hyperplasia and also in different models to determine its beneficial effects in humans who suffered from benign prostatic hyperplasia.

CONCLUSION

Oral administration of Naringin for 21 days showed dose dependant inhibition of prostate enlargement when compared to that of positive control group. The preventive effect is likely due to inhibition of oxidative damage caused by free radical generation. Further experimental studies are required to confirm the present findings before deciding whether they are meaningful enough to be explored in humans with benign prostatic hyperplasia.

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