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Evaluation of Concurrent Administration of Testosterone and Nettle Extract on Prostate Gland of Rat

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Abstract: Prostatic hyperplasia results in obstructive lower urinary tract symptoms. Plant extracts are frequently used to treat it. This study investigated whether nettle *(Urtica Dioica)* extract (UDE) could improve prostate hyperplasia using testosterone in rats. Male Wistar rats were randomly divided in four groups of 5 rats: first group was kept without treatment, second group injected with almond oil (oil group), third group injected subcutaneously with testosterone 3mg/kg/day and fourth group injected subcutaneously with testosterone 3mg/kg/day accompanied orally treated with UDE 50mg/kg/day. Treatments lasted for 30 days. At completion of the study, rats were sacrificed, and the prostates were removed, cleaned, and weighed. The prostate weight ratio (prostate weight/rat body weight) was calculated. Prostate tissue was examined by light microscope. The prostate weight ratio and acini lumen diameter was significantly lesser in control group than other groups. Also nettle decreased histomorphological changes of prostate that induced by testosterone. In conclusion, UDE orally administered was effective for reducing established prostate hyperplasia.

Key words: Prostate Hyperplasia · *Urtica Dioica* Extract · Histomorphologic Changes · Rats

INTRODUCTION

Prostate gland is the largest accessory sex gland and has a key role in male mammal's sexual physiology. Prostate discharges provide good conditions for fertilization by increasing of spermatozoa motility and survival, spermatozoa nuclear density, spermatozoa maturation and decreasing of environmental shock effects on spermatozoa [1]. Prostate is a tubuloalveolar gland and secretary units are deposited in a fibromuscular stroma. Because of its anatomical location which is around urinary bladder neck and proximal portion of urethra, disorders related this gland have an adverse effects on the low urinary tract [2] and clinically are important. Prostate development, grow and function are dependant to androgens [3]. Testosterone is the major androgen in blood circulating that converts to more potent androgen dihydrotestosterone by 5α reductase in target tissues like prostate. DHT is bounded to its cytoplasmic receptor and the complex entered nucleus and is bounded to nuclear receptor and activated the genes which are involved in prostate growth. The common disorders related to this gland in middle age and aging men are prostate cancer and benign prostatic hyperplasia which disrupt normal balance of gland growth and enlarge the gland [4].

In past selective treatment for prostate enlargement was surgery mostly. During recent years drug therapy has been used for treatment of early stages prostate disorders [5]. As surgery and drug therapy are expensive and also there are possible side effects. So it is necessary to make low cost and safer therapeutic agents. Phytotherapy is much common in many countries for prostate hyperplasia treatment (BPH).

Stinging nettle is one of the plants which have extensive therapeutic usages. Stinging nettle (*Urtica dioica*) is an annual plant from Urticacae family and was considered as a therapeutic plant in world since past. The nettle root contains fatty acids, sterols, flavonoids, proteins, polysaccharides and lectins [6, 7]. Its antiviral, anti-inflammatory and anti analgesia effects have been determined. It has a mild estrogenic property. There are other reports showing its benefits of extract. Root or other portions are used in different conditions including prostate hyperplasia, diabetes, rheumatoid arthritis, high

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blood pressure and allergic rhinitis, lymphocytic stimulation, prevention of platelet aggregation and lowering blood fat [6, 8-10]. In Iran it grows mostly in Mazandaran and Golestan area and has edible usage in these areas. In Iran Safarinejad [11] reported that stinging nettle has benefit effects in improving clinical signs of BPH [11]. As reported testosterone administration induces an experimental model of prostate enlargement which its clinical signs are comparable with BPH signs [4, 12], also regarding nettle applications in prostate enlargement treatment, the present study was purposed to evaluation of concurrent administration effect of testosterone and stinging nettle aqueous extract root on the rat ventral prostate gland weight, volume and histological structure.

MATERIALS & METHODS

Adult male Wistar rats with weighing 200-260 g were provided from Laboratory Animals Grow and Care Center of Ahvaz Jondishapour Medical Science University. All animals were kept in a room temperature maintain at 23±1°C and a regular 12 h light:12 h dark cycle. They were kept one week before use and water and commercial pelleted diet were *ad libitum*. After one week adaptation to laboratory conditions animal weighed and were randomly divided into 4 following groups (5 animals in each group):

- Group was kept without treatment (control group).
- Group which received almond oil 3 mg/kg daily subcutaneously (oil group).
- Testosterone enanthate (TE) group which received TE diluted in almond oil 3mg/kg daily subcutaneously.
- TE and nettle aqueous extract root (net) (from Pourcina Co. Iran) which treated by 3 mg/kg of TE and 50 mg/kg (net) by gavage daily. All treatments were done at 11 am for 30 days.

Animals were conducted in accordance with Iranian Humane Care and Ethical Animal Welfare. Animals were euthanized 24 hours after the last treatments and the body weight was recorded.

Histological Studies: The abdominal cavity was opened and the ventral prostate bilateral lobs were removed fast. Trimmed of fat and connective tissue cautiously and their weight and volume were recorded and fixed in buffer formalin 10%. After fixation the samples were passaged to preparing histological sections. Passage process included washing with running water, dehydration with ethanol increased solutions, clearing with xylene solution and embedding in paraffin. After blocking samples with melted paraffin, the blocks were trimmed and 5-6 micrometer (μ) sections were prepared by rotator microtome. Then sections were stained by hematoxylin & eosin staining (H&E) and were mounted. 5 slides from each sample were studied histologically and micrometrically.

To study and comparison of prostate gland histological changes following testosterone administration and nettle possible protective effects in considered groups structural and micrometrical factors including glandular units surface density, luminal diameter of units which have nearly circular shape, parenchyma: inter acinar space ratio, ratio of acini with folded epithelium to whole acini in field, epithelial height in outer and inner acini were evaluated. Also cellular hyperplasia, structural and morphological changes of acinar epithelial cells and connective tissues were studied. Micrometrical studies were done by means of graded lenz and calibrated slide in optical microscope (Olympus, Japan).

Data Analysis: Calculation was performed using the SPSS statistical package. Data analysis was performed by one-way ANOVA followed by LSD post Hoc. All results were shown as mean ± S.E.M. In all comparisons, $P < 0.05$ was considered as the criterion of significance.

RESULTS

Prostate mean wet weight in TE group was more than control group ($P<0.001$), oil and TE+Net groups ($P \leq o.oo1$) significantly. In TE+Net group, this mean was more than control group significantly ($P<0.05$).

There was no significant difference between weight of body in rats of groups.

TE increased body weight ratio significantly ($P<0.001$). The ratio in oil and TE + Net groups was less than TE group significantly ($P<0.001$) but more than control group significantly ($P<0.05$) (Fig. 1).

Histomorphological structure of prostate in control and oil groups was similar and was consisted of acini covered with simple cylindrical cells. These cells had a basal euchromatin nucleous and a definite nucleolus. Nuclei had a linear arrangement. Cytoplasm of cells was basophilic and was observed purple to blue. In cellular apical cytoplasm there was a clear region which was allocated to Golgy apparatus. There were a few folds in acini walls. Smooth muscle cells were around of acini

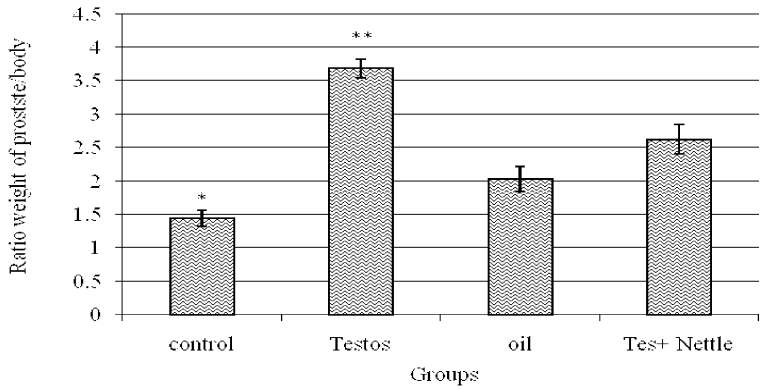

Fig. 1: Mean (±E.S.) of ratio weight of prostat gland to body of rats after 4week treatment in control, testestoron, almond oil and testosteron+ nettle group.* represents significant difference between control to other groups and ** shows significant difference between testostron group to other groups ($p<0.05$). n=5.

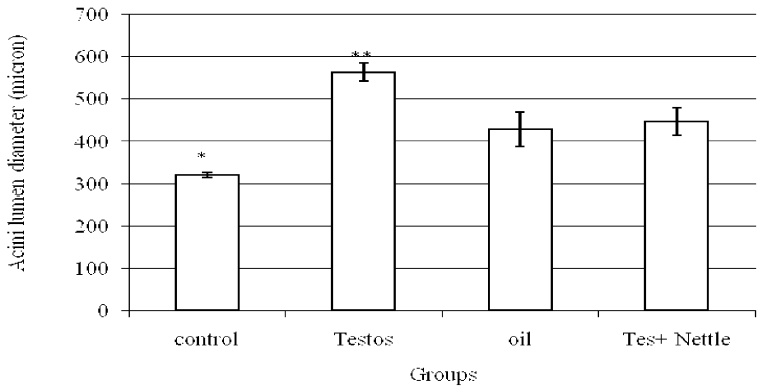

Fig. 2: Mean (±E.S.) of acini lumen diameter after 4week treatment in control, testestoron, almond oil and testosteron+ nettle group.* represents significant difference between control to other groups and ** shows significant difference between testostron group to other groups ($p<0.05$). n=5.

immediately beneath basal membrane. There were connective fibers between or around muscle cells. Acini lumen had filled with an eosinophilic material which was epithelial cells secretion. There was a fine stroma between acini (Fig. 3A). Stroma was a loose connective tissue and had scant cells and fibers. It appeared that stroma was made of intercellular material mostly which were removed during tissue passage.

Acini lumen was dilated and lumen diameter was significantly increased ($p<0.001$) in the TE group with comparison to other groups (Fig. 2) and placed along the side. Acini wall appeared thin (Fig.3B). The epithelial cells height mean was less than control group significantly ($P<0.001$). Epithelial cells were low cylindrical or cubical. It appeared that secretary cells were inactive. These cystic like dilations contained eosinophilic secretions. Inter acinar space is narrow and contained scant stroma .The mean percentage of interacinar space was less than control group significantly ($P<0.001$). There was no significant difference in folded units mean percentage to total units mean percentage ratio between TE and control groups although it appeared less in TE group. In TE group units surface density mean was less than control group significantly ($P<0.001$).

Epithelial cells were tall columnar and were active secretary cells in TE+Net group (Fig.3C). Their height mean was more than TE group significantly ($P\leq0.001$). It was also more than control group significantly ($P<0.05$). The mean of acini lumen diameter was significantly decreased in comparison with TE group ($P<0.001$) (fFg.2). It was more than control group but this difference was not significant ($P>0.05$). Acini lumen was filled with eosinophilic materials. Inter acinar space was narrow and filled with stromal cells and fibers in TE +Net group. There was no difference in inter acinar space mean percentage between TE and TE+Net groups. It was in TE +Net group significantly less than control group ($P<0.001$).

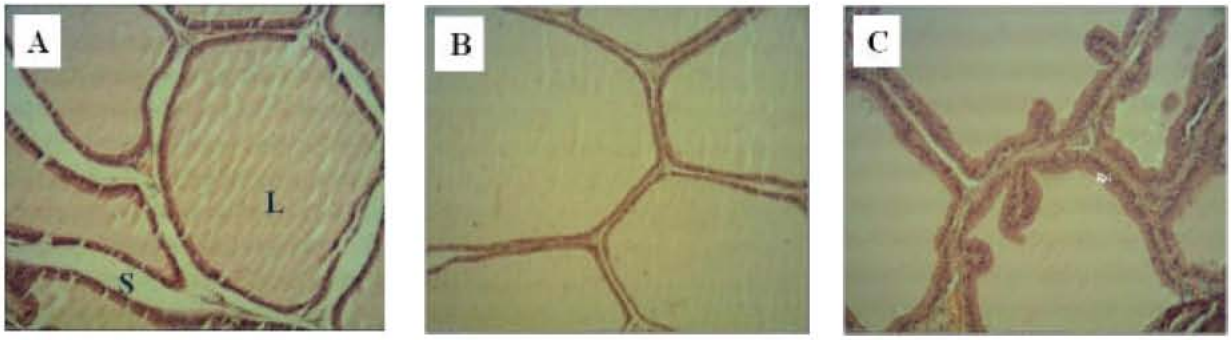

Fig. 3: A. Histological features of the prostat in control and oil groups (× 240, H&E). B. prostat structure after treatment with testosteron. (× 240, H&E). C. treated by testosteron+ nettle. (× 240, H&E). L: lomen, S: space between acini, Epi: Epithelium.

There were many folds in acini wall which protruded into their lumen. The folded units mean percentage to total units mean percentage ratio was more than TE group ($P<0.001$) and control group ($P<0.01$) significantly. There was no significant difference between TE + Net and TE groups in units surface density mean The number of cells and amount of connective tissue had increased in inter acinar space of TE + Net group so that they had filled inter acinar space and there was no empty space.

DISCUSSION

In our study, testosteron administration caused histological changes in prostate of rats which was opposite to some studies[2, 13-15]. Testosteron increased weight and volum of prostate in our study but we did not see hyperplasia. While testosteron was induced BPH in some other studies [2, 13-15]. This conteraversy may be related to different formulation (we used testostoron enantate) or duration of testosteron administration (we aministrated it for 30 days). Testosteron was used for experimental BPH in several studies. For example it was used in Gonzales *et al*. [14] study for Red maca (*Lepidium meyenii*) effect on prostate size in rats ; in Guo *et al*. research for evaluation effect of baicalein on experimental prostatic hyperplasia in rats and mice ; in study of Noa *et al*. [16], Bisson *et al*. [17] and Adesanya *et al*. [12].

Co-administration of nettle extract decreased effect of testosteron on prostate weight and structural changes. Nettle has stroles, flavonoids, polysaccharides and other copounds [6, 7]. These agents interact with testosterone action. Wagner *et al*. [18] reported anti prostatic principle of stinging nettle roots . Koch [8] saw that roots of *Urtica dioica* is benefite in treatment of benign prostaic hyperplasia and associated lower urinary tract symptoms. In other study, the components of stinging nettle roots inhibited experimentally induced prostatic hyperplasia in mice [19]. Effects of the polysaccharide fraction of *Urtica fissa* on castrated rat prostate hyperplasia induced by testosterone propionate [20]. The nettle may decrease production active form of testosterone (dihydrotestosteron) by 5α reductase inhibition. This effect is similar to flutamide which is a nonsteroidal antistestosterone used in the treatment of BPH & prostatic cancer. It blocks the effects of DHT at the testosterone receptor. The trend of antitestosterone effect of flutamide against testosterone induced hyperplasia was observed [4].

The antioxidative effect of nettle may modulate its anti prostatic action. Other antioxidants such as vitamin C supplementation prevent testosterone-induced hyperplasia of rat prostate by down-regulating HIF-1α [15].

CONCLUSION

Nettle decreased histomorphological changes of prostate which induced by testosterone. It may be effective for reducing prostate disorders.

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