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Pumpkin Seed Oil and Phytosterol-F Can Block Testosterone/Prazosin-Induced Prostate Growth in Rats

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Key Words
Prostate growth, rats · Testosterone · Prazosin · Pumpkin seed oil

Abstract
Introduction: This study was undertaken to investigate the effects of pumpkin seed oil alone or combined with Phytosterol-F on testosterone/prazosin-induced (T-P) prostate growth in rats. ***Materials and Methods:*** Forty adult Wistar rats were divided into five groups, including: one control group, rats treated with vehicle only, one group treated with T-P, and two groups of T-P-treated rats, one receiving orally pumpkin seed oil alone and one group receiving orally pumpkin seed oil combined with Phytosterol-F. Two weeks later, the prostatic weight-to-body weight ratio was determined after sacrifice. The total protein concentration was measured by using a protein assay. Some ventral prostatic tissues were histologically examined after hematoxylin-eosin staining. ***Results:*** Histological sections of the ventral prostate showed that the architecture of the prostate glands became hyperplastic in the T-P rats, but not in the control or vehicle-treated animals. As compared with the control or vehicle group, T-P rats had a significantly higher prostatic weight-to-body weight ratio for the ventral prostate ($p = 0.05$ and $p = 0.007$, respectively), but not for the dorsolateral prostate ($p = 0.53$ and $p = 0.73$, respectively). The T-P rats had significantly higher protein levels within both lobes (ventral lobe, $p = 0.02$ and $p < 0.0001$, respectively; dorsolateral lobe, $p = 0.06$ and $p = 0.005$, respectively). As compared with the T-P-alone rats, the TP rats treated with pumpkin seed oil alone or pumpkin seed oil combined with Phytosterol-F had a significantly lower weight ratio for the ventral prostate ($p = 0.01$ and $p = 0.004$, respectively) and significantly lower protein levels within both lobes ($p = 0.03$ and $p = 0.003$, respectively; $p = 0.007$ and $p = 0.002$, respectively). In addition, Phytosterol-F had some additive effect on the total protein synthesis within the ventral prostate ($p = 0.02$). ***Conclusion:*** Pumpkin seed oil alone or combined with Phytosterol-F can block the T-P-induced increases in prostatic weight-to-body weight ratio and protein synthesis.

Introduction

Benign prostatic hyperplasia (BPH) is a common disease among the aged male population. Patients often suffer from lower urinary tract symptoms associated with

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the disease. The treatment options range from conservative, pharmacological treatments (such as α-blockers or 5α-reductase inhibitors) to minimally invasive therapy or surgical prostatectomy. However, more and more people seek for alternative phytotherapy because of unsatisfactory pharmacological outcomes or the fear of a surgical procedure and its associated complications (erectile dysfunction or retrograde ejaculation) [1, 2].

Extracts from pumpkin seeds *(Curcurbita pepo L.)* and fruits of dwarf palms *(Sabal serrulata)* were used in folk medicine as a remedy for micturition problems caused by the prostate [3]. Several clinical studies also reported on the efficacy and safety of plant extracts in treating symptomatic BPH [4–10]. These plant extracts are mainly composed of phytosterols, phytoestrogens, terpenoids, fatty acids, lectins, plant oils, polysaccharides, or flavonoids [11]. Many action mechanisms have been postulated, including anti-inflammatory effects, alteration of the cholesterol metabolism, decrease of sex-hormone-binding globulin, inhibition of 5α-reductase, inhibition of aromatase, antiandrogenic and/or antiestrogenic effects, improvement of detrusor function, interference with growth factors (antiproliferative effects), blockade of α-adrenergic receptors, and free radical scavenger effects [12, 13].

Extracts from pumpkin seeds in combination with dwarf palm extracts were reported to be effective in improving the BPH-induced lower urinary tract symptoms [3]. However, little is known about how pumpkin seed oil (PSO) alone or in combination influences prostatic growth. Experimental prostate growth procedures using a variety of agents, including dihyroxytestosterone or testosterone, estrogen, prazosin (an α_1-adrenergic antagonist), and α_2-agonists or α_2-antagonists, or their combinations, were reported to be able to increase prostate weight [14] and were widely used in animal models for studying their impact on chronic urinary tract obstruction [15] or for studying the effect of various agents or plant extracts [16, 17].

Therefore, the aim of this study was to investigate the effect of PSO alone or combined with Phytosterol-F on prostate growth induced by testosterone and prazosin (T-P).

Materials and Methods

Animals and Agents

A total of 40 3-month-old male Wistar rats were used in this study and categorized into five groups: one control group, one vehicle-only group, one T-P-treated group, and two T-P-treated groups to which the study extracts were administered. The rats were maintained under a dim light, on a 12-hour light/12-hour dark photocycle. They were provided with water and a standard laboratory rat chow, ad libitum. Each day at approximately noon, the animals were weighed, injected with T-P, and fed with the study extracts according to the experimental protocol. Testosterone and prazosin were purchased from Sin-Tong Pharma (Taipei, Taiwan) and Sigma Chemical (St. Louis, Mo., USA), respectively. All study extracts and preparations of formulas were provided by Uni-President Enterprises (Tainan, Taiwan).

Induction of Prostate Growth

The T-P-induced prostate growth has been widely used and validated as an animal model of urinary tract obstruction due to benign prostate enlargement [14, 15]. In this study, testosterone (1.25 mg/kg/day) together with prazosin (30 μg/kg/day) was dissolved in 0.1 ml of sesame oil and injected subcutaneously daily for 14 days.

Treatment of the Two T-P Groups with the Study Extracts

These rats were fed daily with PSO alone (2.5 ml/kg/day) or PSO combined with Phytosterol-F (62.5 mg/kg/day), concomitant with T-P, for 14 days. PSO was extracted from pumpkin seeds and naturally contained many components, including 0.5–1.0% phytosterols. Phytosterol-F, composed of many kinds of phytosterols, including β-sitosterol, campesterol, stigmasterol, and brassicasterol, was added for enhancing the antiproliferative effect on prostate growth. Because of the low solubility within the PSO, Phytosterol-F was initially dissolved into few amounts of sesame oil at 140–145°C and poured with PSO to a final concentration of 2.5%. The dosage of Phytosterol-F was determined according to the recommended amount of *Serenoa repens* extract (Permixon®) given to humans.

Determinations of Prostate Weight-to-Body Weight Ratio and Total Protein Levels and Histological Section

All tissue preparations were performed by an independent investigator and coded for histology in a blinded fashion. The animals were killed by an overdose of sodium pentobarbital, and the entire prostate was carefully removed and weighed. The ventral and dorsolateral lobes were separated and weighed. The prostate weight-to-body weight ratio was calculated as the weight of each lobe divided by the rat body weight.

After weighing, about 50 μg prostate tissue of each lobe was collected for protein extraction and total protein assay. The protocols for protein extraction and assaying were according to the manufacturer's procedures. Briefly, the obtained prostate tissue was rinsed in cold saline for removing the blood and homogenized on ice for 3 min in a microhomogenizer. The homogenate was suspended with lysis buffer (1.0% Tween 20, 1 *M* NaCl, 1 × phosphate-buffered saline, and 0.1% sodium azide) containing as protease inhibitor 1 m*M* phenylmethylsulfonyl fluoride on ice for 20 min and then sonicated for 5 min, followed by centrifuging for 10 min at 10,000 *g* and 4°C using a microcentrifuge. The supernatant fractions were collected and stored at –70°C for determination of the total protein concentration. The concentration of protein in the preparations was measured by using a Bio-Rad (Munich, Germany) protein assay.

Some ventral prostate tissues from the control and the T-P-treated rats were obtained for histological section. Tissues were placed in 10% neutral-buffered formalin for a minimum of 2 days

prior to using standard procedures for paraffin embedding, sectioning (4 μm), and staining with hematoxylin and eosin. Morphological changes of the glandular structure were evaluated.

Statistics

Data were entered into computer data files and analyzed using Prism® version 3.0 (GraphPad Software, San Diego, Calif., USA). Data analysis was conducted using an unpaired t test. Any difference with $p < 0.05$ was considered to be statistically significant.

Results

Body Weight

The weight of each rat was determined before sacrifice. There was no significant difference among the five groups, indicating that neither T-P treatment nor the treatment with the study agents could influence the rat body weight.

Establishment of a Rat Model for Experimental Prostatic Growth

Histological sections of the ventral prostate showed that the architecture of the prostate glands became hyperplastic in the T-P-treated rats, but not in the control or the vehicle-only group (fig. 1).

With respect to prostate weight change, the T-P-treated rats had a higher ventral prostate-to-body weight ratio in comparison with the control ($p = 0.007$) or the vehicle-only ($p = 0.05$) group. However, no significant changes were found for the dorsolateral prostate (T-P vs. control, $p = 0.73$; T-P vs. vehicle-only, $p = 0.53$; fig. 2). As far as the protein changes are concerned, T-P-treated rats had significantly higher total protein levels within both prostate lobes in comparison with the control or the vehicle-only group (ventral lobe, $p < 0.0001$ and $p = 0.02$, respectively; dorsolateral lobe, $p = 0.005$ and $p = 0.06$, respectively; fig. 3). Taken together, these data suggest that T-P treatment can induce differential changes within the rat prostate lobes, including histological hyperplasia and increased prostate-to-body weight ratio and total protein levels.

Effect of PSO Alone or Combined with Phytosterol-F

With respect to the prostate-to-body weight ratio, both the rats treated with PSO and the animals treated with PSO plus Phytosterol-F had a significantly lower ventral prostate-to-body weight ratio than had the T-P-treated rats (T-P vs. PSO, $p = 0.01$; T-P vs. PSO + Phytosterol-F, $p = 0.004$), but no significant change was found for the dorsolateral prostate-to-body weight ratio ($p = 0.10$ and $p = 0.65$, respectively; fig. 2). With respect to the total protein levels, both the PSO-treated rats and the animals treated with PSO plus Phytosterol-F had significantly lower values within each lobe than had the T-P-treated rats (T-P vs. PSO, $p = 0.05$ and $p = 0.007$, respectively; T-P vs. PSO + Phytosterol-F, $p = 0.009$ and $p = 0.002$, respectively; fig. 3). Taken together, these data demonstrate that the administration of PSO alone or PSO plus Phytosterol-F can block the T-P-induced changes of the rat prostate in terms of prostate weight-to-body weight ratio and total protein levels.

As for the additive effect of Phytosterol-F, the animals treated with PSO plus Phytosterol-F had significantly lower total protein levels within the ventral prostate lobes than had the PSO-treated rats ($p = 0.02$). However, the difference within dorsolateral lobes did not reach statistical significance ($p = 0.17$). Moreover, no significant difference existed between the two groups regardless of ven-

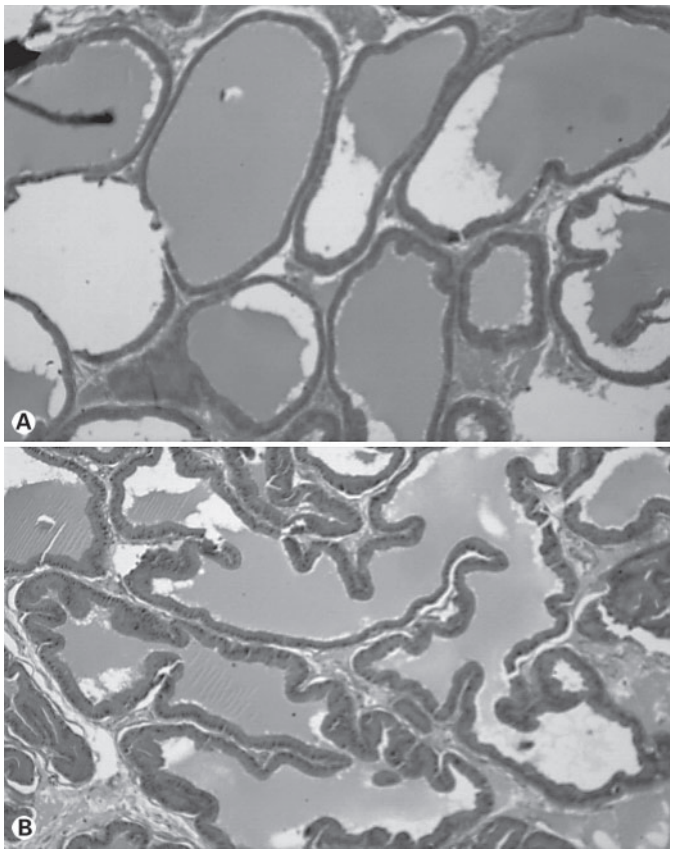

Fig. 1. Histological sections of the ventral prostate. **A** Control rat (×20). **B** Hyperplastic architecture of the prostate gland in a T-P-treated rat (×20).

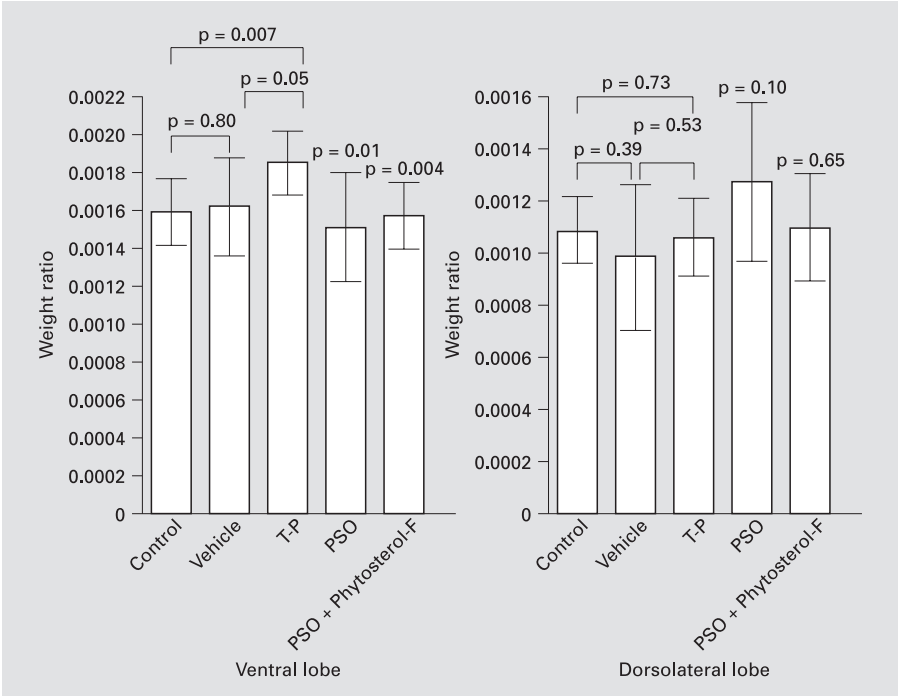

Fig. 2. Prostate weight-to-body weight ratio (weight ratio) in each group. A significant increase of the ratio was noted in the ventral lobe after T-P administration (p = 0.05 and p = 0.007), but not in the dorsolateral lobe (p = 0.53 and p = 0.73). The increase in the ventral prostate was significantly blocked either by PSO monotherapy (p = 0.01) or by PSO + Phytosterol-F treatment (p = 0.004). However, the treatment effect was not found in the dorsolateral lobe (p = 0.10 and p = 0.65). In addition, there was no statistical difference between PSO-treated and PSO + Phytosterol-F-treated groups in terms of prostate weight-to-body weight ratio.

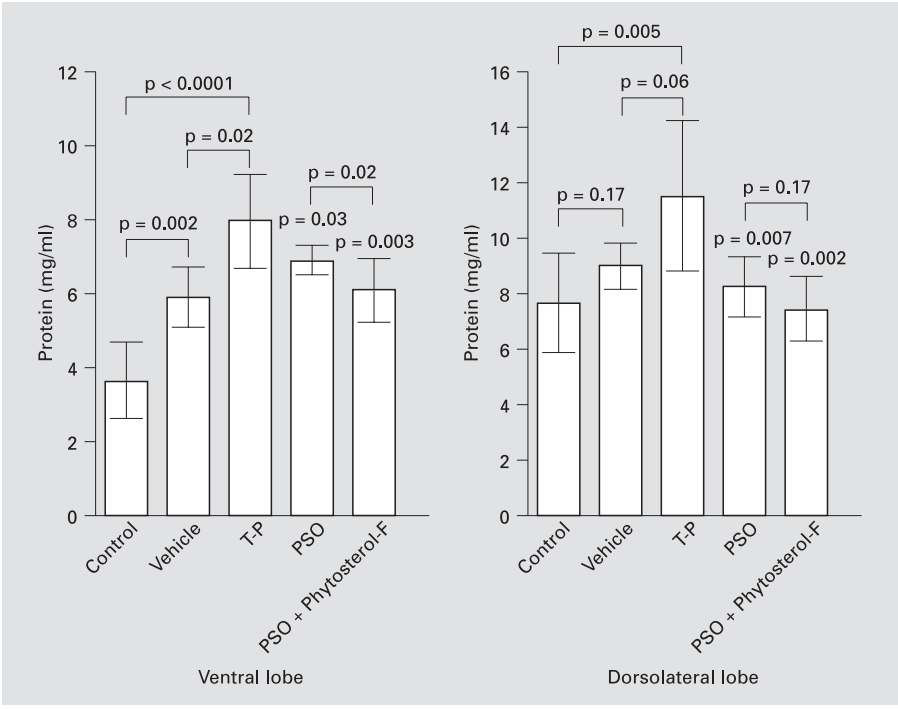

Fig. 3. Total protein level in each group. Significant increases of the protein levels were noted within both lobes after the T-P administration (ventral lobe, p = 0.02 and p <0.0001; dorsolateral lobe, p = 0.06 and p = 0.005). The increases of total protein levels within both lobes were significantly blocked either by PSO alone (p = 0.03 and p = 0.003) or by PSO + Phytosterol-F (p = 0.007 and p = 0.002). In addition, there was some additive effect in terms of protein change from Phytosterol-F within ventral lobes (p = 0.02), but statistical significance was not observed in dorsolateral lobes (p = 0.17).

tral or dorsolateral lobes in terms of weight reduction (p = 0.61 and p = 0.20, respectively). These data show that the combination of Phytosterol-F produced a limited additive effect on the T-P-induced changes of the rat prostate.

Discussion

The hormone-dependent prostate growth in humans has been known since many decades. Significant prostate enlargement starts early during the 3rd decade and con-

tinues slowly throughout life. However, the prostate remains small, if androgen deprivation started in youth, such as after bilateral orchiectomy [18]. Within the prostatic epithelial cells, androgen is able to increase the protein synthesis [19, 20]. Simultaneous administration of dihydroxytestosterone and prazosin to testis-intact rats can increase the prostate-to-body weight ratio and cause the lower urinary tract symptom-like phenotype [15]. In this study, we established a testis-intact animal model mimicking the neurohormone-associated prostate growth in humans, induced by the administration of T-P. Histological sections from the ventral prostate lobe showed hyperplasia of the architecture of the prostate glands. Our data also demonstrated that there was an increased ventral but not dorsolateral lobe prostate-to-body weight ratio and an increased protein synthesis within both lobes after T-P administration. Despite the fact that the increased prostate weight and the increased protein synthesis do not fully translate into BPH-associated voiding symptoms, these changes can be used for monitoring the hormone-associated effects on the prostate growth.

Interestingly, our data demonstrate that some findings were not in conformity with weight and protein changes. The protein synthesis can be up- or downregulated within a few days under the influence of testosterone; in contrast, size or weight of the prostate increased or decreased slowly and gradually with age [18–20]. Therefore, as a parameter for evaluating the androgen effect within a short period, the change of the total protein level would be more sensitive than the weight change.

Moreover, our data also revealed that there were differential effects between the two prostate lobes under the T-P or PSO treatment with and without Phytosterol-F in terms of weight and protein changes. Indeed, each lobe of the prostate has different proportions of androgen receptors and adrenergic receptors [21]. Many previous studies [19–21] showed that the rat ventral prostate is androgen associated, but the dorsolateral prostate is not. Despite of the lack of a weight change, the protein synthesis in the dorsolateral prostate lobe is also increased under the T-P treatment. This implies that there might be factors other than androgen affecting growth of the dorsolateral prostate lobe, such as adrenergic signaling.

Since PSO is rich in many antioxidants and beneficial nutritional supplements such as essential fatty acids, β-carotenes, lutein, γ- and β-tocopherols, phytosterols, and selenium, it is postulated that PSO is of benefit in prostatic diseases. Our results demonstrated that PSO monotherapy or PSO combined with Phytosterol-F can significantly block the T-P-induced changes in the rat prostate in terms of prostate weight-to-body weight ratio and protein levels. From our findings, PSO exhibits some antiandrogenic or antiadrenergic effects at least. Although the whole mechanisms are still unclear, it is worthy to further investigate the effects of PSO. Since PSO also exerts the ability to control serum lipid levels and blood pressure, it is suitable for elderly men with BPH and simultaneous cardiovascular disease [22, 23]. To enhance the treatment effect, Phytosterol-F is added to PSO to increase the proportion of phytosterol. Our data demonstrated that there was some additive effect on protein synthesis from Phytosterol-F despite a lack of effect on the weight reduction. However, it is difficult to clarify which effect Phytosterol-F exerts – antiandrogenic or antiadrenergic.

There are several limitations in this study. First, regardless of testis intact or testis absent, in rats the responses to T-P are not equal to the induction of BPH in humans. Therefore, these results on the inhibition of prostate growth in rats cannot be translated into clinical practice. Second, the parameters weight ratio and protein levels, used in this study, only roughly reflect some changes after induction or therapy. Indeed, many factors may affect these two parameters, such as nutritional status or stress from manual feeding. It might also explain why there was a wide scale of error bars in this study. Third, the effect of Phytosterol-F-alone therapy was not investigated in this study. We could not clarify the effect of Phytosterol-F. Finally, the dosage used in this study was calculated according to the amount of all forms of phytosterols contained in the extract of *S. repens* (Permixon) for humans. Therefore, such a translation might not be suitable for rats.

In conclusion, a 14-day T-P treatment increased the total protein concentration in both lobes of the rat prostate and the weight ratio in the ventral lobe. These changes could be blocked by either PSO monotherapy or PSO combined with Phytosterol-F. Phytosterol-F can induce some additive blockade of the protein synthesis in the ventral lobe. Further study might be required for investigating the molecular mechanisms of these extracts. Nevertheless, these results obtained in rats can not be totally translated into the human situation.

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