

Full Length Research Paper

Effects of *Platycodon grandiflorum* methanolic extract on testosterone-induced benign prostatic hyperplasia in Wistar rats

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Accepted 8 May, 2012

Benign prostatic hyperplasia (BPH) is highly prevalent in the male population over the age of 60 years, manifesting as prostatic enlargement and distinctive changes in tissue histomorphology. In this study, we investigated whether a *Platycodon grandiflorum* methanolic extract (PGME) improved BPH in a testosterone propionate (TP)-induced model of BPH in rats. Castration was performed via the scrotal route under sodium pentobarbital anesthesia, and BPH was induced in the rats with a subcutaneous injection of TP (3 mg/kg) given every consecutive day for 4 weeks after castration. The control group of castrated rats received subcutaneous injections of corn oil. Experimentally, induced rat model of BPH, PGME led to significant reductions in prostate weight and dihydrotestosterone levels in the serum and prostate. Histologically, BPH was evident in the ventral lobe of the prostate, and PGME treatment significantly reduced the severity of the lesion. Therefore, PGME was effective in reducing TP-induced BPH in a rat model, and may be useful for the clinical treatment of patients with BPH.

Key words: Benign prostatic hyperplasia (BPH), dihydrotestosterone, testosterone, *Platycodon grandiflorum*.

INTRODUCTION

Benign prostatic hyperplasia (BPH) is a multifactorial disease and the commonest noncancerous form of abnormal prostate cell growth affecting older man (Gossel and Wuest, 2004). As the prostate enlarges, it can constrict the urethra, inducing lower urinary tract symptoms (LUTS) such as a weak urinary stream, incomplete bladder emptying, nocturia, dysuria and bladder outlet obstruction (Pais, 2010; Roehrborn, 2011). Currently, the prevalence of BPH is age-dependent, with initial development usually occurring after 40 years of age and as estimated 50% of men exhibit the symptoms related with BPH by the age of 50 (Roehrborn and Rosen, 2008).

Although the etiology of BPH is not completely understood, it involves hormonal changes in aging men. The development and growth of the prostate gland

depends on androgen stimulation, mainly by dihydrotestosterone (DHT), an active metabolite formed by the enzymatic conversion of testosterone by steroid 5α -reductase. According to previous studies, increased conversion of testosterone to DHT with aging, which is catalyzed by prostate 5α -reductase, is a crucial alteration in development of BPH. Elevated prostatic DHT level promotes excessive prostatic epithelial and stromal cell growth, causing hyperplasia (Carson and Rittmaster, 2003). So, various drugs have been developed with a focus on reduction of DHT level. Particularly, finasteride, as a 5α -reductase inhibitor has been commercially used for treatment of BPH. 5α -reductase inhibitors suppressed the development of BPH via inhibiting the conversion of testosterone to DHT (Foley and Kirby, 2003; Pais, 2010). However, these drugs are limited because of their side effects, including decreased libido, ejaculatory or erectile dysfunction, and nasal congestion (Bullock and Andriole, 2006). To reduce their side effects and reduce the development of BPH, alternative natural materials such

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as saw palmetto and coconut oil have been developed (Bisson et al., 2007; Pais, 2010)

In Asian countries, the root of *Platycodon grandiflorum* has been widely used as a traditional drug since ancient times. More specifically, it is often prescribed as a folk remedy and herbal medicine for coughs, sputum, asthma, and tonsillitis. Recent studies have shown that materials derived from the *Platycodon* root are effective against asthma, have anti-inflammatory effects, and act against fatty liver (Ahn et al., 2005; Choi et al., 2009; Khanal et al., 2009). However, no study has examined the efficacy of *P. grandiflorum* on testosterone-induced BPH in rats. Therefore, in this study, we investigated whether oral dosing with *P. grandiflorum* prevents testosterone-induced hyperplasia in rats.

MATERIALS AND METHODS

Preparation of *platycodon grandiflorum* methanolic extract (PGME)

Roots of *P. grandiflorum* were purchased in October, 2008 from HMAX (Chungbuk, Republic of Korea). A voucher specimen has been deposited at the Korea Institute of Oriental Medicine (KIOM), Daejeon, Republic of Korea. The fresh *P. grandiflorum* was washed three times with tap water to remove salts, epiphytes, and sand, and was stored at -20°C . The frozen samples were lyophilized and homogenized in a grinder before extraction. The dried roots of *P. grandiflorum* (300 g) were extracted three times with 70% MeOH (3 L) with sonication for 1 h. The extract solution was filtered through filter paper and evaporated to dryness (41.31 g). The yield of the dried extract from the crude starting material was 13.77%. The concentrate was freshly dissolved in phosphate-buffered saline before use.

Animals

Specific-pathogen-free male Wistar rats, aged 10 weeks, that had been routinely serologically screened for the relevant respiratory pathogens, were purchased from Jung Ang Co. Ltd (Seoul, Korea). The rats were maintained in an animal facility under standard laboratory conditions for 1 week before the experiments, and maintained on a 12 h light–dark cycle in a room with controlled temperature and humidity. All experimental procedures were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals, and all animal handling followed the dictates of the National Animal Welfare Law of Korea.

Experimental BPH model and drug administration

Adult male Wistar rats, weighing 250 to 350 g, were used for the *in vivo* experiments. After one week of acclimatization, all rats were castrated while anesthetized by an intraperitoneal injection of pentobarbital three days before the beginning of the experiments to exclude the influence of intrinsic testosterone (Sun et al., 2008). Castration was performed by removing the testicles and epididymal fat through the scrotal sac, according to a method published previously (Coppenolle et al., 2000). The spermatic cord and blood vessels were ligated with 3-0 sutures and resected.

BPH condition was induced by repeated subcutaneous injection of testosterone propionate for 4 weeks according to previous study (Sun et al., 2008; Rick et al., 2011). Experimental groups were

divided into 5 groups. Normal control group (A) did not receive any treatment. Castrated rats were randomly divided into four groups ($n = 6$): (B) castration group, which received PBS administered orally and corn oil injected subcutaneously; (C) BPH group, which received PBS administered orally and testosterone propionate (TP, 3 mg/kg body weight) injected subcutaneously; (D) PGME group, which received PGME (200 mg/kg body weight) administered by oral gavage and TP (3 mg/kg body weight) injected subcutaneously; (E) positive control group, which received finasteride (10 mg/kg body weight) administered orally and TP (3 mg/kg body weight) injected subcutaneously. The positive control drug, finasteride, is a well-known 5α -reductase inhibitor used for BPH treatment. All rats were treated once a day for four weeks. Body weight was measured weekly during the experiment. The application volume was calculated in advance, based on the most recent recorded body weight of individual animals.

Blood and tissue samples

After a treatment period of 4 weeks and an overnight fast, the rats were anesthetized with sodium pentobarbital (100 mg/kg body weight, i.p.). Blood samples were collected and centrifuged at $2000 \times g$ for 10 min. The prostate was collected from each rat and weighed. All prostatic specimens from each group were fixed with 10% buffered formalin for 24 h. The tissues were then embedded in paraffin, cut into 4 μm sections, and stained with hematoxylin (Sigma MHS-16)–eosin (Sigma HT110-1-32) solution. The tissues were then mounted and coverslipped using Dako mounting medium (Dakocytomation, Denmark, CA).

Measurement of DHT and testosterone in prostates and serum

The prostate tissue was homogenized in lysis buffer containing protease inhibitors (50 mM Tris-HCl [pH 7.4], 150 mM NaCl, 1 mM EDTA, 0.5% NP-40, 0.1% SDS, 1 mM EGTA, 100 $\mu\text{g}/\text{mL}$ PMSF, 10 $\mu\text{g}/\text{mL}$ pepstatin A, and 100 μM Na_3VO_3). The homogenates were centrifuged at $12,000 \times g$ for 25 min at 4°C , and the protein concentrations in the supernatant fractions were determined using Bradford reagent (Bio-Rad, Hercules, CA). Enzyme-linked immunosorbent assays (ELISAs) were performed according to the manufacturer's instructions. DHT and testosterone levels in the serum and prostates were measured with ELISA kits (Cayman, USA). The values are expressed per milligram of protein.

Measurement of alanine aminotransferase (ALT) and aspartate aminotransferase (AST) levels in serum

ALT and AST levels were determined to assess liver and kidney function using commercial kits (Beckman Coulter, Inc., Fullerton, CA, USA) and an autoanalyzer (Beckman CX4, CA, USA).

Image capture and photomicrography

Photomicrographs were captured with a Photometric Quantix digital camera running a Windows program and montages were assembled in Adobe Photoshop 7.0. The images were cropped and corrected for brightness and contrast, but were not otherwise manipulated.

Statistical analysis

Data are expressed as means \pm standard deviations (SD). Statistically significant differences were determined with Student's

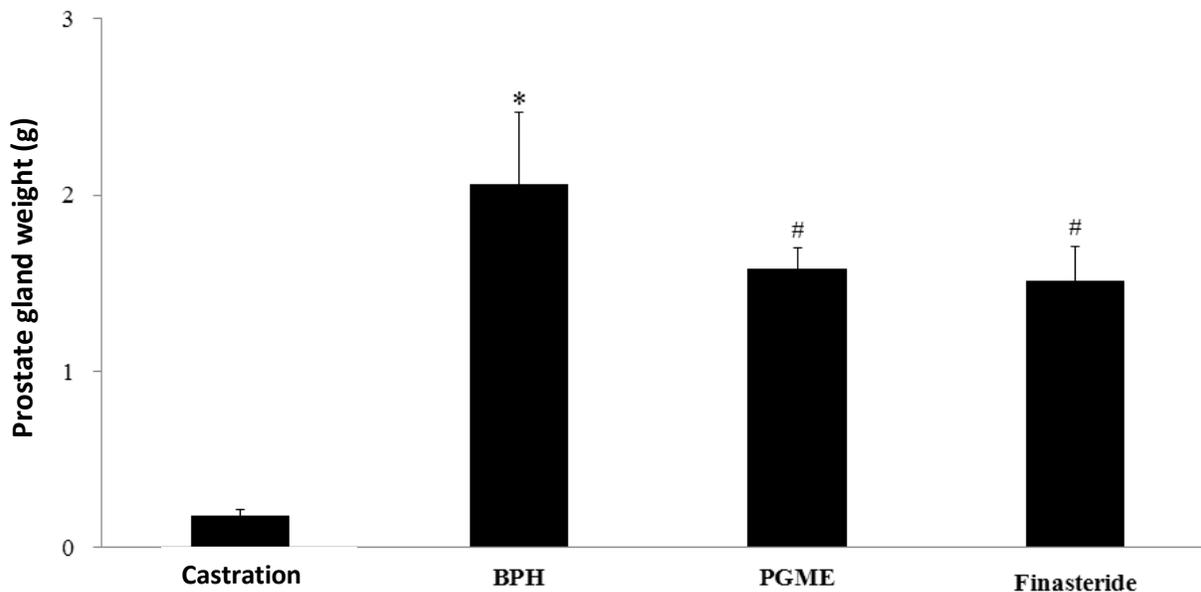


Figure 1. Effects of PGME on prostate weight. The rat model of benign prostate hyperplasia (BPH) was induced with daily injections of testosterone for 4 weeks after castration. BPH group showed a significant increase in the prostate weight compared with castration group. In contrast, PGME group and Finasteride group significantly decreased compared with BPH group. Values are expressed as means \pm SD ($n = 6/\text{group}$). #Significant difference at $p < 0.01$ compared with the castration group. *Significant difference at $p < 0.05$ compared with the BPH group.

two-tailed t test when independent means were compared, using Microsoft Excel. The critical level for significance was set at $p < 0.05$.

RESULTS

Effect of PGME on prostate weight

As shown in Figure 1, the prostate weights of the rats in the various groups were statistically significantly different at the end of the study. The castration group (0.18 ± 0.04 g) markedly decreased the prostate weights when compared with normal control group (1.44 ± 0.12 g). In contrast, the BPH group (2.06 ± 0.41 g) significantly increased the prostate weight compared with the castration group. The finasteride group (1.51 ± 0.20 g) significantly reduced the prostate weights compared with the BPH group. Also, PGME group (1.58 ± 0.12 g) was observed the significant reduction in the prostate weight in comparison to the BPH group, which is very similar to the finasteride group.

Effect of PGME on testosterone and DHT levels in serum

In testosterone levels in serum, there was no significant difference between all groups except for the normal control group (Table 1). In DHT levels in serum, the BPH group statistically increased more than the normal control group (8.79 ± 1.20 ng/mL) and castration group ($6.24 \pm$

1.13 ng/mL). Conversely, finasteride group (14.45 ± 3.62 ng/mL) markedly decreased the DHT levels in serum compared with the BPH group. PGME group (10.07 ± 2.12 ng/mL) also significantly increased the DHT levels in serum as well as the result of finasteride group.

Effect of PGME on DHT levels in prostate

As shown in Figure 2, the DHT level of BPH group (61.56 ± 7.87 ng/mL) significantly increased more than the normal control group (31.96 ± 0.60 ng/mL) and castration group. In contrast, the finasteride group (28.23 ± 6.77 ng/mL) significantly increased the DHT level in prostate compared with the BPH group. The PGME group (34.77 ± 9.70 ng/mL) also observed the significant reduction in the DHT level of prostate in comparison to the BPH group, which was similar to the result of finasteride group.

Effect of PGME on the histomorphological changes in prostate tissue

The prostate of castration group was observed the atrophy of glandular and the reduction of epithelial thickness of prostate compared with the normal control. In contrast, the prostate of BPH group showed the increased thickness of epithelial in prostate caused by epithelial cell hyperplasia. The finasteride group was observed the reduction in epithelial thickness of prostate compared with BPH. The PGME group was observed the

Table 1. The effects of PGME on DHT and testosterone levels in serum.

Group	Dose (mg/kg)	Testosterone (pg/mL)	DHT (ng/mL)
NC	-	133.07 ±20.1	8.79 ± 1.20
Castration	-	38.34 ± 0.3	6.24 ± 0.50
BPH	-	204.04 ±39.1#	19.16 ± 3.49#
PGME	100	174.99 ± 31.3	10.58 ± 2.22*
Finasteride	10	170.52 ± 28.1	14.45 ± 3.61

#P < 0.05 vs. castration group, *P < 0.05 vs. BPH group.

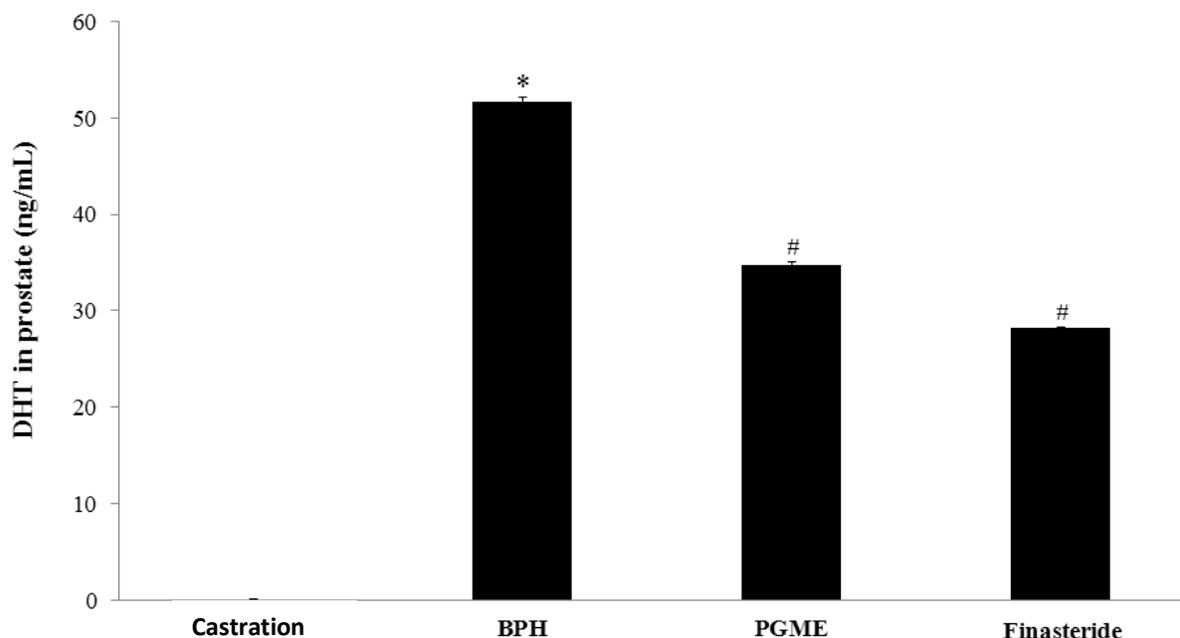


Figure 2. Effects of PGME on DHT levels in prostate. DHT levels in prostate were evaluated using ELISA. BPH group markedly increased DHT level in prostate while PGME and Finasteride groups significantly reduced in comparison to BPH group. Values are expressed as means ± SD (n = 6/group). #Significant difference at p < 0.01 compared with the castration group. *Significant difference at p < 0.05 compared with the BPH group.

reduction in epithelial thickness of prostate compared with the BPH group, as well as the finasteride group (Figure 3).

Cytotoxicity of PGME in a BPH model

PGME did not affect the activities of the serum toxicity marker enzymes, ALT and AST, indicating normal liver and kidney function (Figure 4).

DISCUSSION

We examined the effects of PGME in a testosterone-induced rat model of BPH and compared them with the effects of finasteride, which is currently used to treat BPH.

Treatment with PGME for 4 weeks significantly inhibited the development of testosterone-induced prostatic hyperplasia, which was evident in the reduction in the elevated prostate weight/body weight ratio, reduced DHT levels in the prostate and serum, and histopathological evidence.

The increased prostate weight is used as one of crucial markers of BPH according to previous study (Pais, 2010). BPH is characterized by stromal and epithelial cells hyperplasia, resulting in prostate enlargement. In previous studies, animals with BPH had a significant increase in prostate weight compared with normal control animals, whereas those of animals treated with finasteride or others herbal remedies for the management of BPH had meaningfully reduced compared with BPH animals (Bisson et al., 2007; Pais, 2010). For these reason, many studies have evaluated the inhibitory

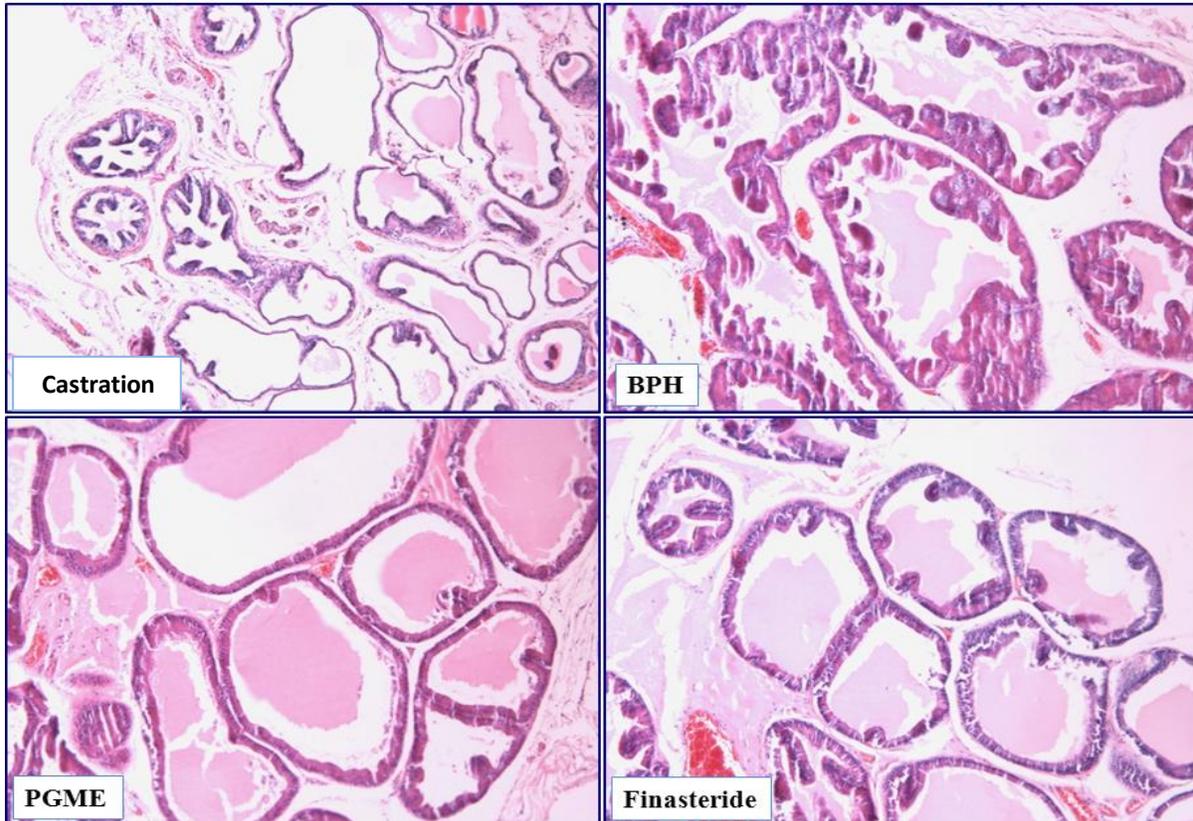


Figure 3. Effects of PGME on prostate hyperplasia. Histological examination of prostate tissue was performed 24 h after the final testosterone injection. Prostate tissues were fixed, sectioned to 4 μ m, and stained with hematoxylin–eosin solution (magnification $\times 200$). BPH group was observed the hyperplasia of epithelial cell. In contrast, PGME and Finasteride groups reduced the hyperplasia of epithelial cell, indicating decrease in epithelial layer thickness when compared with BPH group.

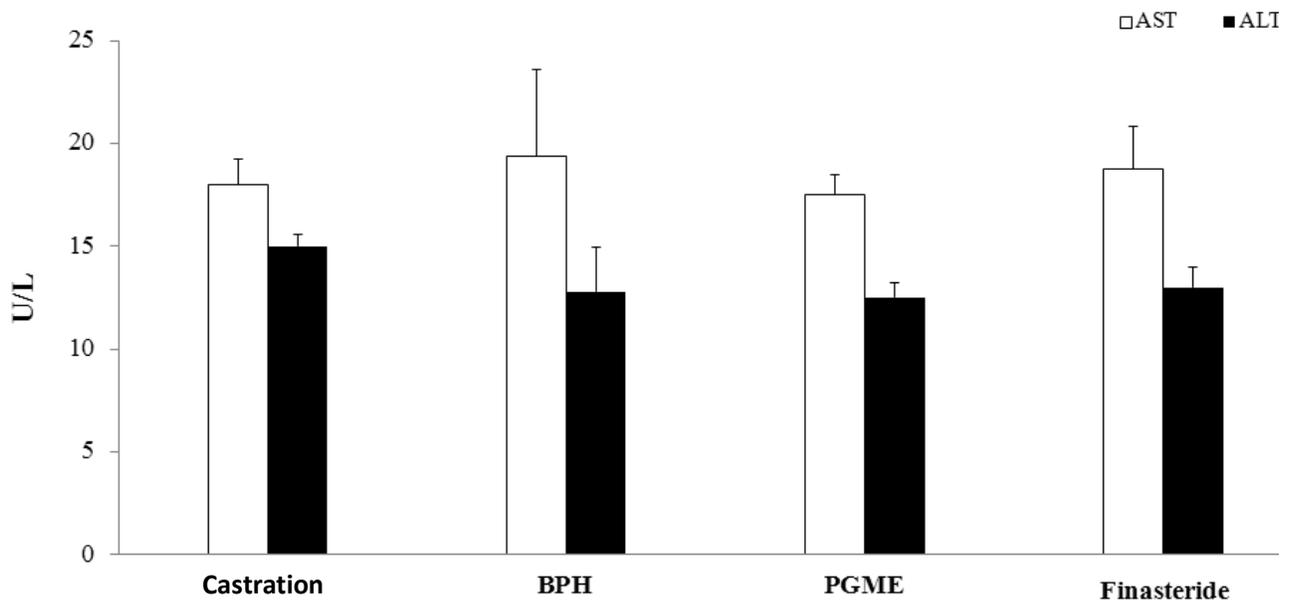


Figure 4. Effects of PGME on serum AST and ALT levels. Serum was collected after the final testosterone injection was given to the rats. Each sample was analyzed using commercial AST/ALT detection kits. There were no significant differences of serum AST and ALT levels between all groups.

effects of various materials on the development of BPH by measuring prostate weight (Jang et al., 2010; Veeresh Babu et al., 2010). In present study, the animals with BPH showed significantly increased prostate weight compared with the normal control animals; however, PGME-treated animals showed the significant reduction in these measures compared with the BPH animals. These findings were consistent histomorphologic examination of prostate. BPH animals showed epithelial hyperplasia with an increase in epithelial thickness compared with the normal control animals. In contrast, PGME-treated animals showed the reduction in epithelial hyperplasia with decrease in epithelial thickness. Based on these results, PGME treatment effectively inhibited the prostatic hyperplasia induced by testosterone.

The two main classes of drugs used as BPH treatments are inhibitors of α 1-adrenoceptor inhibitors, which inhibit smooth muscle cell contraction (Furuya et al., 1982), and inhibitors of type II 5α -reductase, an enzyme responsible for the conversion of testosterone to the more potent androgen dihydrotestosterone (DHT) (Griffiths and Denis, 2000). Steroid 5α -reductase converts testosterone to DHT, an active form of androgen, in the prostate. Increased production of DHT results in the development of prostatic hyperplasia (Pais, 2010). Because DHT has a 10 times higher affinity for the androgen receptor than testosterone, DHT easily binds to androgen receptor, which stimulates the transcription of growth factors that are mitogenic for the epithelial and stromal cells for prostate (Carson and Rittmaster, 2003). The importance of DHT in prostatic hyperplasia was demonstrated by previous studies in which an inhibitor of 5α -reductase was administered to experimental animals with BPH (Roehrborn, 2011). For instance, at clinical doses, finasteride is selective for 5α -reductase and achieves about 70% suppression of serum DHT and 68%–86% suppression of intraprostatic DHT (Span et al., 1999). These findings are in agreement with our results. In present study, PGME reduced the DHT levels in the prostate and serum relative to those in rats with testosterone-induced BPH. Thus, these results indicate that PGME inhibits the development of BPH via down-regulation of DHT. Interestingly, although testosterone level in finasteride-treated group was not observed a significant difference, it was decreased compared with the BPH animals. Before performing present study, we expected the increase in testosterone level according to previous study (Pais, 2010; Roehrborn, 2011). This finding is unlike to previous studies and is unexpected. Many researchers have conducted the studies on relationship between drug treatment and testosterone level in BPH condition. In most previous studies, administration of finasteride showed that the testosterone level is increased compared with that of BPH animals in many studies due to inhibit the transformation testosterone to DHT. On the other hand, some previous studies showed that the testosterone level is similar to

that of BPH animals following drug treatment as well as the result of present study (Gasco et al., 2007). However, we did not find the clear reason why testosterone level is not changed in present study.

Conclusion

Oral administration of PGME in a BPH rat model significantly decreased the prostate weight, prostatic epithelial hyperplasia, and DHT levels in the serum and prostate. These results indicate that PGME may effectively inhibit the development of BPH. A further study is required to explore the effects of the major active components of *Platycodon grandiflorum* and the molecular mechanisms underlying their action.

ACKNOWLEDGEMENT

This research was supported by a grant from 'The Evidence-based Medicine for Herbal Formulae' from the Korea Institute of Oriental Medicine (KIOM).

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