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ORIGINAL ARTICLE

# Jaeumganghwa-tang, a Traditional Herbal Formula Inhibits the Development of Benign Prostatic Hyperplasia in Rats

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**Abstract** Benign prostate hyperplasia (BPH) is the most common proliferative disorder affecting older men and results in prostate enlargement and lowered urinary tract symptoms. Jaeumganghwa-tang (JGT), an oriental traditional herbal formula, has been used in China (Zi-yin-jiang-huo-tang in Chinese), Japan (Jiin-koka-to in Japanese), and Korea for many years. Effects of JGT on prostate dihydrotestosterone (DHT) level and prostatic hyperplasia were investigated using a rat model, in which BPH was induced using testosterone propionate (TP). Rats were divided into five groups. One group was used as a normal, and four groups received subcutaneous injections of TP for 4 weeks to induce BPH. JGT (200 or 400 mg/kg) was administered daily for 4 weeks by oral gavage concurrently with TP injections, and rats were sacrificed at scheduled times. Prostates were weighed, and histopathologic examination was conducted. DHT levels in serum and the prostate were measured, and the expression of proliferating cell nuclear antigen (PCNA) protein was investigated using Western blotting. BPH animals showed increases in absolute and relative weights of the prostate, levels of DHT in serum and the prostate, and expression of PCNA in the prostate, whereas JGT-treated animals showed significant reductions in these indices compared with the BPH animals. Administration of JGT attenuated TP-induced epithelial hyperplasia. These findings indicate that JGT inhibits the development of BPH, an effect closely associated with a reduction in DHT level.

**Keywords** benign prostatic hyperplasia · dihydrotestosterone · Jaeumganghwa-tang · traditional herbal formula

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## Introduction

Benign prostate hyperplasia (BPH) is the most common proliferative disorders affecting older man and is characterized by hyperplasia of the stromal and epithelial cells of the prostate, resulting in prostate enlargement and lower urinary tract symptoms such as a weak urinary stream, incomplete bladder emptying, frequent urination, nocturia, urgency, and dysuria (Preuss and Adderly, 1998; Bhargava et al., 2004). The prevalence of BPH is age-dependent, with initial development usually occurring after 40 years of age (Roehrborn and Rosen, 2008). An estimated 50% of men exhibit histologic evidence of BPH by the age of 50, and 75% of men exhibit histologic evidence of BPH by 80. Currently, the two main medications used for BPH worldwide are α1-adrenergic receptor antagonists such as doxazosin, terazosin, and tamsolusin, and 5α-reductase inhibitors such as finasteride and dutasteride. Alpha-blockers are the most common choice for initial therapy in the USA and Europe (Black et al., 2006; Hutchison et al., 2007; Roehrborn et al., 2007). They relax smooth muscle in the prostate and the neck of the bladder, thereby counteracting any obstruction to the flow of urine. On the other hand, the 5α-reductase inhibitors modulate hormone levels (Roehrborn et al., 2004). In aging men, increased conversion of testosterone to dihydrotestosterone (DHT), which is catalyzed by prostate 5α-reductase, is pivotal in development of BPH, because elevated prostate DHT concentration promotes excessive cellular growth, causing hyperplasia (Andriole et al., 2004). In addition, 5α-reductase inhibitors inhibit production of DHT. Although α1-blockers and 5α-reductase inhibitors are effective treatments for

**Table 1** Crude components of JGT

| Scientific name | Latin name | Amount (g) | Company of purchase | Source |
|---|---|---|---|---|
| *Paeonia lactiflora* | Paeoniae Radix | 4.875 | Omniherb | Hwasun, Korea |
| *Angelica gigas* | Angelicae Gigantis Radix | 4.5 | Omniherb | Pyeongchang, Korea |
| *Rehmannia glutinosa* | Rehmanniae Radix Preparata | 3.75 | Omniherb | Jangheung, Korea |
| *Asparagus cochinchinensis* | Asparagi Tuber | 3.75 | HMAX | China |
| *Atractylodes japonica* | Atractylodis Rhizoma Alba | 3.75 | Omniherb | China |
| *Liriope platyphylla* | Liriopis Tuber | 3.75 | Omniherb | Yeongcheon, Korea |
| *Rehmannia glutinosa* | Rehmanniae Radix Crudus | 3.00 | Omniherb | Jangheung, Korea |
| *Citrus unshiu* | Citri Unshius Pericarpium | 2.625 | Omniherb | Jeju, Korea |
| *Anemarrhena asphodeloides* | Anemarrhenae Rhizoma | 1.875 | Omniherb | China |
| *Phellodendron amurense* | Phellodendri Cortex | 1.875 | Omniherb | China |
| *Glycyrrhiza uralensis* | Glycyrrhizae Radix et Rhizoma | 1.875 | HMAX | China |
| *Zingiber officinale* | Zingiberis Rhizoma Crudus | 3.75 | Omniherb | Yeongcheon, Korea |
| *Zizyphus jujube* | Zizyphi Fructus | 3.75 | Omniherb | Yeongcheon, Korea |
| Total amount | | 43.125 | | |

BPH, their use is limited due to such side effects as ejaculatory dysfunction, decreased libido, and upper respiratory tract infection (Gormley et al., 1992; Bullock and Andriole, 2006).

Jaeumganghwa-tang (JGT), an oriental traditional herbal formula, has been used for clinical treatment of respiratory diseases and kidney diseases in China, Japan, and Korea. JGT is a mixture of 13 crude herbs (Table 1), and each crude herb is traditionally used for treatment of inflammatory, allergic, and proliferative diseases. Recent studies reported that JGT has protective effects against allergic reactions and inflammation, and modulates the immune response (Kim et al., 2004; Jung et al., 2010). In addition, each crude herb has been shown to have pharmacological effects in *in vivo* and *in vitro* experiments. *Angelicae gigantis radix* (Kil et al., 2008), *Asparagi radix* (Wiboonpun et al., 2004), *Phellodendri cortex* (Park et al., 2007), and *Glycyrrhizae Radix* (Yokozawa et al., 2005) exert antioxidant and anti-inflammatory effects. In particular, *Atractylodis rhizome* (Kang et al., 2011) and Paeoniae radix (Sun et al., 2008) have been shown to exert antiproliferative effects on cancer cell lines by inducing apoptosis. However, no studies have been published on the protective effects of JGT and its crude herbs against BPH. Therefore, we investigated the effects of JGT on prostate DHT level and prostatic hyperplasia using a rat model in which BPH was induced by testosterone.

## Materials and Methods

**Chemical and reagents.** 5-HMF and hesperidin were purchased from Sigma-Aldrich (St Louis, MO) and Chengdu Biopurify Phytochemicals Ltd. (Chengdu, China), respectively. Albiflorin, paeoniflorin, berberine, and glycyrrhizin were the products of Wako (Osaka, Japan). Liquiritin and nodakenin were purchased from NPC BioTechnology Inc. (Daejeon, Korea). The purity of all reference standards was >98.0%. High performance liquid chromatography (HPLC)-grade methanol, acetonitrile, and water were obtained from J.T. Baker (Phillipsburg, NJ). Glacial acetic acid was of analytical reagent grade, procured from Junsei (Tokyo, Japan).

**Preparation of sample solutions.** JGT was prepared in our laboratory from a mixture of chopped crude herbs purchased from Omniherb (Yeongcheon, Korea) and HMAX (Jecheon, Korea). Before performing the study, identity of each crude herb was confirmed by a pharmacist and professors at College of Oriental Medicine. JGT was prepared as described in Table 1 and extracted in distilled water at 100°C for 2 h. The extract was evaporated to dryness and freeze-dried (yield; 20.8%). Lyophilized JGT extract was weighed (200 mg), poured into a 20-mL flask, and distilled water added to the volumetric mark. After dissolving, the extract was filtered through a 0.2-μm syringe filter, and 10 μL aliquots of filtrate were injected into the HPLC column.

**HPLC analysis of JGT.** A methanol standard stock solution containing components 5-HMF, albiflorin, paeoniflorin, berberine, liquiritin, nodakenin, hesperidin, and glycyrrhizin (all at 1,000 μg/mL) was prepared and diluted to the appropriate concentration range for establishing calibration curves. Analysis was performed using a Shimadzu LC-20A HPLC system (Shimadzu Co., Kyoto, Japan), consisting of a solvent delivery unit, an on-line degasser, a column oven, an autosampler, and a PDA detector. The data processor employed LC solution software (Version 1.24). The analytical column used was a Gemini C18 (250 mm × 4.6 mm; particle size 5 μm, Phenomenex, Torrance, CA). The mobile phases consisted of solvent A (1.0%, v/v, aqueous acetic acid) and solvent B (acetonitrile with 1.0%, v/v, acetic acid). The gradient flow was as follows: (A)/(B)=95/5 (0 min) (A)/(B)=30/70 (40 min) (A)/(B)=0/100 (45 min; hold for 5 min) (A)/(B)=95/5 (55 min; hold for 5 min). The column temperature was maintained at 40°C. The analysis was carried out at 1.0 mL/min with PDA detection at 190–400 nm. The injection volume was 10 μL.

**Animals.** Male 12-week-old Wistar rats weighing 250–350 g (Central Lab. Animal. Int. Seoul, Korea) were housed in a room

maintained at 18–23°C and at a relative humidity of 40–60% with an alternating 12 h/12 h light/dark cycle and were offered a standard laboratory diet and water *ad libitum*. All experimental procedures were carried out in accordance with the NIH Guidelines for the Care and Use of Laboratory Animals, and animal handling followed the dictates of the National Animal Welfare Law of Korea.

**Induction of BPH and treatment.** BPH was induced by subcutaneous injection of testosterone propionate (TP, 3 mg/kg body weight, s.c.) for 4 weeks. After one acclimatization, the rats were divided into five groups: (A) normal control group, which received PBS orally with corn oil (s.c.), (B) BPH group, which received PBS orally and TP (s.c.), (C) positive control group, which received finasteride (10 mg/kg body weight) by oral gavage and TP (s.c.), and (D and E) JGT groups, which received 200 and 400 mg/kg body weight, respectively of JGT orally and TP (s.c.). Finasteride (Sigma, Saint Louis, MI), a 5α-reductase inhibitor, was used as a positive control. The effective dose of finasteride was based on previous reports (Yamashita et al., 1996; Huynh, 2002). All animals were treated once daily for 4 weeks and body weight was measured weekly. The application volumes were 5 mL/kg body weight for oral administration (PBS, finasteride, and JGT) and 3 mL/kg body weight for subcutaneous injection (corn oil and TP) and were calculated in advance based on the most recently recorded body weights of individual animals.

After the last treatment, all animals were fasted overnight and anesthetized using pentobarbital (100 mg/kg body weight, i.p.). Blood samples were drawn from the caudal vena cava, and the serum was separated by centrifugation. Serum was stored at at –80°C for hormone assays. The prostate were removed immediately and weighed. Relative prostate weight was calculated as the ratio of prostate weight to body weight. The percentage inhibition of the increase in prostate weight induced by JGT was determined according to a previously described method (Veeresh Babu et al., 2010). Briefly, the ventral lobe of the prostate was divided in half. One half was fixed using 10% neutral-buffered formalin and embedded in paraffin for histopathologic examination and the other was stored at –80°C for the hormone analysis.

**Preparation of prostate homogenates.** Prostate tissue was homogenized (1/10 w/v) in Tissue Lysis/Extraction reagent containing protease inhibitors (Sigma) using homogenizer. Homogenates were centrifuged at 12,000 g for 25 min at 4°C, and protein concentrations in the supernatant fractions were determined using Bradford reagent (Bio-Rad, Hercules, CA).

**Measurements of DHT levels in serum and prostate.** Levels of DHT in serum and prostate were measured using enzyme-linked immunosorbent assay kit purchased from ALPCO Diagnostics (Salem, NH). Values are expressed as per mg protein for the prostate and per mL for serum.

**Western blotting.** Equal amounts of total lung protein (30 μg) were heated at 100°C for 5 min then loaded onto 12% SDS-PAGE gels, followed by transfer to nitrocellulose membranes at 100 V for 2 h. The membranes were blocked for 1 h with Tris-buffered saline containing 0.05% Tween-20 (TBST) plus 5% skim milk and were incubated with antiproliferating cell nuclear antigen (PCNA, 1:1000 dilution; Santa Cruz Biotechnology, Santa Cruz, CA) and anti-β-actin (1:1000 dilution; Cell Signaling Technology, Danvers, MA) overnight at 4°C. The membranes were washed three times with TBST and then incubated with a 1:10,000 dilution of horseradish peroxidase-conjugated secondary antibody (Jackson ImmunoResearch, West Grove, PA) for 1 h at room temperature. The membranes were again washed three times with TBST and were then developed using an enhanced chemiluminescence kit (ECL, Amersham Biosciences, Little Chalfont, UK).

**Histopathologic examination.** To assess morphological changes in the prostate, tissue embedded in paraffin was cut into 4-μm thick sections and stained with hematoxylin (Sigma MHS-16) and eosin (Sigma HT110-1-32). The tissues were subsequently mounted and cover slipped using mounting medium (Invitrogen, Carlsbad, CA) and were then examined microscopically (Nikon, Japan). In addition, measurement of prostate epithelial thickness was performed using an image analyzer (Molecular Devices, Inc., Sunnyvale, CA).

**Statistical analysis.** Data are expressed as means ± SE. Statistical significance was determined using analysis of variance. When tests showed a significant difference among groups, data were analyzed using a multiple comparison procedure and Dunnett's test (Dunnett, 1964). The significance levels were set at $p < 0.05$ and $p < 0.01$.

## Results

**HPLC analysis of JGT.** Three-dimensional chromatogram was obtained using HPLC-PDA detector. Under optimized chromatography conditions, eight components were eluted before 40 min in sample analysis by using mobile phases consisting of solvent A (1.0%, v/v, aqueous acetic acid) and solvent B (acetonitrile with 1.0%, v/v, acetic acid) (Fig. 1). The retention times of the components were 8.12 (5-HMF), 14.80 (albiflorin), 15.55 (paeoniflorin), 16.32 (berberine), 17.18 (liquiritin), 18.03 (nodakenin), 19.04 (hesperidin), and 33.86 min (glycyrrhizin).

**JGT reduces TP-induced increase in prostate weight.** TP induced a significant increase in absolute and relative prostate weights in the BPH group compared with the normal control group, whereas were markedly decreased in the finasteride group compared with the BPH group (Table 2). JGT-treated animals also exhibited significant decreases in absolute and relative prostate weights compared with the BPH group: JGT inhibited the TP-induced increase in prostate weight by 42.94% in the 200 mg/kg JGT group and by 36.22% in the 400 mg/kg JGT group, which were similar to results of the finasteride-treated group.

**JGT decreases DHT level in serum.** Serum DHT level was markedly increased in the BPH group (356.5±33.30 pg/mL, $p < 0.01$) compared with the normal controls (161.38±32.09 pg/

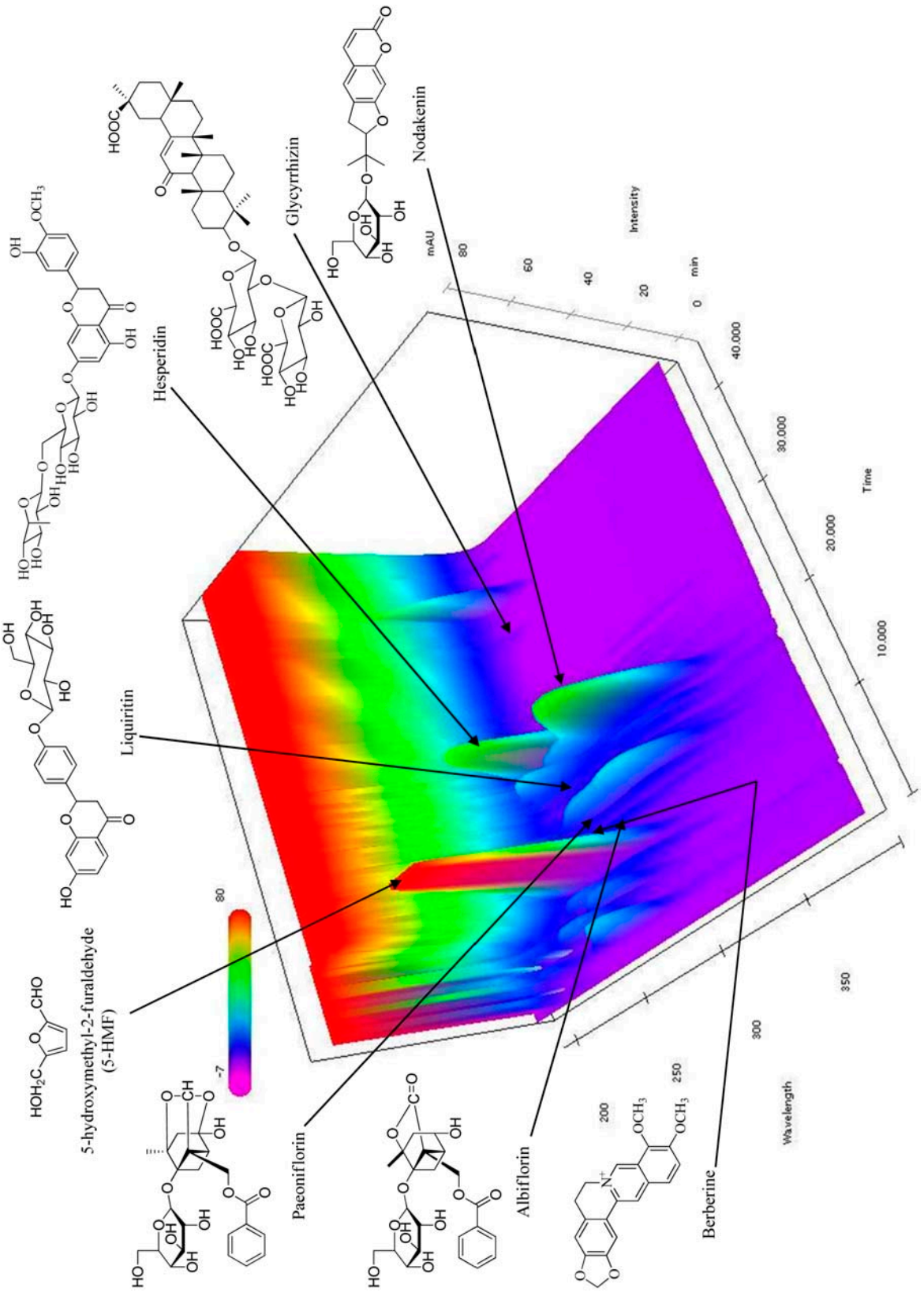


**Fig. 1** Three-dimensional chromatogram of JGT by HPLC-PDA.

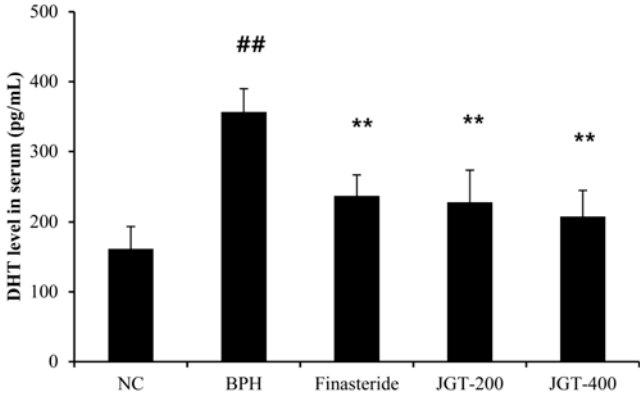


**Fig. 2** Effects of JGT on serum DHT level. NC: corn oil (s.c.) + PBS (p.o); BPH: testosterone (s.c.) + PBS (p.o.); Fin: testosterone (s.c.) + finasteride (10 mg/kg, p.o.); JGT-200, 400: testosterone (s.c.) + JGT (200 and 400 mg/kg, respectively, p.o). ##Significant difference at $p$ <0.01 compared with the normal control. **Significant difference at $p$ <0.01 compared with the BPH.

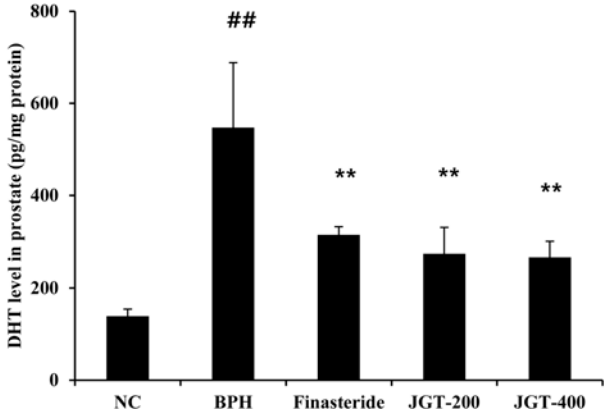


**Fig. 3** Effects of JGT on the DHT level in the prostate. NC: corn oil (s.c.) + PBS (p.o); BPH: testosterone (s.c.) + PBS (p.o.); Fin: testosterone (s.c.) + finasteride (10 mg/kg, p.o.), JGT-200, 400: testosterone (s.c.) + JGT (200 or 400 mg/kg, respectively, p.o). ##Significant difference at $p$ <0.01 compared with the normal control. **Significant difference at $p$ <0.01 compared with the BPH.

mL) whereas was significantly decreased in the finateride-treated group (236.6±29.88 pg/mL, $p$ <0.01) compared with the BPH group. Serum DHT levels were 227.41±45.74 pg/mL in the 200 mg/kg JGT group ($p$ <0.01) and 207.62±36.53 pg/mL in the 400 mg/kg JGT group ($p$ <0.01), and were significantly decreased compared with the BPH group (Fig. 2).

**JGT decreases DHT level in the prostate.** Prostate DHT levels in the BPH group (547.6±140.84 pg/mg protein) were markedly higher than those in the normal controls (Fig. 2). However, prostate DHT levels in the finasteride-treated group (315.5±17.98 pg/mg protein, $p$ <0.01) were significantly lower than those in the BPH group. Similar to the finasteride-treated group, prostate DHT levels were 274.8±56.94 pg/mg protein in the 200 mg/kg JGT group and 266.8±34.93 pg/mL in the 400 mg/kg JGT group,

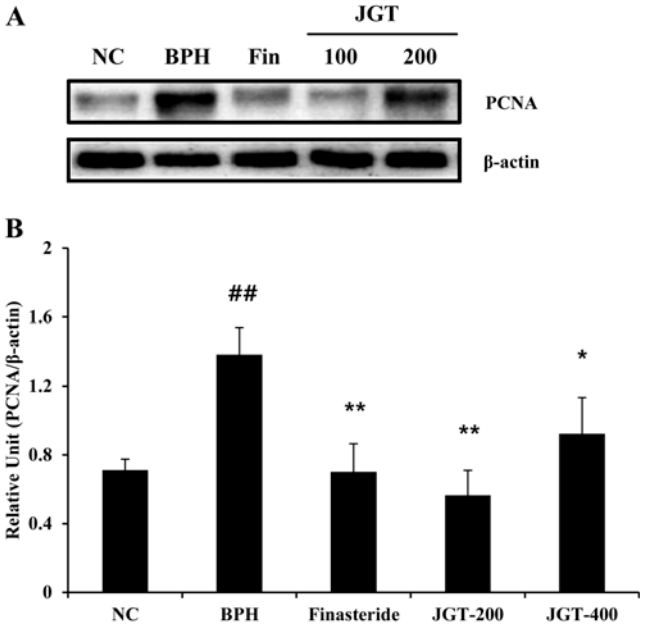


**Fig. 4** Effects of JGT on the expression of PCNA protein. (A) Image of a gel, (B) relative units of PCNA expression (ratio of PCNA to β-actin). NC: corn oil (s.c.) + PBS (p.o); BPH: testosterone (s.c.) + PBS (p.o.); Fin: testosterone (s.c.) + finasteride (10 mg/kg, p.o.); JGT-200, 400: testosterone (s.c.) + JGT (200 and 400 mg/kg, respectively, p.o).

which were significantly lower than those in the BPH group (Fig. 3).

**JGT reduces the expression of PCNA protein in the prostate.** Expression of PCNA protein was increased in the BPH group compared with the normal control group and decreased in the finasteride-treated group compared with the BPH group. Expression of PCNA protein was also reduced in the JGT-treated groups compared with the BPH group (Fig. 4A and 4B).

**JGT attenuates epithelial hyperplasia in the prostate.** Prostate epithelial cell layers were thicker in the TP-induced BPH animals than in the normal controls (Fig. 5A). Finasteride-treated animals showed mild epithelial hyperplasia compared with the BPH animals. JGT-treated animals also showed a reduction in the thickness of the epithelial cell layers of the prostate compared with BPH animals, which was similar to the reduction observed in finasteride-treated animals (Fig. 5B).

## Discussion

The effects of JGT on prostate weight and DHT level in a rat model of TP-induced BPH were evaluated. BPH animals showed increases in prostate weight and in serum and prostate DHT levels compared with normal controls. In histopathologic examination, prostate epithelial cell hyperplasia was observed in BPH animals. In JGT-treated animals, prostate weight and serum and prostate DHT levels were significantly lower than those of the BPH

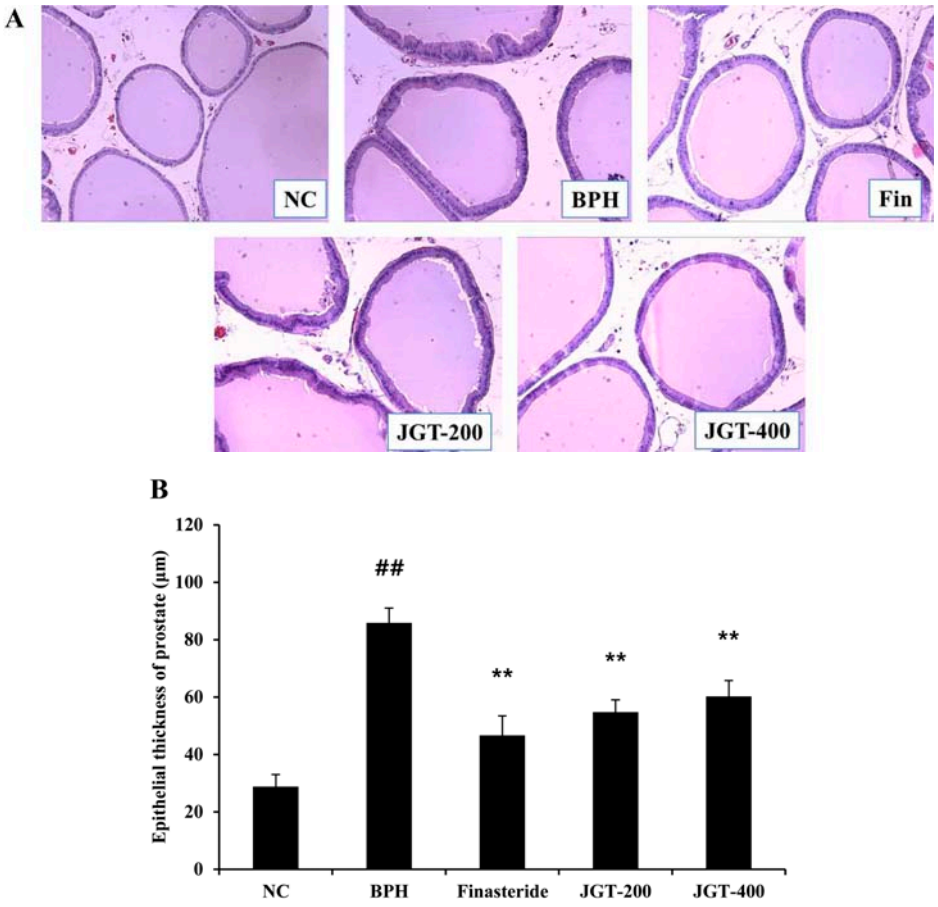


**Fig. 5** Effects of JGT on prostatic hyperplasia. (A) Histology of prostate, (B) epithelial thickness of prostate. Histopathologic examination of prostate tissue was performed 24 h after the final testosterone injection. Prostate tissues were fixed, sectioned at 4-μm thickness, and stained with hematoxylin and eosin solution (magnification ×200); NC: corn oil (s.c.) + PBS (p.o); BPH: testosterone (s.c.) + PBS (p.o.); Fin: testosterone (s.c.) + finasteride (10 mg/kg, p.o.), JGT-200, 400: testosterone (s.c.) + JGT (200 and 400 mg/kg, respectively, p.o).

animals and had less prostate epithelial cell hyperplasia than the BPH animals. In addition, the level of PCNA, a marker of cell proliferation, was lower in the prostate of JGT-treated animals than in those of the BPH animals.

BPH is characterized by stromal and epithelial cell hyperplasia, resulting in prostate enlargement. For this reason, an increase in prostate weight is used as an indicator of BPH (Arruzazabala et al., 2006; Veeresh Babu et al., 2010). Previous studies showed that animals with BPH had significantly increased prostate weights compared with normal controls, whereas animals treated with finasteride and other remedies for the management of BPH had markedly decreased prostate weight compared with BPH animals (Arruzazabala et al., 2007; Veeresh Babu et al., 2010). In the present study, BPH animals exhibited a significant increase in relative prostate weight compared with the normal controls. In contrast, administration of JGT significantly decreased prostate weight compared with the BPH animals. These results were consistent with the results of histopathologic examination of the prostate. BPH animals showed epithelial cell proliferation in the prostate, whereas animals treated with JGT showed mild histomorphological alterations. Therefore, these results indicate that JGT effectively inhibits the induction of prostate enlargement by TP. In addition, PCNA expression was consistent with the effects of JGT in BPH animals. PCNA is widely used to assess cellular proliferation in benign and malignant prostatic hyperplasia. PCNA is a cofactor for DNA ploymerase delta (Shivji et al., 1992; Essers, 2005). PCNA has been shown to be correlate directly with the proliferative state of various tissues. Previous studies showed that PCNA is a valuable marker of proliferation in the prostate tissue, and its protein expression is markedly increased in experimental animals with BPH (Lai et al., 2004; Zhong et al., 2008; Li et al., 2010). In our study, BPH animals showed an increase in the expression of PCNA protein. In contrast, JGT significantly decreased the expression of PCNA protein compared with BPH animals, indicating a reduction in prostatic hyperplasia.

DHT, a metabolite of testosterone, plays a critical role in the development of BPH. DHT is synthesized primarily in the prostate from circulating testosterone by 5α-reductase and acts in

**Table 2** Effects of JGT on body and prostate weights

| Groups | Prostate weights | | % Inhibition | Body weights (g) | |
|---|---|---|---|---|---|
| | Absolute (g) | Relative (g) | | Initial | Final |
| NC | 1.44±0.10 | 0.35±0.02 | | 273.5±8.50 | 416.7±12.75 |
| BPH | 2.97±0.55$^{\#\#}$ | 0.75±0.16$^{\#\#}$ | | 278.3±14.27 | 400.1±31.33 |
| Finasteride | 1.95±0.28$^{**}$ | 0.51±0.04$^{**}$ | 58.83% | 276.0±13.01 | 380.3±46.58 |
| JGT-200 | 2.32±0.29$^{*}$ | 0.58±0.07$^{*}$ | 42.94% | 277.3±13.93 | 403.7±18.35 |
| JGT-400 | 2.31±0.16$^{**}$ | 0.60±0.05$^{*}$ | 36.22% | 278.1±13.45 | 384.2±20.96 |

NC: corn oil injection (s.c) + PBS (p.o.); BPH: testosterone (s.c) + PBS (p.o.); Finasteride: testosterone (s.c) + finasteride (10 mg/kg, p.o.), JGT-200 and -400: testosterone (s.c) + JGT (200 and 400 mg/kg, respectively, p.o.) .

$^{\#\#}$ $p$ <0.01 when compared with the normal control.

$^{*,**}$ $p$ <0.05 or $p$ <0.01 when compared with the BPH group.

an autocrine fashion on the stromal cells of the prostate. It binds to nuclear androgen receptors and signals the transcription of growth factors that are mitogenic for epithelial and stromal cells, resulting in prostate stromal and epithelial cell hyperplasia (Carson and Rittmaster, 2003). DHT has tenfold higher affinity for androgen receptors than testosterone and has 15–30 fold higher affinity for androgen receptors than adrenal androgen, because it dissociates more slowly from the androgen receptor than adrenal androgen (Andriole et al., 2004). 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 et al., 2004). Because DHT is produced from testosterone by 5α-reductase, many researchers have reduced DHT level by inhibiting 5α-reductase. Finasteride, 5α-reductase inhibitor, is an elective drug for treatment of BPH. Finasteride decreases both plasma and prostatic DHT concentrations, resulting in a reduction in prostate weight, and ultimately providing relief from lower urinary tract symptoms caused by BPH (Suzuki et al., 1998; Andriole et al., 2004). In the present study, BPH animals showed increases in DHT levels in serum and the prostate, whereas finasteride significantly reduced these levels as described previously (Suzuki et al., 1998). JGT also decreased DHT levels in serum and prostate. These findings indicate that JGT inhibits the development of BPH in rats and suggest that this effect is closely associated with the reduction in DHT level in BPH animals.

Oral administration of JGT to BPH rats significantly decreased prostate weight, hyperplasia, PCNA expression, and DHT levels in serum and prostate. These findings indicate that JGT effectively inhibits the development of BPH via reduction in DHT level. Therefore, JGT could be useful for the treatment of BPH.

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