Quisqualis indica Improves Benign Prostatic Hyperplasia by Regulating Prostate Cell Proliferation and Apoptosis

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Quisqualis indica (QI) has been used for treating disorders such as stomach pain, constipation, and digestion problem. This study was aimed to evaluate the therapeutic efficacy of QI extract on treating benign prostatic hyperplasia (BPH) in LNCaP human prostate cancer cell line and a testosterone-induced BPH rat model. LNCaP cells were treated with QI plus testosterone propionate (TP), and androgen receptor (AR) and prostate specific antigen (PSA) expression levels were assessed by Western blotting. To induce BPH, the rats were subjected to a daily subcutaneous injection of TP (3 mg/kg) for 4 weeks. The rats in treatment group were orally gavaged with QI (150 mg/kg) together with the TP injection. In-vitro studies showed that TP-induced increases in AR and PSA expression in LNCaP cells were reduced by QI treatment. In BPH-model rats, the prostate weight, testosterone in serum, dihydrotestosterone (DHT) concentration and 5α-reductase type 2 mRNA expression in prostate tissue were significantly reduced following the treatment with QI. TP-induced prostatic hyperplasia and the expression of proliferating cell nuclear antigen (PCNA) and cyclin D1 were significantly attenuated in QI-treated rats. In addition, QI induced apoptosis by up-regulating caspase-3 and -9 activity and decreasing the B-cell lymphoma 2 (Bcl-2)/Bcl-2-associated X protein (Bax) ratio in prostate tissues of BPH rats. Further investigation showed that TP-induced activation of AKT and glycogen synthase kinase 3β (GSK3β) was reduced by QI administration. Therefore, our findings suggest that QI attenuates the BPH state in rats through anti-proliferative and pro-apoptotic activities and might be useful in the clinical treatment of BPH.

Key words benign prostatic hyperplasia; dihydrotestosterone; Quisqualis indica; testosterone

Benign prostatic hyperplasia (BPH) is a common urogenital disorder that affects up to 85% of males who are over 50 years old.1 BPH is characterized by the increased proliferation of epithelial and stromal cells of the prostate.2 BPH generally causes lower urinary tract symptoms (LUTS), such as incomplete bladder emptying, bladder obstruction, bloody urination, and frequent urination.3

The etiology of BPH is not entirely clear. However, the development and incidence of BPH are associated with dysregulation of androgens, and with increased proliferation and decreased apoptosis of cells in the prostate gland.4-9 Testosterone and dihydrotestosterone (DHT) have key roles in the development and growth of the entire male genital tract, and they stimulate differentiation of the prostate gland.10,11 The adrenal glands and testes synthesize testosterone, and prostatic 5α-reductase type 2 converts it to DHT.12 DHT then binds to the androgen receptor (AR), which is transported to the nucleus, where it regulates genes important for prostate growth and differentiation.4 BPH development and progression are associated with activation of the AKT pathway, a major growth factor-mediated signal transduction pathway.6 Over-stimulation of the AKT pathway increases the activity of B-cell lymphoma 2 (Bcl-2) and cyclin D1, leading to increased proliferation and decreased apoptosis of cells, and transformation to the malignant state.8,9,13,14

Quisqualis indica (QI), commonly known as Chinese honeysuckle or Rangoon creeper, is a vine of the Combretaceae family that grows in tropical Asian countries, such as China, the Philippines, Bangladesh, Myanmar, India, and Malaysia.15 Different parts of this plant have compounds such as quisqualic acid, the alkaloid trigonelline, the flavonoid rutin, the amino acids L-proline and L-asparagine, and two forms of the enzyme cysteine synthase (isoenzymes A and B).15 Practitioners of traditional Asian medicine have used QI to treat stomach pain, constipation, and digestion problems.16 Recent studies have shown that QI has anti-inflammatory, antioxidant, antimicrobial, cytotoxic, insecticidal, anti-obesity, anti-diabetic, immunomodulatory, and hypolipidemic properties.17-21 However, the pharmacological effects of QI on BPH have not yet been established. We investigated the potential therapeutic effects of QI on BPH by using a human prostate cancer cell line and a rat model of testosterone propionate (TP)-induced BPH.

MATERIALS AND METHODS

Preparation of QI Extract Seeds of QI were obtained from a local herbal market in Ansan, Korea, authenticated by Dr. Yeon, and deposited at the herbarium of the HUONS Research Center (Voucher No. HU033/SKJA150427). The dried seeds were homogenized to a fine powder, and 100 g of this...
(powder was extracted by reflux with 1 L of 70% ethyl alcohol at 80°C for 1 h. The resulting fraction was vacuum-dried and stored at 4 °C until use.

For identification of amino acids, including quisqualic acid, all amino acids were subjected to pre-column derivatization with O-phthaldialdehyde and ethanethiol, as described previously. Free amino acids were measured using HPLC, performed on an Agilent 1200 series system (Agilent Technologies, CA, U.S.A.). Separation of QI extract was performed at 30°C with a ZORBAX Eclipse Plus C 18 column (4.6×250 mm, 5 µm particle size; Agilent Technologies). A sodium acetate buffer (solvent A; 0.01 M, pH 4.6) with methanol (solvent B) used as the mobile phase, and the programmed gradient runs were 0–5 min (20% B), 5–30 min (50% B), and 30–33 min (100% B), followed by a 1 mL/min flow rate equilibration with 20% B for 10 min. Absorbance was measured at 338 nm. The QI extract contained various amino acids, including asparagine, quisqualic acid, arginine, and glutamic acid (Supplementary Fig. 1). The administered extract was standardized by the quisqualic acid content (≥0.63%), using a validated HPLC assay, as described by the Hong Kong Chinese Materia Medica Standards (HKCMMS) Office.

Cell Culture The human prostate cancer cell line (LNCaP) was obtained from the American Type Culture Collection (ATCC, Manassas, VA, U.S.A.). These cells were grown in RPMI-1640 medium (Gibco, CA, U.S.A.) with 10% fetal bovine serum (FBS; Gibco), 100 U/mL penicillin, and 100 mg/mL streptomycin (Gibco), and maintained in a 5% CO₂ incubator at 37°C.

Cell Treatments LNCaP cells were seeded onto 6-well plates (5×10⁵ cells/well) in 1 mL of RPMI-1640 medium with 10% FBS, 100 U/mL penicillin and 100 mg/mL streptomycin. One day later, the medium was replaced and the cells were incubated in medium containing 100 nmol TP (Tokyo Chemical Ins. Co., Tokyo, Japan) and QI (10, 25 or 50 µg/mL) for an additional 72 h. Following two washes with phosphate-buffered saline (PBS), the cells were harvested for preparation of whole-cell extracts.

Cell Viability Assay The cytotoxic effect of QI extract on LNCaP cells was evaluated using the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay as previously described. In brief, cells were plated at 1×10⁴ cells per well in 96-well plates in 100 µL RPMI-1640 medium, and QI was added (concentration range: 25 to 3200 µg/mL). After incubation at 37°C for 72 h, the medium was replaced with 10 µL of the MTT reagent (100 µL/mL, Daellab Service, Seoul, South Korea), followed by incubation under the same conditions for 2 h. Absorbance was determined at 450 nm using a microplate reader. Cell viability was calculated as:

\[
100\% \times \frac{\text{OD}_{450_{\text{nm}}}}{	ext{OD}_{450_{\text{nm}}} \text{ of control group}}
\]

Animals Eight-week-old male Sprague-Dawley (SD) rats were purchased from Orient Bio (Seongnam, South Korea). Animals were maintained under standard laboratory conditions (22±2°C; relative humidity, 50±5%; 12 h light/dark cycle), and were allowed to consume a standard rodent chow and sterilized tap water ad libitum. All animal protocols were approved by the Animal Experimental Ethics Committee of Chungnam National University (Daejeon, South Korea).

Testosterone-Induced BPH Rats were acclimatized for 1 week prior to randomized division into 4 groups (6 rats/group): (a) normal control (NC) group (oral PBS and subcutaneous injection of corn oil); (b) BPH group (oral PBS and subcutaneous injection of TP [3 mg/kg body weight (BW); Tokyo Chemical Ins. Co.]; (c) positive control group (oral finasteride [10 mg/kg BW; Sigma, St. Louis, MO, U.S.A.]) and subcutaneous injection of TP [3 mg/kg BW]; and (d) QI group
(oral QI [150 mg/kg BW] and subcutaneous injection of TP [3 mg/kg BW]). All rats received treatments once per day for 4 weeks, and body weights were measured once per week. The most recent body weight was used to determine the volume of QI delivered as 5 mL/kg BW. We conducted a preliminary trail with three doses of QI (50, 150 and 300 mg/kg) to identify the optimum dose for treatment of BPH. Based on these experiments, we chose a dose of 150 mg/kg, because this dose reduced BPH-related parameters (data not shown).

At the end of the experiment, rats were fasted overnight, anesthetized by intraperitoneal injection of pentobarbital (100 mg/kg BW), and dissected to obtain blood from caudal vena cava. This blood was centrifuged, and serum was stored at −80°C until further analysis. The prostate of each rat was also carefully recovered and weighed. The prostatic index and percent inhibition were calculated as previously described: % inhibition = [100 − (treated group − negative control)/(positive control − negative control) × 100].[25] The ventral prostate of each rat was fixed overnight in 10% neutral buffered formalin (NBF), and the rest of the prostate was snap-frozen in liquid nitrogen for protein assays.

Hematoxylin and Eosin (H&E) Staining Prostate tissues of each rat were stained with H&E (Sigma) and examined under a microscope (Nikon ECLIPSE Ni-U, Tokyo, Japan) at 200× magnification. Images were captured from 10 randomly selected fields per rat, and epithelial thickness was measured using ImageJ software (ImageJ v46a; NIH, U.S.A.).

Immunohistochemistry (IHC) IHC was performed using a Vectastain Elite ABC Kit (Vector Laboratories, CA, U.S.A.) according to the manufacturer’s instructions. After antigen retrieval processing, the sections were blocked in normal serum and then incubated with anti-proliferating cell nuclear antigen (PCNA; Abcam, MA, U.S.A.) antibody at 4°C. After overnight, the sections were incubated in biotinylated secondary antibody and developed using a diaminobenzidine (DAB) peroxidase substrate kit (Vector Laboratories). PCNA-positive nuclei were counted in 10 randomly selected fields (×400) from each rat. Data are expressed as PCNA-positive nuclei per 100 cells.

Terminal Deoxynucleotidyl Transferase–Mediated Nick End Labeling (TUNEL) Staining Prostate epithelial cell apoptosis was detected using an in situ apoptosis detection kit (Millipore, MA, U.S.A.). Positive cells were counted in 10 randomly selected fields (×400).

Determination of Testosterone and DHT Levels The serum and prostate concentrations of DHT and testosterone were determined using commercial ELISA (enzyme-linked immunosorbent assay) kits (ALPCO Diagnostics, NH, U.S.A.), according manufacturer’s instructions.

Protein Extraction and Western Blot Analysis LNCaP cells or tissues were homogenized in RIPA lysis buffer (Cell Signaling Technology, CA, U.S.A.) supplemented with protease inhibitors (Roche, Mannheim, Germany) and phosphatase inhibitors (Roche). For immunoblotting, proteins (30–40 µg) were separated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) on 10% gels, and then transferred to nitrocellulose membranes at 40 V for 2 h. For experiments using LNCaP cells, membranes were probed with anti-androgen receptor (AR; Cell Signaling Technology), anti-prostate specific antigen (PSA; Cell Signaling Technology), or anti-β-actin (loading control; Sigma) antibodies in Tris-buffered saline with 0.05% Tween-20 (TBST) and 5% skim milk. Prostate tissues were probed with antibodies against PCNA, cyclin D1, caspase-3, caspase-9, AKT, phosphorylated-AKT (p-AKT), glycogen synthase kinase 3β (GSK3β), phosphorylated-GSK3β (p-GSK3β; Cell Signaling Technology), B-cell lymphoma 2 (Bcl-2), Bcl-2-like protein (Bax; Santa Cruz Biotechnology, CA, U.S.A.), or β-actin (Sigma). After washing, membranes were developed using an enhanced chemiluminescence kit (Thermo Scientific, MA, U.S.A.).

**RESULTS**

Effect of QI on the Viability of Human Prostate Cancer Cells To examine the potential cytotoxicity of QI on LNCaP cells, the MTT assay was performed. The cell viability was not substantially different between cells treated with QI (25 to 3200 µg/mL) and control cells. However, the exposure to 3200 µg/mL concentration of QI significantly decreased the viability by 72.5% (Fig. 1A), indicating that the high concentration of QI inhibits cell growth by its toxicity. Based on these results, we used the low concentration of QI (≤1600 µg/mL) to avoid the cytotoxic effects.

Effect of QI on Expression of AR and PSA in Prostate Cancer Cells Androgens promote the development and progression of prostate cancer, and PSA is an androgen-responsive gene that is widely used to assess progression of human prostate cancer.[26] Finasteride is a type 2 5α-reductase inhibitor that downregulates the conversion of testosterone to DHT and...
interferes the binding of DHT to AR.\textsuperscript{27} Thus, we examined the effect of QI treatment on AR and PSA expression in LNCaP cells. The Western blotting results showed that TP treatment significantly increased the expression of AR and PSA, and that co-treatment with finasteride or QI (50 \( \mu \)g/mL) significantly attenuated this effect (Fig. 1B). These findings suggest that QI suppresses androgen signaling in prostate cancer cells.

**Effect of QI on Prostate Weight in a Rat Model of BPH**

Next, we determined the therapeutic potential of QI for treatment of BPH by using a rat model of TP-induced BPH. In BPH group, treatment of TP resulted in a significant increase in prostate weights (absolute and relative) compared to the NC group. In comparison to the BPH group, finasteride and QI treatment significantly reduced the TP induced enlargement of the prostate gland of rats (Table 1). Further, the prostate growth inhibition ratio in the QI group was found to be 37.9\%, a value similar to that (36.2\%) for the finasteride-treated group (Table 1), indicating QI has potential for treatment of BPH.

**Effect of QI on Histological Changes in the Prostate of Rats with BPH**

Histological analyses of prostate tissues indicated the rats in the NC group had normal cell morphology, but those in the TP group had significant glandular hyperplasia, with multiple layers of epithelial cells, and a decreased glandular luminal area (Fig. 2A). Notably, finasteride and QI treatment reversed the effects of TP (Fig. 2A). As shown in Fig. 2B, rats in the TP-induced BPH group exhibited a significant increase in the epithelial thickness of their prostates compared with those in the NC group. However, treatment with finasteride or QI significantly reduced this increase in epithelial thickness. These findings indicate that the reduced prostate weight after QI treatment results from the attenuation of the abnormal histological effects in the prostate of BPH rats.

**Effect of QI on Testosterone, DHT, and 5\( \alpha \)-Reductase Type 2 in Rats with BPH**

Serum testosterone acts on the prostate, which produces growth factors that regulate cell growth, survival, and apoptosis.\textsuperscript{29} In our study, TP injection significantly increased the level of serum testosterone, and that finasteride and QI partially reversed this effect (Fig. 3A). The prostate produces DHT, which has a major effect on BPH progression, by \( 5\alpha \)-reductase-mediated conversion of circulating testosterone.\textsuperscript{10} The level of DHT was significantly higher in BPH group compared to the NC group. However, treatment with finasteride and QI significantly reduced the DHT level in BPH induced rats (Fig. 3B). In addition, mRNA expression of \( 5\alpha \)-reductase type 2 was up-regulated in TP-induced BPH group compared to the NC group and this level was suppressed by both finasteride and QI treatment (Fig. 3C). These data suggest that QI reduces the androgen concentrations in BPH induced rats.

**Effect of QI on Prostate Cell Proliferation in Rats with BPH**

Next, we investigated the anti-proliferative effect of QI by measuring expression of PCNA in the prostate. Rats in the NC group had very low expression of prostatic epithelial PCNA, but rats in the TP group had an increased number of PCNA-positive cells (Figs. 4A, B). Finasteride and QI partially reversed the effect of TP. Consistent with these results, Western blotting results indicated the TP group had significantly increased levels of PCNA and cyclin D1 compared to the NC group, and treatment with QI and finasteride significantly attenuated this effect (Figs. 4C, D). These data suggest that QI attenuates BPH through anti-proliferative activity.

**Effect of QI on Apoptosis in Rats with BPH**

To determine whether the inhibitory effect of QI on prostate growth

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Table 1. Effect of QI on Prostate Weights

<table>
<thead>
<tr>
<th>Group</th>
<th>Treatment</th>
<th>Prostate weight</th>
<th>% inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Absolute (g)</td>
<td>Relative (%)</td>
</tr>
<tr>
<td>NC</td>
<td>Corn oil/PBS</td>
<td>0.595±0.036</td>
<td>0.151±0.009</td>
</tr>
<tr>
<td>TP</td>
<td>TP/PBS</td>
<td>1.532±0.089**</td>
<td>0.434±0.019**</td>
</tr>
<tr>
<td>TP+Fina</td>
<td>TP/Finasteride</td>
<td>1.193±0.047**</td>
<td>0.339±0.013**</td>
</tr>
<tr>
<td>TP+QI</td>
<td>TP/QI</td>
<td>1.177±0.050**</td>
<td>0.334±0.017**</td>
</tr>
</tbody>
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The % inhibition was calculated by using absolute prostate weight as follow: 100\%−[(treated group−negative control)/(positive control−negative control)\times100]. Abbreviations: NC, corn oil-injected and PBS-treated rats; TP, TP (3 mg/kg)- and PBS-treated rats; TP+Fina, TP (3 mg/kg)- and finasteride (10 mg/kg)-treated rats; TP+QI, TP (3 mg/kg)- and QI (150 mg/kg)-treated rats. Results are expressed as the means±S.E.M. *\( p<0.01 \) compared with the NC group; **\( p<0.01 \) compared with the TP group.
was attributable to apoptosis, we examined its effect on apoptosis in BPH-model rats using TUNEL staining. The number of TUNEL-positive cells in the prostate was reduced in the BPH group compared with the NC group (Figs. 5A, B). However, an increased number of positive cells were observed in finasteride and QI treated groups (Figs. 5A, B). Moreover, Western blotting revealed significantly increased expression of cleaved caspase-3 and -9 with finasteride and QI treatment compared with the BPH group (Fig. 5C). In addition, the expression of anti-apoptotic protein, Bcl-2 was decreased,
Fig. 5. Effect of QI Administration on Prostate Cell Apoptosis in BPH-Model Rats

(A) Representative photomicrographs of prostate tissue showing apoptotic cells (Arrows), determined by TUNEL staining (magnification, ×400). (B) Number of TUNEL-positive cells in the prostate. TUNEL-positive nuclei were counted in 10 randomly selected fields (magnification, ×400) from each rat; results are expressed as TUNEL-positive nuclei/100 cells. The expression levels of caspase-3 and -9 (C) and Bcl-2 and Bax (D) were determined by Western blotting; β-actin was used as an internal control. The results were obtained in two different gels by calculating the average of six independent prostates per group. Abbreviations: NC, corn oil-injected and PBS-treated rats; TP, TP (3 mg/kg)- and PBS-treated rats; TP + Fina, TP (3 mg/kg)- and finasteride (10 mg/kg)-treated rats; TP + QI, TP (3 mg/kg)- and QI (150 mg/kg)-treated rats. Data are expressed as the means ± S.E.M. *p < 0.05 and **p < 0.01 compared with the NC group; *p < 0.05 and **p < 0.01 compared with the TP group.

Fig. 6. Effects of QI on the AKT/GSK3β Pathway in Prostate Tissues

The results were obtained in two different gels by calculating the average of six independent prostates per group. Abbreviations: NC, corn oil-injected and PBS-treated rats; TP, TP (3 mg/kg)- and PBS-treated rats; TP + Fina, TP (3 mg/kg)- and finasteride (10 mg/kg)-treated rats; TP + QI, TP (3 mg/kg)- and QI (150 mg/kg)-treated rats. Data are expressed as the means ± S.E.M. *p < 0.05 and **p < 0.01 compared with the NC group; *p < 0.05 and **p < 0.01 compared with the TP group.
β-prostatic AKT and GSK3 administration substantially increased the phosphorylation of suppresses the development of BPH by regulating AKT/GSK3. As shown in Fig. 6, TP treatment significantly reduced Bcl-2/Bax ratio in BPH induced rats (Fig. 5D), indicating QI prevents adverse effects. The present study assessed the protective role of QI on BPH using a human prostate cancer cell line and a rat model of BPH. Our in-vitro results showed that QI treatment significantly inhibited the TP-induced increases of AR and PSA expression in LNCaP cells. In agreement, the TP-induced increases in prostate weight, serum testosterone, prostatic DHT concentration, and 5α-reductase type 2 mRNA expression in prostate tissue were significantly attenuated in QI-treated rats. A 4-week toxicity study in rats further showed that QI had no adverse effects at a dose of 2000 mg/kg.

### DISCUSSION

Despite significant progress in available treatments, BPH remains the most common prostatic disease affecting older men. Given the many side effects of surgery and pharmacological therapy, and the long latency of BPH, natural products from plants have emerged as alternative treatments because they may provide therapeutic efficacy with fewer adverse effects. The present study assessed the protective role of QI on BPH using a human prostate cancer cell line and a rat model of BPH. Our in-vitro results showed that QI treatment significantly inhibited the TP-induced increases of AR and PSA expression in LNCaP cells. In agreement, the TP-induced increases in prostate weight, serum testosterone, prostatic DHT concentration, and 5α-reductase type 2 mRNA expression in prostate tissue were significantly attenuated in QI-treated rats. A 4-week toxicity study in rats further showed that QI had no adverse effects at a dose of 2000 mg/kg.

Overall enlargement of the prostate is a sign of BPH, which is characterized by increased proliferation of epithelial and stromal cells in the prostate. Hyperplasia of these cells leads to increased prostate weight and compression of the urethral canal, thereby blocking urine flow. Previous studies indicated that animals with experimentally induced BPH have significantly greater prostate weight than controls, and that finasteride treatment decreases BPH. In line with these previous reports, our present findings revealed that TP injection contributed to a significant increase in prostatic weights of BPH induced rats compared to the control rats. In contrary, the rats treated with QI showed significantly decrease prostate weights compared with BPH induced rats. Our histopathological examinations of rat prostate tissues are consistent with this result. More specifically, rats in the TP group had greater epithelial hyperplasia and epithelial thickness than rats in the TP/QI group. These findings suggest that QI effectively inhibited the development of prostatic hyperplasia and BPH.

Prostate DHT levels increase as BPH progresses, and prostatic 5α-reductase converts testosterone into DHT. DHT increases hyperplasia of prostatic stromal and epithelial cells, resulting in the enlargement of prostate. Therefore, 5α-reductase inhibitors are effective treatments for men with BPH. Finasteride is a type 2 5α-reductase inhibitor that down-regulates plasma and intra-prostatic DHT concentrations, and reduces prostate size, epithelial cell height, and the synthesis of DHT. In agreement with previous studies, our results indicate that finasteride significantly reduced testosterone and...
DHT levels in the serum and prostates of rats, and that QI had a similar effect. In addition, QI and finasteride also decreased the expression of 5α-reductase type 2 mRNA in the prostate. These results indicate that the QI-induced reduction of prostatic epithelial hyperplasia is closely associated with the reduced DHT level.

BPH is characterized by an increased number of prostatic stromal and epithelial cells due to uncontrolled proliferation, and increased prostate volume. Our in vitro data showed that QI inhibited cell viability in prostate cancer cells at the high concentration (≥3200 µg/mL). We further evaluated the mechanism responsible for the inhibitory effects of QI on BPH by measuring the expression of PCNA in the prostate tissue of TP-treated rats. PCNA expression is significantly elevated in patients with BPH or prostate cancer, and the extent of cell proliferation is related to the clinic grade of prostate cancer. Animals with experimentally induced BPH also have significantly increased prostatic PCNA expression. We found that rats with BPH that were given QI treatment had significantly reduced expression of PCNA and cyclin D1, as well as fewer PCNA-positive cells. This indicates that QI protected against BPH development due to its anti-proliferative activity. Our data also revealed that QI increased the apoptosis of prostate epithelial cells. Moreover, treatment with QI decreased the expression of the anti-apoptotic protein, Bcl-2, and increased the expression of the pro-apoptotic protein, Bax. Hence, the QI-treated rats have a decreased Bcl-2/Bax ratio, as well as increased levels of prostatic caspase-3 and -9. Various diseases, including BPH, are characterized by disruption of processes that regulate cell proliferation and cell death. Epithelial cells in normal prostate tissue express little or no Bcl-2 or caspase-3, but hyperplastic changes are associated with increased levels of Bcl-2 and a decreased levels of Bax in the prostate. The Bcl-2/Bax ratio is an important measure of cell fate, and an increased ratio (resulting from aberrant expression of these proteins) is common in BPH. Therefore, agents that promote prostate cell apoptosis have potential as treatments for BPH. We hypothesize that the pro-apoptotic activity of QI, together with its anti-proliferative activity, were responsible for the suppression of prostate cell proliferation and reduction in prostate volume.

We also investigated the effects of QI on the AKT/GSK3β pathway to elucidate the mechanism by which QI inhibits TP-induced cell proliferation in the prostate. Activation of the AKT pathway is known to play key roles in cell proliferation, apoptosis and survival in various cell types. AKT increases cyclin D1 levels by stimulating its mRNA translation. Phosphorylation/inactivation of GSK3β by AKT also results in elevated levels of cyclin D1, thus allowing progression through the G1 phase of the cell cycle. Additionally, AKT inactivates Bad and caspase-9, two pro-apoptotic proteins, whereas inhibition of AKT signaling induces cell death by reducing the expression of Bcl-2 and enhancing the expression of Bax or Bad. Our results show that QI treatment decreased the TP-induced activation of AKT and GSK3β. We also found that these QI-induced decreases in p-AKT and p-GSK3β levels paralleled reduced cyclin D1 expression and a decrease in the Bcl-2/Bax ratio. These results suggest that the QI-induced increase in apoptosis and suppression of prostate cell proliferation may be mediated via alterations in the AKT pathway.

The two most widely accepted classes of medications for treatment of BPH are 5α-reductase inhibitors and alpha-1 (α1)-adrenergic receptor antagonists. However, the utility of these drugs is limited by their side effects, which include decreased libido, ejaculatory or erectile dysfunction, and nasal congestion. Our results show that oral administration of QI to rats, at a dose of 2000 mg/kg/d for 4 weeks, produced no clinical signs or evident treatment-related adverse effects. However, further studies of potential chronic toxicity and genotoxicity are needed to establish the safe oral dose of QI.

In conclusion, the present study provides the first evidence that QI protects against TP-induced BPH in rats, as indicated by its ability to reduce prostate size and hyperplasia. These effects can be attributed, at least in part, to the decreased prostatic levels of DHT and the anti-proliferative and pro-apoptotic activities of QI. Use of QI as a therapeutic agent for the treatment of BPH warrants further exploration.

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Conflict of Interest The authors declare no conflict of interest.

Supplementary Materials The online version of this article contains supplementary materials.

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