Title: A phytosterol enriched refined extract of Brassica campestris L. pollen significantly improves benign prostatic hyperplasia (BPH) in a rat model as compared to the classical TCM pollen preparation Qianlie Kang Pule'an Tablets

Article Type: Original Article

Section/Category: Urology

Keywords: Brassica campestris L., Brassicaceae, pollen, TCM, phytosterols, benign prostatic hyperplasia

Abstract: In Qinghai Province, the Brassica campestris L. pollen preparation Qianlie Kang Pule’an Tablets (QKPT) is traditionally used for BPH therapy. However, in QKPT the content of supposedly active phytosterols is relatively low at 2.59 %, necessitating high doses for successful therapy. Therefore, a phytosterol enriched (4.54 %) refined extract of B. campestris pollen (PE) was developed and compared with QKPT in a BPH rat model. Six groups of rats (n=8 each), namely sham operated distilled water control, castrated distilled water control, castrated QKPT 2.0 g/kg, castrated PE 0.1 g/kg, castrated PE 0.2 g/kg, and castrated PE 0.4 g/kg were intragastrically treated with the respective daily doses. Testosterone propionate (0.3 mg / day) was administered to all castrated rats, while the sham operated group received placebo injections. After 30 days, the animals were sacrificed and prostates as well as seminal vesicles excised and weighted in order to calculate prostate index (PI), prostate volume index (PVI), and seminal vesicle index (SVI), defined as organ weight in g per 100 g body weight. Compared with sham-operated controls, PI (P < 0.01), PVI (P < 0.01), and SVI (P < 0.01) were all significantly increased in all castrated rats. After treatment with PE at 0.4 and 0.2 g/kg or QKPT at 2.0 g/kg per day, both indices were significantly reduced (P < 0.01) as compared to the castrated distilled water control. For PE at 0.1 g/kg per day only PI was significantly reduced (P < 0.05). At the highest PE concentration of 0.4 g/kg per day both PI and SVI were also significantly reduced when compared to the QKPT group (P < 0.05). Both PE and QKPT demonstrated curative effects against BPH in the applied animal model. In its highest dose at 0.4 g/kg per day, PE was clearly superior to QKPT.
Dear Prof Dr Wagner:

I herewith state in my function as corresponding author that all of my co-authors – Ruwei Wang, Yuta Kobayashi, Yu Lin, Hans Wilhelm Rauwald, Ling Fang, and Hongxiang Qiao – support the submission of our manuscript in its present form for publication in Phytomedicine.

On behalf of all authors,
Sincerely yours,
Kenny Kuchta

---
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Dear Prof Dr Wagner:

Enclosed you find our manuscript entitled “A phytosterol enriched refined extract of *Brassica campestris* L. pollen significantly improves benign prostatic hyperplasia (BPH) in a rat model as compared to the classical TCM pollen preparation Qianlie Kang Pule’an Tablets” (Ruwei Wang, Yuta Kobayashi, Yu Lin, Hans Wilhelm Rauwald, Ling Fang, Hongxiang Qiao, Kenny Kuchta) with the request for publication as an Original Paper in Phytomedicine.

In our present manuscript, we have used a rat model to examine the urological effects of Qianlie Kang Pule’an Tablets, an adaptogenic TCM preparation for symptoms of weakened renal qi, made of *Brassica campestris* L. pollen from Qinghai Province, China. In this traditional preparation the whole pollen grains are retained with intact pollen grain walls, reducing the percentage content in pharmacologically active phytosterols and slowing down their absorption, thus necessitating unfavourably high doses for patients. Therefore, a phytosterol enriched refined extract of *Brassica campestris* L. pollen was newly developed and investigated in the same rat model, where it displayed superior efficacy as compared to the classical TCM preparation.

It would be a great pleasure for us, if our manuscript could find your interest!

Sincerely yours, Kenny Kuchta

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Original Paper

A phytosterol enriched refined extract of Brassica campestris L. pollen significantly improves benign prostatic hyperplasia (BPH) in a rat model as compared to the classical TCM pollen preparation Qianlie Kang Pule’an Tablets

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Keywords
Brassica campestris L., Brassicaceae, pollen, TCM, phytosterols, benign prostatic hyperplasia
Abstract

In Qinghai Province, the *Brassica campestris* L. pollen preparation Qianlie Kang Pule’an Tablets (QKPT) is traditionally used for BPH therapy. However, in QKPT the content of supposedly active phytosterols is relatively low at 2.59 %, necessitating high doses for successful therapy. Therefore, a phytosterol enriched (4.54 %) refined extract of *B. campestris* pollen (PE) was developed and compared with QKPT in a BPH rat model. Six groups of rats (n=8 each), namely sham operated distilled water control, castrated distilled water control, castrated QKPT 2.0 g/kg, castrated PE 0.1 g/kg, castrated PE 0.2 g/kg, and castrated PE 0.4 g/kg were intragastrically treated with the respective daily doses. Testosterone propionate (0.3 mg / day) was administered to all castrated rats, while the sham operated group received placebo injections. After 30 days, the animals were sacrificed and prostates as well as seminal vesicles excised and weighted in order to calculate prostate index (PI), prostate volume index (PVI), and seminal vesicle index (SVI), defined as organ weight in g per 100 g body weight. Compared with sham-operated controls, PI (P < 0.01), PVI (P < 0.01), and SVI (P < 0.01) were all significantly increased in all castrated rats. After treatment with PE at 0.4 and 0.2 g/kg or QKPT at 2.0 g/kg per day, both indices were significantly reduced (P < 0.01) as compared to the castrated distilled water control. For PE at 0.1 g/kg per day only PI was significantly reduced (P < 0.05). At the highest PE concentration of 0.4 g/kg per day both PI and SVI were also significantly reduced when compared to the QKPT group (P < 0.05). Both PE and QKPT demonstrated curative effects against BPH in the applied animal model. In its highest dose at 0.4 g/kg per day, PE was clearly superior to QKPT.
Introduction

Benign prostate hyperplasia (BPH) is one of the most common diseases among elderly men in East Asia and exhibits gradually increasing incidence rates. It is most commonly treated with α-adrenergic receptor blockers such as phenoxybenzamine or terazosin; 5α-reductase inhibitors such as finasteride; and male hormone inhibitors such as cyproterone or medroxyprogesterone; but also with TCM based phytopharmaceuticals such as Qianlie Kang Pule'an Tablets (QKPT) (Huang 2001).

QKPT, made of Brassica campestris L. pollen from Qinghai Province, is an adaptogenic TCM preparation for symptoms of weakened renal qi, commonly used in the treatment of chronic prostatitis, prostatic hyperplasia, incontinence, as well as for soreness and weakness of waist and knees. Its safety and efficacy has been demonstrated both in animal experiments (Cai et al. 1997, Gao et al. 2006) and several clinical studies (Yan and Wang 2008, Xie et al. 1988, Du and Zhang 2007). Furthermore, similar adaptogenic effects have also been described for standardized pollen extracts from European Phytomedicine (Juzyszyn et al. 1997).

The main phytochemical constituents of B. campestris L. pollen are diverse fatty acids, flavonoids, N-containing compounds, as well as phytosterols like e.g. β-sitosterol, stigmasterol, and campesterol (Yang and Yang 2009). Besides some reports on the activity of pollen flavonoids on BPH related gene expression (Han et al. 2007), phytosterols are commonly regarded as the most important contributors to its pharmaceutical activity concerning BPH (Di Silverio et al. 1998, Dreikorn 2002) although their content is relatively low – typical less than 1 % (Weirauch and Gardner 1978). Curative effects of these pollen constituents on malign prostate hyperplasia and pro-apoptotic effects on prostate cancer cells have also been reported (Ifere et al. 2009, Scholtysék et al. 2009, Shenouda et al. 2007, Wu and Lou 2007). Beyond prostate diseases, phytosterols were demonstrated to exert positive effects not only on unrelated forms of cancer (Awad et al. 2000, Zhao et al. 2009) but also on blood fat and cholesterol levels (Racette et al. 2010, Chen et al. 2009, Tikkanen et al. 2001).

Amongst the diverse group of the phytosterols, β-sitosterol has widely been described as exceptionally active in several clinical studies on BPH (Preuss et al. 2001, Prager et al. 2002, Klippel et al. 1997). In several further publications, this clinical activity has been theorized to be due to a partial anti-androgenic actively of this individual phytosterol (Gutendorf and Westendorf 2001, Mellanen et al. 1996, Wu et al. 2003), which has not been reported in the same extent from other member of this group of compounds. In this context, β-sitosterol has been identified as a 5α-reductase antagonist, inhibiting the conversion of testosterone to the
even more active androgen dihydrotestosterone, thus facilitating a reduction in prostate size and an improvement of BPH symptoms in both animal models and clinical studies with human subjects (Prager et al. 2002, Cabeza et al. 2003).

However, although the efficacy of the classical TCM pollen preparation QKPT is well supported by clinical research, one persistent problem with this therapy approach is the fact that in the traditional preparation the whole pollen grains are retained with intact pollen grain walls, reducing the percentage content in phytosterols and slowing down their absorption, thus necessitating unfavourably high doses for patients.

In the present study, a phytosterol enriched pollen refined extract (PE) was newly developed based on QKPT. It was subsequently compared to the original product in a BPH rat model, demonstrating a favourable effect of this improved preparation as compared to the traditional TCM product.
Materials and Methods

Drug material

Pollen was obtained from a bee farm in the midst of flowering *B. campestris* fields in Chengdong district, Xining city, Qinghai province, China. The pollen was collected from the flowers by the bees and later harvested from their hives.

The classical TCM preparation Qianlie Kang Pule'an Tablets (QKPT) was prepared from this pollen using a traditional apothecary hand press for tableting. Each individual Qianlie Kang Pule'an Tablet consists of 0.5 g of the above-mentioned pollen without any additional ingredients.

In contrast, for the preparation of the refined extract PE, 10 kg pollen was broken down using a CWP-250 grinder (Chao Wei, Dezhou, China). Subsequently, the crushed pollen was extracted with 60 L 95% ethanol for 2 h. After this first extraction step was completed, the crushed pollen was extracted one again in identical fashion. The two liquid extracts were combined and evaporated to dryness under reduced pressure, yielding 2.0 kg of the examined pollen extract PE. Thus, 1.0 g of PE is equal to 5.0 g pollen.

Photometric assay on total phytosterol content

For preparing the photometric test solution, 500 mg of the respective sample were accurately weighed into a 100 ml Erlenmeyer flask and suspended in 50 ml chloroform, after which the flask was tightly plugged. During the subsequent 60 min of sonication, the flask was constantly cooled to prevent a build-up of pressure in the sealed flask. After filtration through a syringe filter, 10 ml of the solution were precisely pipetted into a 100 ml round bottom flask and the chloroform solvent removed under reduced pressure. The dry residue was re-dissolved in 20 ml of a solution of 10 % sodium hydroxide in ethanol. After 2 h of incubation at 95°C on the water bath under reflux, the solution was cooled to room temperature and diluted with 40 ml of distilled water, transferred into a separating funnel, and extracted 3 times with 20 ml of n-hexane each. The 60 ml of n-hexane solution were combined and extracted twice with 40 ml of distilled water each. Subsequently, all aqueous phases were discarded, while the n-hexane phase was reduced to ca. 30 ml under reduced pressure. This solution was completely transferred into a 50 ml volumetric flask that was filled to exactly 50 ml, yielding the photometric test solution.

1.0 ml of the photometric test solution was filled into a 10 ml test tube. The n-hexane solvent was volatilized above a hot water bath. Afterwards, 0.6 ml of a 5 % solution of vanillin in glacial acetic acid and 0.8 ml of perchloric acid were added. After intensive stirring, the mixture was placed on a water bath at 70°C for
30 min and subsequently cooled to room temperature under cold, flowing water. Thereafter, 4.6 ml acetic acid were added and after mixing, the absorbance was measured at 542 nm using a UV2450 UV spectrophotometry (Shimadzu, Kyoto, Japan), calibrated against pure n-hexane as a negative control experiment.

As a photometric reference solution, 5 mg of β-sitosterol – cordially provided by the Chinese National Institute for Food and Drug Control (batch No. 110851-200605) – were accurately weighed into a 50 ml volumetric flask and dissolved in n-hexane at exactly this volume. The further measurement was performed identically to the above described procedure for the test solution.

The phytosterol content of the sample was calculated as β-sitosterol according to the following formula:

\[
Pp\% = \frac{M0}{A0} \cdot \frac{A}{M} \cdot 100\%
\]

Cp % = Content of phytosterols in the sample
M0 = Weight of β-sitosterol in the photometric reference solution
A0 = Absorbance of the β-sitosterol photometric reference solution
A = Absorbance of phytosterols in the photometric test solution
M = Weight of the sample in the photometric test solution

**HPLC assay on β-sitosterol content**

For preparing the HPLC test solution, 1000 mg of the respective sample were accurately weighed into a 100 ml round bottom flask and suspended in in 20 ml of a solution of 10 % sodium hydroxide in ethanol. After 2 h of incubation at 95°C on the water bath under reflux, the solution was cooled to room temperature and diluted with 40 ml of distilled water, transferred into a separating funnel, and extracted four times with 20 ml each of light petroleum. The 80 ml of light petroleum solution were combined and extracted twice with 20 ml of distilled water each. Subsequently, all aqueous phases were discarded, while the light petroleum phase was reduced to dryness under reduced pressure. The dry residue was dissolved in 5 ml of isopropanol which were subsequently transferred into a 25 ml volumetric flask. In order to accumulate all small traces of β-sitosterol from the sample, the round bottom flask was washed with additional 5 ml of isopropanol three times, which were all pooled in the above-mentioned 25 ml volumetric flask. This flask was finally exactly filled to 25 ml with isopropanol, yielding the HPLC test solution.

An Agilent 1260 HPLC apparatus (Agilent, USA) was used with an Agilent SB C18 (150 mm × 4.6 mm, 5
μm) column (column temperature 40°C), and methanol / acetonitrile / water (25/65/10, v/v/v) as the mobile phase. The flow rate was kept constant at 1 mL/min. The injection volume was set at 20 μL of HPLC test solution and the detection wavelength at 210 nm. All HPLC solvents were purchased at HPLC grade quality from Merck (Darmstadt, Germany).

As an HPLC reference solution, 5 mg of β-sitosterol – cordially provided by the Chinese National Institute for Food and Drug Control (batch No. 110851-200605) – were accurately weighed into a 25 ml volumetric flask and dissolved in isopropanol at exactly this volume. The further measurement was performed identically to the above described procedure for the HPLC test solution.

The β-sitosterol content of the sample was calculated according to the following formula:

$$C_s\% = \frac{m_0 \cdot a}{a_0 \cdot m} \cdot 100\%$$

Cs % = Content of β-sitosterol in the sample
m0 = Weight of β-sitosterol in the HPLC reference solution
a0 = Peak area of the β-sitosterol peak in the HPLC reference solution
a = Peak area of the β-sitosterol peak in the HPLC test solution
m = Weight of the sample in the HPLC test solution

**Animals and experimental treatments**

Male Sprague Dawley rats weighing 200~230 g were kept at 22~24°C under a 12 h dark-light cycle. Rats had free access to drinking water and a standard diet of rodent chow. All surgeries were performed under pentobarbital sodium anaesthesia and sterile conditions. Castration of animals was performed through the scrotal route, removing the testes of 40 rats. In the 8 rats of the sham operated distilled water control group, the same surgical operation was performed except that the testes were left in place unaltered. Starting one week after this operation, the castrated rats were each subcutaneously administered with testosterone propionate 0.3 mg/day during the 30 day timeframe of the trial, while the sham operated group received placebo injections of the pure oil solvent of the testosterone propionate preparation. At the same time, the rats were randomly divided into 5 groups of 8 animals each: castrated distilled water control, castrated QKPT 2.0 g/kg, castrated PE 0.1 g/kg, castrated PE 0.2 g/kg, and castrated PE 0.4 g/kg (with these PE doses corresponding to daily pollen doses of 0.5, 1.0, and 2.0 g/kg, respectively), all of which were intragastrically treated with the respective daily doses. Twenty-four hours after the last administration, rats were killed and
prostates and seminal vesicle were removed.

**Prostate index, prostate volume index, seminal vesicle index**

The prostates and seminal vesicles were dissected and weighed immediately and the volume of the prostates was measured. The three indexes were calculated as follows:

PI: prostate index (g / 100 g) = wet weight of the prostate (g) / body weight (g) x 100

PVI: prostate volume index (ml / kg) = volume of the prostate (ml) / body weight (g) x 1000

SVI: seminal vesicle index (g / 100 g) = wet weight of the seminal vesicle (g) / body weight (g) x 100

**Microscopic examination**

Prostates were fixed in 10% formalin solution, embedded in paraffin, and sectioned using a RM2255 microtome (Leica, Wetzlar, Germany). The sections were stained with haematoxylin and eosin for microscopic observation of pathological changes in the prostates, using a Nikon 55i microscope (Nikon, Tokyo, Japan). The glandular cavity diameter and epithelium height of the prostates were measured using a micrometre in five random visual fields, after which the average value was calculated for statistical analysis.

**Statistical analyses**

All data are expressed as mean ± SD. Statistical analyses were performed using both one-way variance analysis (ANOVA) and Dunnett's t-test. Values of $P < 0.05$ were considered as statistically significant.
Results and Discussion

Phytochemical Examination

In the above described photometrical testing systems, the overall content of phytosterols in the classical TCM pollen preparation Qianlie Kang Pule'an Tablets (QKPT) was determined as 2.59 %, whereas the content of β-sitosterol, the most active individual compound of this class, was measured by HPLC as 0.057 % of the QKPT drug. In the newly developed phytosterol enriched B. campestris L. pollen refined extract (PE), the overall content of phytosterols was found to be 4.54 %, with the individual content of β-sitosterol enriched to 0.101 % of PE. Instead of the significant enrichment of both the class of phytosterols in total, which were enriched by 75 %, and β-sitosterol, which was enriched by 77 %, the relative content of β-sitosterol amongst the phytosterol phase remained relatively stable at 2.2 %.

Effect of PE on prostates

As shown in Table 1, both PI and PVI were significantly increased in castrated rats (P < 0.01) as compared to the sham operated distilled water control group. After treatment with PE at 0.4, 0.2, and 0.1 g/kg or QKPT at 2 g/kg, both indexes were markedly reduced (P < 0.01 and P < 0.05, respectively) as compared to the castrated distilled water control group. In addition, also when comparing the results of the QKPT 2.0 g/kg group with those of the 0.4 g/kg PE group, which contains an equivalent amount of pollen, PI was significantly reduced (P < 0.05) in the high-dose PE group. While PVI also displayed a decreasing tendency under 0.4 g/kg PE treatment, the difference as compared to the QKPT 2.0 g/kg group was not statistically significant in this case.

Effects of PE on seminal vesicles

Our data (Table 2) demonstrate that SVI was significantly increased (P < 0.01) in the castrated animals as compared to the sham operated distilled water control group. However, the effect could be reduced by treatment with testosterone propionate. SVI values for the castrated QKPT 2.0 g/kg, castrated PE 0.4 g/kg, and castrated PE 0.2 g/kg groups, were all significantly lower than those of the castrated distilled water control group (P < 0.01), demonstrating PE treatment to reduce SVI values in a dose-dependent manner. When further comparing the results of the QKPT 2.0 g/kg group with those of the 0.4 g/kg PE group, which contains an equivalent amount of pollen, the latter exhibited significantly better SVI readings (P < 0.05).
**Effect of PE on pathological changes in hyperplastic prostates**

In the present study, a classification standard for the severity of the hyperplastic state of the prostates of test animals was designed. As shown in Table 3, most of animals in the castrated distilled water control group suffered from serious pathological changes in their prostates. However, these symptoms were significantly reduced by treatment with PE at 0.4 and 0.2 g/kg ($P < 0.01$, $P < 0.05$). In the castrated distilled water control group (Fig. 1B), the prostatic epithelial cells proliferated to significantly higher levels than in the sham operated distilled water control group (Fig. 1A), and the prostatic glandular cavities were also markedly enlarged. In the three PE groups (Fig. 1D-F) and in the QKPT 2.0 g/kg group (Fig. 1C) both epithelial cell proliferation and glandular cavity enlargement were significantly reduced. Concerning a quantitative analysis of these effects, the results in Table 4 demonstrate that both maximal glandular cavity diameters and glandular epithelium height in the distilled water control group were significantly larger than those in the sham operated distilled water control group ($P < 0.01$). In comparison with the distilled water control group, the glandular cavity diameters in the PE 0.4 and 0.2 g/kg groups as well as in the QKPT 2.0 g/kg group were significantly reduced ($P < 0.01$). A similarly significant reduction of glandular epithelium height in the PE 0.4 g/kg group was also observed ($P < 0.05$). In addition, the effects of high-dose PE (0.4 g/kg) on pathological changes of hyperplastic prostates were somewhat favourable in comparison with those of QKPT (2.0 g/kg), which contains an equivalent amount of pollen. However, this effect was not sufficiently statistically significant.

**Conclusion**

These results demonstrate that the classical TCM preparation Qianlie Kang Pule’an Tablets (QKPT) can prevent testosterone propionate induced pathological changes of the prostate gland in the present experimental setup. In the newly developed pollen refined extract (PE) these effects were further enhanced. Correspondingly, the effect of high-dose PE on BPH in the 0.4 g/kg group of the present animal model was significantly superior to the effect observed in the QKPT 2.0 g/kg group for several of the above-mentioned biological marker values, although the effective dose of pollen was equivalent in the two groups.

It could therefore be shown that the newly developed refined extract (PE), prepared from *Brassica campestris* pollen after mechanical fragmentation of the pollen walls, constitutes a significant improvement with better curative effect, lower dose, and higher bioavailability than the traditional TCM preparation Qianlie Kang Pule’an Tablets.

This marked improvement in activity may be explained by the significantly increased dosage of
\(\beta\)-sitosterol – 0.101 % in PE as opposed to only 0.057 % in QKPT – in the refined extract and will form a point of continuation for further studies.
Literature


Yang BC, Yang YF. Advances in studies on material basis of pollen used for treatment of prostatic diseases.

Chinese Traditional and Herbal Drugs, 2009, 40: 144-149.

Figure 1
Histomorphologic changes in the rat prostates stained with haematoxylin and eosin (magnification: x400). Sham operated distilled water control group (A), castrated distilled water control group (B), castrated QKPT 2.0g/kg group (C), castrated high-dose PE 0.4g/kg group (D), castrated mid-dose PE 0.2g/kg group (E), and castrated low-dose PE 0.1g/kg group (F).
Figure 2
Representative chromatograms of both the HPLC reference solution (A), HPLC test sample solution for PE (B), and HPLC test sample solution for QKPT (C). The respective β-sitosterol peaks are marked with an integration line. Experimental details see text.
Table 1

Effect of PE on volume and wet weight of hyperplastic prostates in rats. All values are expressed as mean ± SD (each group n = 8).

<table>
<thead>
<tr>
<th>Group</th>
<th>BW (g)</th>
<th>wet weight of the prostate (g)</th>
<th>PI (g/100g BW)</th>
<th>volume of the prostate (ml)</th>
<th>PVI (ml/kg BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operated</td>
<td>451.9±27.0</td>
<td>0.514±0.065</td>
<td>0.114±0.011</td>
<td>0.431±0.131</td>
<td>0.950±0.273</td>
</tr>
<tr>
<td>Castrated control</td>
<td>379.9±37.3###</td>
<td>0.720±0.131##</td>
<td>0.190±0.033##</td>
<td>0.691±0.115##</td>
<td>1.836±0.356##</td>
</tr>
<tr>
<td>QKPT 2.0g/kg</td>
<td>389.6±14.7</td>
<td>0.520±0.029**</td>
<td>0.134±0.009**</td>
<td>0.531±0.096**</td>
<td>1.368±0.275*</td>
</tr>
<tr>
<td>PE 0.4g/kg</td>
<td>411.8±14.4$$</td>
<td>0.496±0.054**</td>
<td>0.120±0.014**$</td>
<td>0.488±0.119**</td>
<td>1.184±0.285**</td>
</tr>
<tr>
<td>PE 0.2g/kg</td>
<td>408.6±27.4</td>
<td>0.565±0.040**</td>
<td>0.139±0.012**</td>
<td>0.563±0.083*</td>
<td>1.377±0.174**</td>
</tr>
<tr>
<td>PE 0.1g/kg</td>
<td>390.0±22.2</td>
<td>0.610±0.040*</td>
<td>0.157±0.009*</td>
<td>0.581±0.080*</td>
<td>1.493±0.211*</td>
</tr>
</tbody>
</table>

#P < 0.05; ##P < 0.01 as compared with sham operated distilled water control group

*P <0.05; **P <0.01 as compared with castrated distilled water control group

$P <0.05; $$P <0.01 as compared with castrated QKPT 2.0g/kg group

BW = body weight
Table 2

Effect of PE on the wet weight of the seminal vesicle in BPH rats. All values are expressed as mean ± SD (each group n = 8).

<table>
<thead>
<tr>
<th>Group</th>
<th>BW (g)</th>
<th>wet weight of the seminal vesicle (g)</th>
<th>SVI (g/100g BW)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operated</td>
<td>451.9±27.0</td>
<td>1.682±0.203</td>
<td>0.372±0.040</td>
</tr>
<tr>
<td>Castrated control</td>
<td>379.9±37.3##</td>
<td>2.194±0.320##</td>
<td>0.581±0.088##</td>
</tr>
<tr>
<td>QKPT 2.0g/kg</td>
<td>389.6±14.7</td>
<td>1.962±0.203</td>
<td>0.503±0.047**</td>
</tr>
<tr>
<td>PE 0.4g/kg</td>
<td>411.8±14.4*$$</td>
<td>1.788±0.305*</td>
<td>0.434±0.066**$$</td>
</tr>
<tr>
<td>PE 0.2g/kg</td>
<td>408.6±27.4</td>
<td>1.957±0.125</td>
<td>0.480±0.028**</td>
</tr>
<tr>
<td>PE 0.1g/kg</td>
<td>390.0±22.2</td>
<td>2.008±0.280</td>
<td>0.512±0.082</td>
</tr>
</tbody>
</table>

#P < 0.05; ##P < 0.01 as compared with sham operated distilled water control group

*P < 0.05; **P < 0.01 as compared with castrated distilled water control group

$P < 0.05; $$P < 0.01 as compared with castrated QKPT 2.0g/kg group

BW = body weight
Figure 3

Bar chart of the measured values for the seminal vesicle index (SVI) among the individual groups of experimental animals (n = 8) in the present study, displaying high responsibility of the physiological effects of the examined pollen preparations.

## = P < 0.01 as compared with sham operated distilled water control group

** = P < 0.01 as compared with castrated distilled water control group

$ = P < 0.05 as compared with castrated QKPT 2.0g/kg group

---

Sham Castrated QKPT 2.0g/kg PE 0.4g/kg PE 0.2g/kg PE 0.1g/kg

0.00 0.05 0.10 0.15 0.20 0.25 0.30 0.35 0.40 0.45 0.50 0.55 0.60 0.65 0.70

SVI [g/100g BW]
Figure 4
Bar chart of the measured values for the prostate index (PI) among the individual groups of experimental animals (n = 8) in the present study, displaying high responsibility of the physiological effects of the examined pollen preparations.

## = $P < 0.01$ as compared with sham operated distilled water control group

* $P < 0.05$; ** = $P < 0.01$ as compared with castrated distilled water control group

$ = P < 0.05$ as compared with castrated QKPT 2.0g/kg group
Table 3
Effect of PE and QKPT on pathological changes of hyperplastic prostate in BPH rats (each group n = 8).

<table>
<thead>
<tr>
<th>Group</th>
<th>Degree of Prostatic hyperplasia</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>—</td>
</tr>
<tr>
<td>Sham operated</td>
<td>8</td>
</tr>
<tr>
<td>Castrated control</td>
<td>0</td>
</tr>
<tr>
<td>QKPT 2.0g/kg *</td>
<td>2</td>
</tr>
<tr>
<td>PE 0.4g/kg **</td>
<td>4</td>
</tr>
<tr>
<td>PE 0.2g/kg *</td>
<td>4</td>
</tr>
<tr>
<td>PE 0.1g/kg</td>
<td>1</td>
</tr>
</tbody>
</table>

#P < 0.05; ##P < 0.01 as compared with sham operated distilled water control group

*P <0.05; **P <0.01 as compared with castrated distilled water control group

—    → No interstitial inflammatory cell infiltration, and no epithelial hyperplasia

+    → Mild prostate epithelial hyperplasia

++   → Prostate epithelial hyperplasia, glands enlarged

+++  → Significant proliferation of prostate epithelium, glands significantly enlarged,
       prostatic glandular cavities markedly enlarged
Table 4

Effect of PE on glandular cavity diameter and glandular epithelium height of prostates in BPH rats. Values are expressed as mean ± SD (each group n = 8).

<table>
<thead>
<tr>
<th>Group</th>
<th>Glandular cavity diameter (μm)</th>
<th>Glandular epithelium height (μm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sham operated</td>
<td>160.0±14.6</td>
<td>13.9±2.0</td>
</tr>
<tr>
<td>Castrated control</td>
<td>266.3±35.9##</td>
<td>24.0±3.3##</td>
</tr>
<tr>
<td>QKPT 2.0g/kg</td>
<td>232.1±22.2*</td>
<td>21.5±3.8</td>
</tr>
<tr>
<td>PE 0.4g/kg</td>
<td>217.8±19.8**</td>
<td>19.9±4.1*</td>
</tr>
<tr>
<td>PE 0.2g/kg</td>
<td>226.0±29.0*</td>
<td>21.8±4.2</td>
</tr>
<tr>
<td>PE 0.1g/kg</td>
<td>237.6±15.7</td>
<td>21.4±2.8</td>
</tr>
</tbody>
</table>

#P < 0.05; ##P < 0.01 as compared with sham operated distilled water control group

*P <0.05; **P <0.01 as compared with castrated distilled water control group