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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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ABSTRACT

In Qinghai Province, the *Brassica campestris* L. pollen preparation Qianlie Kang Pule'an Tablet (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 volume index (PVI) as well as prostate index (PI) 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, testosterone treated 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 tamsulosin; 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* 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 have been demonstrated both in animal experiments (Cai et al. 1997, Gao et al. 2006)

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and in 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* 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 – typically 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, Scholtysek 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 members of this group of compounds. In this context, β-sitosterol has been identified as a 5α-reductase inhibitor, 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 the patients.

In this 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 (Fig. 1) in Chengdong district, Xining city, Qinghai Province, PR China. The pollen was collected from the flowers by the bees (Fig. 1) and later harvested from their hives.

The classical TCM preparation 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. Ready-made QKPT can be obtained from Zhejiang Conba Pharmaceutical (Lanxi, PR China; yaojb@conbagroup.com).

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 once 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 were 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 redissolved 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 three 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.

One millilitre 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:

$$C_p\% = \frac{M_0}{A_0} \cdot \frac{A}{M} \cdot 100\%$$

where C_p % = content of phytosterols in the sample; M_0 = weight of β-sitosterol in the photometric reference solution; A_0 = absorbance of the β-sitosterol photometric reference solution; A = absorbance of phytosterols in the photometric test solution; and 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 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