Inhibition of Spontaneous Canine Benign Prostatic Hyperplasia by an *Urtica fissa* Polysaccharide Fraction

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**Abstract**

In this study, we investigated the inhibition of spontaneous canine benign prostatic hyperplasia by a crude polysaccharide fraction extracted from *Urtica fissa* roots and stems. After oral administration of *U. fissa* polysaccharide fraction for 3 months, the dog prostatic volume reduced significantly when compared to that before treatment using CT examination. The high-dosage *U. fissa* polysaccharide fraction (120 mg/kg body weight/day) and finasteride (0.5 mg/kg body weight/day) treatments showed both almost 30% reduction of the initial prostatic volume. At the end of the administration of *U. fissa* polysaccharide fraction, the prostates were excised, and the volumes were measured by water displacement. The prostatic volume showed significant decrease by 11%, 15%, and 21% for the 30, 60, and 120 mg/kg/day *U. fissa* polysaccharide fraction treatment groups, respectively, compared to the control group. Histological observation found that *U. fissa* polysaccharide fraction inhibited the dog prostatic epithelial cells proliferation and enlarged glandular lumen diameter. The crude polysaccharide fraction of *U. fissa* is a possible new candidate for the treatment of benign prostatic hyperplasia.

**Supporting information** available online at [http://www.thieme-connect.de/products](http://www.thieme-connect.de/products)

**Introduction**

Benign prostate hyperplasia (BPH) is the most common disease among aged men. More than 40% of men over 60 years of age suffer from BPH in China [1]. Phytotherapeutics are very popular for the treatment of BPH, especially in Europe [2]. One of the most widely used plants is stinging nettle (*Urtica dioica*, Urticaceae family) [3]. Various water extracts from *U. dioica* roots are widely used for treatment of benign prostatic hyperplasia [3, 4]. *Urtica* plants have also been used as folk medicine with a long history in China for treatment of eczema, rheumatism, and inflammation. But so far, *Urtica* plants have not been listed in the China Pharmacopoeia as authentic medicinal herbs; only *U. fissa* and *U. cannabina* L. were approved as Sichuan Province regional medicinal herbs by the China Ministry of public health. None of the *Urtica* plant extraction products has been approved and entered the market in China. Our studies intended to isolate the pharmacological fraction from *U. fissa* as a standard product and evaluate its therapeutic efficacy for BPH.

The main active compounds of *Urtica* remain undetermined, although many compounds were reported in *Urtica* water or aqueous alcohol extracts [5–11]. Some macromolecular materials, such as polysaccharide and glycoprotein, are considered as the pharmacological components [12–14]. In our previous study, the water extract of *U. fissa* roots and stems was subjected to ultrafiltration with molecular weight cut off 5000 to remove small molecular components including fatty acids, sterols, flavonoids, monosaccharides or oligosaccharides, and a crude polysaccharide fraction (UFP) was obtained. We evaluated UFP inhibitory effects on castrated rat prostate hyperplasia induced by testosterone propionate. Treatment with UFP at 62.5 mg/kg body wt/day induced a decrease in the prostatic volume index by 32%, wet prostatic weight index by 17%, and dry weight index by 23%, respectively. Histological examination showed that proliferation of prostatic epithelial cells and fibrotic tissues was significantly inhibited [15]. In the present study, we further investigated the UFP inhibitory effects on spontaneous canine benign prostatic hyperplasia.
Results

Before experiment, the prostate volumes of 47 dogs were initially screened by palpation. Thirty dogs whose prostate volume was larger than 18 cm³ by CT examination were included in further experiments. They were randomly divided into five groups with no significant difference in age, body weight, and the prostate volume among groups. After 3 months orally administration of finasteride and UFP, the prostate volumes of the dogs were estimated again by CT. As shown in Table 1, the prostatic volume in the control group increased slightly to 102% of the initial volume with aging, but the prostatic volumes of all treated groups including oral administration of finasteride and UFP showed a significant reduction compared to the initial volumes. The high dosage UFP (120 mg/kg body weight/day) and finasteride (0.5 mg/kg body weight/day) treatments showed both almost 30% reduction of the initial prostatic volume.

At the end of experiment, dogs were sacrificed, and prostates were excised. Prostatic volume sizes were measured by water displacement as shown in Fig. 1, and the values were similar to those of the second CT scanning (Table 1). Both prostatic volume values showed that administration of UFP for 3 months resulted in a dose-related reduction. The mean prostatic volume was reduced by 11%, 15%, and 21% for 30, 60, and 120 mg/kg/day in the UFP treatment groups, respectively. A similar decrease of 12%, 17%, and 21% in mean prostatic weight was also found for 30, 60, and 120 mg/kg/day UFP treatments, respectively, compared to the control group.

Histological changes in the prostates after UFP treatment are shown in Fig. 2. As canine spontaneous BPH progressed in the control group, prostatic glandular epithelial cell proliferated with a mastoid morphology. Many papillas had protruded into glandular cavities, and the shape of the glandular lumen was irregular. More fibrotic tissues appeared in the prostatic glandular interstices. Inflammatory lymphocytes infiltrates were also seen in the perirectal region. After finasteride (0.5 mg/kg/day) and all dose UFP treatments, the glandular epithelial cell proliferation

<table>
<thead>
<tr>
<th>Groups</th>
<th>Age (years)</th>
<th>Weight (kg)</th>
<th>Prostate volume (cm³)</th>
<th>Before treatment</th>
<th>After treatment</th>
<th>Volume change (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>9.3 ± 2.1</td>
<td>14.5 ± 2.1</td>
<td>14.05 ± 1.73</td>
<td>14.41 ± 1.76</td>
<td>+ 0.36 ± 0.70</td>
<td>(102.69)</td>
</tr>
<tr>
<td>Finasteride (0.5 mg)</td>
<td>8.0 ± 1.1</td>
<td>13.7 ± 0.9</td>
<td>14.68 ± 2.73</td>
<td>10.34 ± 2.08</td>
<td>− 4.34 ± 1.12</td>
<td>(70.47)</td>
</tr>
<tr>
<td>UFP (30 mg)</td>
<td>8.2 ± 2.0</td>
<td>14.0 ± 1.1</td>
<td>13.59 ± 2.90</td>
<td>12.79 ± 3.15</td>
<td>− 0.80 ± 0.58</td>
<td>(93.34)</td>
</tr>
<tr>
<td>UFP (60 mg)</td>
<td>8.7 ± 1.8</td>
<td>14.9 ± 2.5</td>
<td>13.83 ± 1.53</td>
<td>12.45 ± 2.06</td>
<td>− 1.39 ± 0.62</td>
<td>(89.52)</td>
</tr>
<tr>
<td>UFP (120 mg)</td>
<td>7.5 ± 1.4</td>
<td>14.0 ± 1.3</td>
<td>15.08 ± 2.74</td>
<td>11.45 ± 2.40</td>
<td>− 3.63 ± 1.05</td>
<td>(75.72)</td>
</tr>
</tbody>
</table>

Six dogs in each group; * p < 0.05; after 3 months treatment vs. before treatment. * p < 0.05, ** p < 0.01, *** p < 0.001: treatment groups vs. control group.
was inhibited, and the glandular lumen became wider because of the reduction of the epithelial projection into the lumen. Phenol-sulfuric acid assay was used to determine the total sugar content; a good linearity of calibration curve between glucose concentration and absorbance was found (y = 6.58 × 10^{-3} x + 0.0135) in the glucose concentration range from 36 to 180 µg/ml with a correlation coefficient of 0.9994. The total sugar content in UFP was determined to be 58.8%.

The monosaccharide fingerprint of acid hydrolyzed UFP was performed in HPLC. As shown in Fig. 3, six sugars including galacturonic acid, glucose, galactose, rhamnose, and arabinose were presented in HPLC chromatogram. The relative percentage content was calculated as 5: 14: 15: 10: 4 using the area normalization method.

Molecular weight of UFP was determined by the high performance gel permeation chromatography (HPGPC). The calibration curve Log (Mn) = - 0.535Ve + 8.83 was made by retention time of dextran standards vs. MW. Its linearity was found in the range from 2500 to 1.3 × 10^6 with a correlation coefficient of -0.9957. Four peaks corresponding to > 1.0 × 10^6, 7.0 × 10^4, 6.8 × 10^3, and < 2.5 × 10^2 Da, respectively, appeared in the chromatogram of UFP as shown in Fig. 4. This result indicated that UFP was not homogenous with a wide molecular weight range.

Discussion

In this study, the aqueous extract of *U. fissa* was ultrafiltrated in order to remove the monosaccharides, oligosaccharides, and other water-soluble small molecule weight compounds and obtain a crude polysaccharide fraction. The inhibitory effect of the polysaccharide fraction of *U. fissa* was investigated using spontaneous canine BPH model since it is reported to closely resemble human BPH and to be the best available animal model for the evaluation of medication for human BPH [16]. First, when we compared the prostatic volume change before and after 3 months treatment with the crude polysaccharide fraction of *U. fissa* by CT examination, the prostatic volume in the respective canine groups were significantly reduced, especially at 60 mg/kg and 120 mg/kg dosages, while the prostatic volume of the control group increased slightly with aging. After prostates were excised at the end of 3 months UFP administration, prostatic volumes were measured by water displacement and also showed a dose-dependent reduction. Histological observation suggested that UFP reduce the prostatic size by inhibiting the prostatic epithelial cells proliferation and extending glandular lumen diameter. All these results indicated that UFP caused a significant inhibition of spontaneous canine BPH. Meanwhile, its tolerability was excellent, and no serious adverse effects were found. In our previous study [15], UFP could also inhibit the epithelial cell proliferation of castrated rat prostate hyperplasia induced by testosterone propionate in vivo. But the mechanism of UFP is unknown at present.

As we know, it is hard to evaluate the quality of a polysaccharide fraction by one method, e.g., HPLC. We proposed a series of methods to control the quality of UFP, including quantitation of polysaccharide content, fingerprint profiling of monosaccharide composition, and molecular weight determination. Phenol-sulfuric acid assay is often used to quantify the total sugar. In our study, a good linearity of the calibration curve between glucose concentration and UV absorbance at 490 nm was obtained, and the total sugar content in UFP was quantified and
reached 58.8%, indicating that polysaccharide is the main component in the crude fraction.

A polysaccharide is composed of a variety of monosaccharides. Monosaccharide composition analysis is important for quality control of polysaccharide products. Although many analytical methods such as gas chromatography (GC), HPLC, and thin layer chromatography (TLC) were reported for component analysis of mono-, di-, oligo-, and polysaccharides in food, diets, and herb extracts, HPLC is one of the tools with advantages of simple sample preparation for analysis of crude samples and simultaneous analysis of monosaccharides. Based on our HPLC chromatogram, glucose, galactose, and arabinose were determined as main sugar components in UFP.

HPGPC is a widely used method available for polysaccharide MW determination because of its high efficiency, good reproducibility, and fast speed. Our results indicated that UFP is not homogeneous, with a widely varying molecular weight from $1.0 \times 10^6$ to $2.5 \times 10^7$ Da.

Our previous studies also indicated that UFP was not homogeneous in structural features. We purified several polysaccharides from UFP by anion exchange and gel-filtration chromatography. The structural features of these polysaccharides were characterized, even though their antiprostatic activities were not investigated. These polysaccharides consist of a linear chain of 1,6-linked $\beta$-D-glucopyranosyl, 1,6-linked $\alpha$-D-galactopyranosyl, and 1,5-linked $\beta$-arabinofuranosyl residues; some terminal $\alpha$-D-glucopyranosyl residues were attached to the chain [17, 18] (Supporting Information).

In conclusion, the crude polysaccharide fraction of *U. fissa* is a possible new candidate for the treatment of BPH. But the standardization and quality control of this fraction remains a challenge for human clinical application.

### Materials and Methods

### Drugs

Finasteride (a 5-alpha reductase inhibitor, purity>98%) was purchased from Hangzhou MSD Pharmaceutical Company, Ltd.

### Plant materials

*U. fissa* was collected in September 2005 at the Anshun mountains in the Guizhou Province of China and identified by Prof. Liu Cun-sheng from the Beijing University of Chinese Traditional Medicine. A voucher specimen was deposited under the number 20051121 in our laboratory.

The polysaccharide fraction of *U. fissa* was prepared as described in our previous report [13]. Briefly, *U. fissa* (103 kg) were pre-extracted with 95% ethanol (800 L) to remove color materials. The plant residue was extracted with boiling water twice (800 L). The aqueous extract was ultrafiltered to remove small molecular substances using an ultrafiltration system including a pump and a hollow fiber microporous membrane cartridge with molecular weight cut off of 5000 Dalton (Beijing Xubang Membrane Equipment Co. Ltd.). The concentrated fraction was evaporated under vacuum to obtain the crude polysaccharide fraction (UFP, 1.2 kg).

### Animals

Male beagle dogs, 6–14 years old and 8.3–14.9 kg weight, were provided by the Animal Center of Beijing Institute of Radiation Medicine and maintained under standard environmental conditions (air-conditioned room, 12 h/day controlled lighting, food and water *ad libitum*). Animal care and surgery protocols were approved by the Animal Care Committee of the Institute of Radiation Medicine (approval date: August 3, 2006, approval No. 0010229). All animals were treated in accordance with recognized scientific ethics.

### Estimation of prostate volume by CT scanner

Before the experiment, dogs were initially screened by palpation based on the magnitude and hardness of the prostate. The prostate volume was estimated by Pro-Speed spiral CT scanner (GE) examination with a layer of 5 mm under pentobarbital anesthesia. The transverse diameter, the craniocaudal diameter, and the longest diameter of the sagittal plane of the prostate were measured. According to the Guidelines for New Drugs Preclinical Studies (edited by the Ministry of Health, P.R. China), the prostate volume was calculated as follows: volume of prostate ($cm^3$) = transverse diameter $\times$ craniocaudal diameter $\times$ longest diameter of the sagittal plane $\times \pi/6$. The dogs with prostate volumes larger than 18 cm$^3$ were used in our experiments.

### Dog groups

Thirty dogs with prostate volumes larger than 18 cm$^3$ were randomly divided into five groups (6 dogs in each group). ▶ Group 1: control group, all six dogs were given water orally every day for 3 months. 
▶ Group 2: the animals received finasteride orally for 3 months at a dose of 0.5 mg/kg body weight/day.
▶ Groups 3, 4, and 5: the animals received UFP orally at doses of 30, 60, and 120 mg/kg body weight/day for 3 months, respectively.

At the end of the 3-month treatment, the prostatic volumes of all thirty dogs were estimated again by CT. The reduction in volume induced by the UFP was evaluated in terms of the percentage change of the prostate volume.

### Histological examination

At the end of the 3-month treatment, dogs were sacrificed 24 h after the last drug administration under pentobarbital anesthesia, and prostates were excised. Prostatic wet weight and water displacement volume were measured. Each prostate was fixed in 10% phosphate-buffered paraformaldehyde, embedded in paraffin, mounted on slides, sectioned at 4 µm and stained with hematoxylin and eosin.

### Phenol-sulfuric acid assay for determination of total sugar content

Accurately weighed 12.0 mg glucose was dissolved in distilled water in a 50-mL volumetric flask to a certain volume as stock solution. In each tube, 150, 300, 450, 600, or 750 µL stock solution was added, respectively, and diluted by distilled water to 2 mL, then 1 mL 6% phenol and 5 mL sulfuric acid were added. The tubes were kept in 40°C water bath for 30 min. The absorbance was measured at 490 nm using UV-2100 ultraviolet spectrophotometer (Unica), and the absorbance of a blank (2 mL distilled water plus the reagents) was subtracted from the absorbance of the standard solution. The calibration curve was constructed by glucose concentration ($X$, µg/mL) vs. UV absorbance ($Y$).

Accurately weighed 6.0 mg UFP was dissolved in distilled water in a 10-mL volumetric flask to a certain volume as sample solution. Absorbance was determined as the procedure above. Total sugar content in UFP was calculated by the calibration curve.
HPLC fingerprint of monosaccharide

UFP (12 mg) was mixed with 5 mL of 2 M trifluoroacetic acid (TFA) in a screw-cap vial and hydrolyzed at 110°C for 6 h. After cooling, the hydrolyzed solution was concentrated, dissolved by 2 mL MeOH and evaporated in vacuo to draw off TFA. The residue was dissolved in 500 µL distilled water and the fingerprint analyzed by a HPLC system including P230 HPLC pumps (Elite) and WellChrom K-2301 refractive index detector (Knauer). The column was Carbomix H-NP 10:8% (7.8 x 300 mm). 2.16 mM H2SO4 was used as mobile phase with a flow rate of 0.5 ml/min at 40°C. The monosaccharide standards including galacturonic acid, glucuronic acid, xylose, arabinose, rhamonose, galactose, fructose, mannose, and glucose were purchased from the National Institute for the Control of Pharmaceutical and Biological Products.

HPGPC of molecular weight

Molecular weight of UFP was determined by the HPLC system described above and an EC2000 GPC workstation (Elite). The column was OHpak SB-804 HQ (8.0 mm × 300 mm) and 0.7% Na2SO4 was used as mobile phase with a flow rate of 0.5 ml/min at 40°C. A calibration curve was prepared from the known MW Dextran standards (D-2000000, D-133800, D-41100, D-10000, D-2500) which were purchased from the National Institute for the Control of Pharmaceutical and Biological Products.

Statistical analysis

Data were expressed as mean ± SD and compared using the double-tail Student's t test; p < 0.05 was taken as statistically significant.

Supporting information

The purification details and structural features of U. fissa polysaccharides are available as Supporting Information.

Acknowledgements

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Conflict of Interest

The authors declare no conflict of interest.

References