The Inhibiting Effects of *Urtica dioica* Root Extracts on Experimentally Induced Prostatic Hyperplasia in the Mouse

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Abstract: **Extracts of stinging nettle roots (*Urtica dioica* L., Urticaceae) are used in the treatment of benign prostatic hyperplasia (BPH). We established a BPH-model by directly implanting an urogenital sinus (UGS) into the ventral prostate gland of an adult mouse. Five differently prepared stinging nettle root extracts were tested in this model. The 20% methanolic extract was the most effective with a 51.4% inhibition of induced growth.**

Key words: **Benign prostatic hyperplasia (BPH), *Urtica dioica* L., Urticaceae, scopoletin, 3β-sitosterin, *Urtica dioica* agglutinin (UDA), polysaccharides.**

Introduction

More than 80 per cent of men older than 80 develop a benign prostatic hyperplasia (BPH). In the treatment of BPH grades I and II according to Vahlensiek, the extracts prepared from roots of *Urtica dioica* L., Urticaceae (such as Bazoton©uno) have been used taking into account the observations of Rückle (1). He demonstrated that teas made from stinging nettle roots had some positive effects on the well-being of the patients. The pathogenesis of BPH, however, is still an unresolved problem. A number of hypotheses have been proposed. McNeal (2) suggested that the pathological process of BPH is comparable to a reawakening of embryonic growth potential. During the last few years, growth factors and their receptors have been discussed as being possibly involved in the development of BPH. Other theories were more concerned with the influence of 5α-reductase and with different hormone levels. Experimental support for the hypothesis of the reactivation of embryonic growth potential was provided by Cunha and his co-workers (3). They demonstrated that the embryonic urogenital sinus is a potent inductor for the growth of adult bladder epithelium. Therefore, Chung (4) established an *in vivo* model based upon the growth-promoting effect of fetal tissue on adult mouse prostatic glands. We have adopted this latter model, directly implanting a fetal urogenital sinus (UGS) into the prostate gland of adult mice to evaluate different stinging nettle root extracts. Various compounds were isolated from *U. dioica*, for example, phenylpropane (5), lignans, sterols (6), lectin(s) (7), and polysaccharides (8) (among others). We intended to test five characterised extracts in the mouse model. For solvents, we chose, successively, cyclohexane (→ extract C), ethyl acetate (→ extract E), 1-butanol (→ extract B). We made separate extracts with water (→ extract W), and 20% methanol/water (→ extract M). The amounts of 3β-sitosterin, scopoletin, total protein, total sugar (W, M), and *Urtica dioica* agglutinin (UDA) were analysed in the different extracts.

Materials and Methods

Plant material

Dried roots of *Urtica dioica* L., tested in accordance with DAB 1996, were purchased from H. Finzelbergs Nachfolger GmbH & Co., Andernach, Germany. Voucher specimens are deposited at the Institut für Pharmazeutische Biologie in Marburg.

Extraction

Dried, milled roots (1002.9 g) were macerated for 24 hours at room temperature with 2.0 l of cyclohexane, transferred to a percolation column (r = 8.0 cm) and extracted with additional 6.0 l of cyclohexane. The solution was evaporated in vacuo (2.83 g extract C). The extraction procedure was then continued with 10.0 l of ethyl acetate and 8 l of 1-butanol. After evaporation (in vacuo) we obtained 3.82 g ethyl acetate extract (E) and 3.74 g butanol extract (B). To obtain native polar extracts (containing polysaccharides), we prepared extra aqueous (W) and 20% methanolic-aqueous (M) extracts. Dried, milled roots (1005.8 g) were macerated for 24 hours at room temperature with 3 l of 20% methanol, transferred to a percolation column and extracted with additional 5.0 l of 20% methanol. After lyophilization we obtained 67.68 g extract (M). Dried, milled roots (1019.0 g) were macerated for 24 hours at room temperature with 3 l of water, transferred to a percolation column and extracted with additional 5.0 l of water. After lyophilization we obtained 78.55 g extract (W).

Analysis of main compounds

Materials: column: LiChrospher RP-18 (4-4, 125-4) for sitosterin analysis; LiChrospher RP-8 (4-4, 125-4) for scopoletin analysis. Waters lambda model 481 LC, Waters 600 E system controller, Waters 745 data module, HP pump: HP 1050, HP F det.: HP 1046 a, HP UV det.: HP VWD.

Sitosterin: About 100–200 mg of extract were accurately weighed and treated with 5.00 ml of acetonitrile (LiChrosolv). This solution was treated in a supersonic bath for 10 minutes, then cooled, and passed through a sterile-filter. 20 μl were used for HPLC. Temp: 40 °C, det.: 206 nm, solv. A: water, solv. B: acetonitrile, flow: 1.0 ml gradient: 0 (min) 30 % A 70 % B; 15 min 10 % A 90 % B (linear); 20 (min) 0 % A 100 % B (linear); 30 (min) 0 % A 100 % B (linear).

Scopoletin: About 100–200 mg of extract were accurately weighed and 40 ml of 1 % phosphoric acid solution were added. The sample was heated in a water bath for 20 min at 60 °C, 8.0 ml of acetonitrile were than added. This solution was treated in a supersonic bath for 10 minutes and then cooled. Up to 50.00 ml acetonitrile were added. After centrifugation for 2 min (12000 g), 20 μl were used for HPLC. Temp: 25 °C; UV det.: 342 nm, F det.: Ex 345 nm, Em 431 nm, solv. A: 1 % phosphoric acid solv. B: acetonitrile, flow: 0.80 ml, isocratic 80 % solv. A/20 % solv. B.

Protein (BCA protein assay) (9, 10): CA Protein Assay Reagent A (Pierce) and BCA Protein Assay Reagent B (Pierce) were available. We used the Pierce BCA protein assay with microtiter plates to analyse aqueous solutions of the different extracts (prepared analogously to the solutions used in the pharmacological testing). To standardise this system for use in a very low protein concentration range, we incubated the plates at 37 °C for 30 min and kept all assay conditions constant.

UDA (haemagglutination assay) (11, 12, 13): 200 μl of fresh human blood (blood group 0, rhesus +) were mixed with the same volume of Alsevers solution (dextrose 2.05 g, trisodium citrate · 2 H_2O 0.8 g, NaCl 0.42 g, and citric acid 0.005 g in 100.0 ml water) and centrifuged for 2 min (2000 rpm), after which the erythrocytes were washed three times and suspended in 5 ml of PBS (K_2HPO_4 1.824 g/l, NaH_2PO_4 0.245 g/l, NaCl 8.77 g/l). The cell suspension was diluted with PBS to give an absorbence rate of 2 in a 1 cm cuvette at 620 nm. To one part of a 0.05 % trypsin solution, five parts of the cell suspension were added and incubated for 1 hour at 37 °C (water bath). Then the solution was washed 5 times with PBS containing 0.1 % glutaraldehyde and incubated for 10 minutes at room temperature. Then the trypsinized and glutaraldehyde-fixed erythrocytes were washed four times with PBS. For the haemagglutination assay we used microtiter plates. 50 μl of erythrocyte suspension and 50 μl of either the lectin or the neutralised extract solution were added to each well. The agglutination was controlled visually after 1 hour at room temperature.

Sugar (anthrone reaction) (14): 20.00 mg of extract were dissolved in 50.00 ml of water; 1 ml of this solution was diluted to 10.00 ml. 0.28 g of anthrone were dissolved in 100 ml of dilute (70 %) H_2SO_4. 5 ml of the latter solution were added to suitably-sized stoppered boiling tubes and kept cool by immersion in an ice/salt-water cooling mixture. Onto the surface of the cooled anthrone reagent, 1 ml of extract or standard solutions (< 100 μg of D-galactose) were layered. After 15 min, the solution was well mixed and heated for 10 min at 100 °C. Then the tubes were cooled in an ice-water bath and the absorbance was measured at 620 nm.

Pharmacological testing

Solutions: 1.) 500 mg of hydroxypropyl-β-cyclodextrin in 10 ml of water (negative control). 2.) 36 mg of suramin, 500 mg of hydroxypropyl-β-cyclodextrin in 10 ml of water (positive control). 3.) 200 mg of extract (C, E, B, M, W), 500 mg of hydroxypropyl-β-cyclodextrin in 10 ml of water. When solubility problems were encountered (C, E, B), the solution was heated for 15 min in a 60 °C water bath, and then treated for 15 min in a supersonic bath and centrifuged (4000 g) for 15 min.

Animals: Balb/c mice: Female Balb/c mice were sacrificed by cervical dislocation on day 16 of gestation (15). Day 0 was designated when a vaginal plug first became visible. The foetuses were harvested and the UGS with connecting tissues were microdissected under a stereodissecting microscope. To prepare the UGS, the fetal bladder and the Wolffian and Müllerian ducts were removed. Up to the moment of implantation, within 3 h after isolation, the isolated UGS was stored in Dulbecco's Modified Eagle Medium (DMEM). Adult male Balb/c mice (50–70 days old) were used as hosts for the harvested UGS. After being anaesthetised with Diazepam and Thalamonal® the abdomen of the male mice were opened and the lobes of the ventral prostate were exposed for implantation. one UGS was directly transplanted into one lobe. The contralateral lobe of each prostate was simply pierced with a fine forceps and served as a control experiment (sham-operated). The implantation day was assigned as day one and the implants were allowed to grow and develop for 28 days. During this period of four weeks, the mice were treated (0.25 ml of solution orally; by probang) with the different extracts of *U. dioica* roots (plus 5 % hydroxypropyl-β-cyclodextrin), or with a solution of 5 % hydroxypropyl-β-cyclodextrin only, or with a suramin solution. After 28 days, the animals were sacrificed, the ventral prostates and the sham-operated and UGS-implantated lobes were removed. The wet weight per lobe of mouse prostate gland was determined.

Statistics

At least 7–9 mice constituted a treatment group, the results of which have been calculated as means and standard deviation. Statistical significance has been evaluated by Wilcoxon rank sum test (Mann-Whitney U-test) (8 [7] mice).

Results

We prepared five extracts within a range of apolar to polar solvents. To characterise these extracts the contents of leading or main components were measured (Tables **1** and **2**). For these substances some interesting activities were postulated. Scopoletin, with known anti-inflammatory activity, was analysed because it is often described as a "leading" substance. β-Sitosterin shows some interesting activity on a steroid hormone binding globulin (SHBG) test system (16), but is not specific for stinging nettles and is also found in foodstuffs (in high amounts). *Urtica dioica* agglutinin (UDA), a lectin (protein), has immunostimulating activity and a binding potential to the EGF (epidermal growth factor) receptor (8, 17). The polysaccharide fraction has a proved immunostimulating and anti-inflammatory activity (18).

The only way to feed the daily portion of extract to the mice was in form of a solution fed orally by probang. We used hy-

Table 1 Content of different compounds in different extracts.

Extract	Scopoletin (ppm)	SD	Sitosterin (%)	SD	Protein (%)	SD	UDA (%)	Sugar (%)	SD
cyclohexane	1.84	0.85	6.250	9.4	4.70	2.53	0		
ethyl acetate	49.39		0.518	2.78	2.31	2.87	0		
1-butanol	82.19	3.88	0.491		21.00	3.88	0		
20 % methanol	24.63	6.25	0.016	2.88	49.50	6.58	0.03	16.48	6.40
water	12.32	1.51	0.035	7.10	38.00	0.51	0.06	6.27	13.73

Table 2 Daily dose of various compounds per mouse in different extracts (dd: g/kg).

Extract	Scopoletin	Sitosterin	Protein	UDA	Sugar
cyclohexane	3.07E-07	1.04E-02	7.83E-03	0	
ethyl acetate	8.23E-06	8.63E-04	3.85E-04	0	
1-butanol	1.37E-05	8.18E-04	3.50E-04	0	
20 % methanol	4.11E-06	2.67E-05	8.25E-02	5.00E-05	2.75E-02
water	2.05E-06	5.83E-05	6.33E-02	1.00E-04	1.05E-02

Table 3 Effects of *Urtica dioica* root extracts on experimentally induced mouse prostatic hyperplasia.

Extract	Sham-operated lobe (mg)	SD	Implanted lobe (mg)	SD	Number of mice	Inhibition of prostate growth (%)	p*
cyclohexane	5.11	1.59	29.89	8.44	9	23.5	0.040
ethyl acetate	6.90	3.44	23.00	10.15	8	5.0	1.000
1-butanol	5.25	1.13	23.88	10.71	8	1.3	0.916
20 % methanol	7.56	1.71	11.78	3.61	9	51.3	0.003
water	5.20	1.23	17.79	8.66	8	26.5	0.267
suramin	3.80	0.71	17.97	6.35	7	25.7	0.156
control**	7.40	1.12	24.20	13.07	9	–	–

* (probability of equaling with the control group).
** control = 5 % hydroxypropyl-β-cyclodextrin in water.

droxypropyl-β-cyclodextrin to enhance the solubility of apolar substances, which is proved to be not toxic in the concentration used (blind). Suramin, a well known drug used against certain protozoa infections, is one model substance used nowadays in tests with growth factors due to its inhibiting effect on certain factors (positive control).

β-Sitosterin is the characteristic component of the cyclohexane extract. A high scopoletin and a moderate β-sitosterin content is typical of the ethyl acetate extract. The butanol extract has the highest amount of scopoletin, with a moderate β-sitosterin and a high protein content. The aqueous and methanolic aqueous extracts are characterised by low β-sitosterin and scopoletin and high protein, UDA and sugar contents. The results of the different extracts in the mouse model are shown in Table **3**.

We tested the kind of proliferation induced in our system with specific markers for PCNA (Proliferating Cell-Nuclear-Antigen) and Ki-67-antigen. Tissue sections were taken from both methanolic extract treated and control solution treated animals. Firstly, we were able to prove that the increase of the prostate is a cellular growth and not an edema or an expansion of the extracellular matrix. Secondly, we could confirm a growth inhibiting effect of the 20% methanolic extract of *U. dioica* roots in this mouse model.

Discussion

With the different extracts of *Urtica dioica* roots we have the possibility to inhibit and to promote induced prostate growth in an *in vivo* system. Of therapeutic interest is the inhibiting property of the aqueous and 20% methanolic extracts. Although there is no direct correlation, it is nevertheless interesting to compare the amounts of the main compounds in the different preparations with the growth inhibiting effect of the extracts (Figs. **1** and **2**). There is no correlation between the amounts of sitosterin and scopoletin with the growth inhibiting effect. In contrast, the extract with the highest concentration of sitosterin had a growth promoting activity. Characteristic substances for the active extracts are the polar components like UDA, protein (total), and carbohydrates or sugars (total). At present, UDA, polysaccharides, or other polar components can be considered as active components in this BPH-test system. Still undetermined is how precisely these constituents may act, i.e., directly or indirectly (e.g., receptor, mediator). The questions of resorption and of the local concentration (prostate) of these substances are still unresolved, but first experiments indicate that a resorption of lectins and polysaccharides may be possible (18, 19).

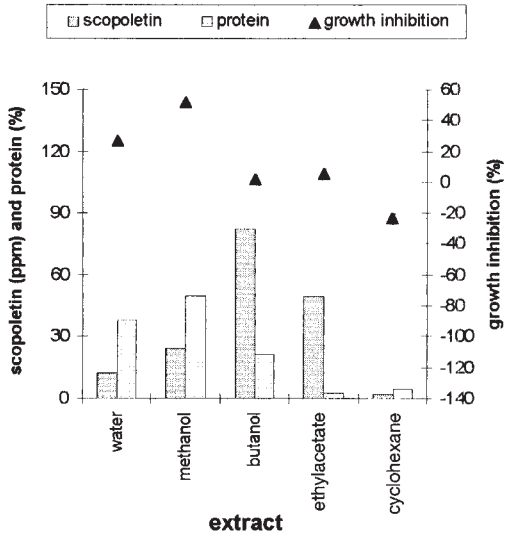

Fig. 1 Comparison between scopoletin and protein contents and growth inhibition in the mouse model.

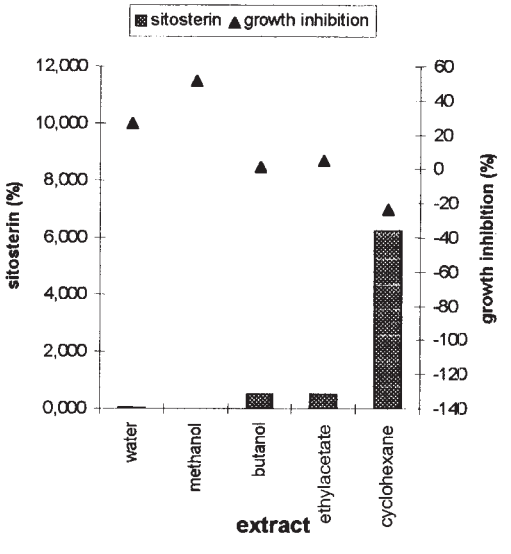

Fig. 2 Comparison between sitosterin content and growth inhibition in the mouse model.

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