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Uromedic® Pumpkin Seed Derived Δ7-Sterols, Extract and Oil Inhibit 5α-Reductases and Bind to Androgen Receptor *in Vitro*

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Abstract

Dihydrotestosterone (DHT) is implicated in the development of benign prostate hyperplasia (BPH). We investigated if Uromedic® pumpkin (variety of *Cucurbita pepo* L. *convar. citrullinina GREB. var. styriaca GREB*) seed soft extract (active ingredients of GRANUFINK® Prosta forte 500 mg), seed oil and isolated Δ7-sterols could inhibit the conversion of [1,2,6,7-^{3}H(N)]-testosterone to DHT by 5α-reductases. Also, we tested competition with [^{3}H]-DHT for binding to the androgen receptor (AR). Pumpkin seed oil and pumpkin seed soft extract were identified as moderately active inhibitors of 5α-R1 and 5α-R2, with almost similar inhibitory capacities (IC_{50} < 5 mg/ml for 5α-R1 and about IC_{50} = 6 mg/ml for 5α-R2). The isolated Δ7-sterols were more active inhibitors (IC_{50} = 0.3 mg/ml for 5α-R1, IC_{50} = 1.0 mg/ml for 5α-R2). All three test compounds bound to the AR dose-dependently, with strong binding by Δ7-sterols (IC_{50} = 0.2 mg/ml) and weaker binding by pumpkin seed oil (IC_{50} = 0.4 mg/ml) and pumpkin seed soft extract (IC_{50} = 1.1 mg/ml). We propose that inhibition of 5α-reductases and competitive binding to the AR are mechanisms of action, by which the Uromedic® pumpkin seed derived test compounds, most specifically Δ7-sterols, counteract DHT and thereby exert clinically positive effects on the prostate, as well as bladder-strengthening effects.

Keywords

5-Alpha-Reductases 5α-R1 and 5α-R2, Androgen Receptor, Dihydrotestosterone, Uromedic® Pumpkin Seed Oil and Soft Extract, (Delta-7) Δ7-Sterols

1. Introduction

Benign prostate hyperplasia (BPH) is commonly found in men, with a worldwide prevalence of from 20% - 62% in men over 50 years [1]. Affected men suffer from the frequent urge to urinate, especially at night, weakened urinary flow, delayed start of urination, intermittency, urine leaking, incomplete bladder emptying as well as difficult or painful urination. The steroidal androgen dihydrotestosterone (DHT) has been implicated in the development and maintenance of BPH.

A common BPH treatment option is the inhibition of the 5-alpha-reductases, enzymes catalyzing DHT formation from testosterone [2] [3]. There are three 5-alpha-reductase isoenzymes: 5α-R1 is expressed in low levels in the prostate, whereas 5α-R2 is highly expressed there. 5α-R3 appears to have a role in malignant tissues [3] [4].

5-alpha-reductase—inhibition has previously been shown for saw palmetto (*Serenoa repens* (Bartr.) Small.) extracts (and for several contained fatty acids) [5] [6], as well as for *Urtica dioica* Linn. extract [7]. 5-alpha-reductase—inhibition was also proposed for *Cucurbita pepo* L. (pumpkin) seed preparations [8].

A further possibility to reduce the pathologic effects of DHT represents the inhibitory competition with DHT for its binding site at the androgen receptor (AR). The AR is activated by binding either testosterone or the more potent androgen dihydrotestosterone. Upon binding, AR undergoes conformational changes and translocates from the cytoplasm to the nucleus, where it dimerizes and regulates transcription by binding to androgen response elements [9]. The AR has two isoforms, AR-A and AR-B [10].

Pumpkin (*Cucurbita pepo* L.) seeds or preparations thereof have long been recognized for their potential of relieving lower urinary tract symptoms related to benign prostatic hyperplasia or to an overactive bladder [11]. The seed soft extract of a special pumpkin breed ("Uromedic® pumpkin", a variety of *Cucurbita pepo* L. *convar. citrullinina GREB. var. styriaca GREB*) [12] [13] is rich in Δ7-sterols, which are typical components in pumpkin seeds [14] and seeds of other Cucurbitaceae, as well as in Amaranthaceae [15]. Δ7-sterols were shown to influence the prostate metabolism [16] [17]. Furthermore, they competitively reduced the binding of DHT to human fibroblasts [18].

Extracts from *C. pepo*, *Prunus Africana* (Hook. f.) Kalkman and *S. repens* displayed antiandrogenic activity in an AR responsive reporter gene assay [19]. However, the molecular effectors appear to vary: in the case of *P. africana*, N-butylbenzenesulfonamide was characterized as active constituent [19], whereas in *S. repens*, certain fatty acids may act by binding—instead—to the alpha-1-adrenergic, muscarinic and 1,4-dihydropyridine receptors, as well as to 5-alpha-reductases [6]. Interestingly, pumpkin seed oil contains high amounts of putatively active fatty acids, *i.e.* oleic acid and linoleic acid [12] [13].

The inhibition of the 5α-reductases as well as the inhibitory binding to the AR

are two possible mechanisms by which the pumpkin seed derived test compounds—Uromedic® pumpkin seed soft extract, seed oil and isolated Δ7-sterols—could exert their positive effects on the prostate and bladder system, especially in relation to BPH. Thus, we tested inhibition of 5*α*-reductase and competitive androgen receptor-binding using radioactively labeled ligands.

2. Materials and Methods

Experimental procedures were performed by "rent-a-lab Dr. Carsten Tober" in Reutlingen, Germany, between April-September 2016.

2.1. Test Compounds and Materials

Test compounds: 1. Pumpkin seed oil of Uromedic® pumpkin seeds (Material 3000000293, Omega Pharma batch: 511062, vendor/batch: 603827/283484), one of the active substances in "GRANUFINK® Prosta" (Omega Pharma Deutschland GmbH). 2. Δ7-sterols isolated from Uromedic® pumpkin seed oil; 3. Pumpkin seed soft extract (DER 15-25:1) from Uromedic® pumpkin seeds; extraction solvent ethanol 92% (m/m) (Material 3000000299, Omega Pharma batch: 531057, vendor/batch: 600516/15000533); active substance of GRANUFINK® Prosta forte 500 mg (Omega Pharma Deutschland GmbH), supplying approximately 30 mg/day Δ7-sterols [14] (Supplementary **Table S1**).

The test compounds were dissolved and stored in DMSO (dimethyl sulfoxide) at 4˚C.

Commercial hexane extracted saw palmetto fruit extract (*Serenoa repens* W. Bartram; DER 7-11:1) served as positive control (5*α*-reductase—inhibition). Finasteride ($C_{23}H_{36}N_2O_2$, 5*α*-reductase—inhibitor) was used to quantify 5*α*-reductase inhibition, and DHT to quantify AR binding.

AR (recombinant rat protein, ligand binding domain, code: A15675) was from ThermoFisher Scientific (Braunschweig, Germany). Dihydrotestosterone (DHT) (code: A83809), testosterone (code: T1500) and finasteride (code: F1293) were from Sigma (Taufkirchen, Germany). [^{3}H]-Dihydrotestosterone ([^{3}H]-DHT, code: NET 453), [1,2,6,7-^{3}H(N)]-testosterone, code: NET370) and Ysi Copper His-Tag SPA Beads (code: RPNQ0096) were from PerkinElmer (Rodgau, Germany). Other chemicals were from Sigma or VWR.

2.2. Preparation of Sources of 5*α*-R1 and 5*α*-R2

Sprague Dawley rats (Elevage Janvier, Le Genest, Saint Isle, France) were sacrificed by decapitation. Liver was dissected on ice, frozen in liquid nitrogen and stored at −80˚C. For the experiments at pH 7.0 (5*α*-R1), rat liver microsomes were prepared as described [20]. For the experiments at pH 5.0 (5*α*-R2), a crude rat liver enzyme preparation was used as described [21].

2.3. 5*α*-Reductase Activity Assays

The enzyme reactions were mainly carried out as described [21]. In a total volume

of 90 µl radioactive (^{3}H-testosterone; 0.5 µCi) and non-radioactive (9.5 µM) testosterone, 0.5 mM beta-NADPH and liver microsomes/crude liver enzyme preparation were incubated in assay buffer (0.1 M Tris-citrate, pH 5.0 or 7.0) in a shaking water bath at 37˚C for 20 or 60 min. The assay was terminated by the addition of 10 µl 2.8 M NaOH. Educt (^{3}H-testosterone) and products (^{3}H-DHT, ^{3}H-alpha-adiol and other metabolites) were separated by HPLC from each other using an adapted [22] and validated method, and using a Beckman System Gold HPLC (Beckman Instruments, San Ramon, CA, USA) fitted with an Onyx monolitic C18 (Phenomenex Ltd. Deutschland, Aschaffenburg, Germany) reversed phase column (100 × 3 mm). Chromatography was performed isocratically for 15 min at a flow rate of 0.5 ml/min with 0.2% NH_4OH in 65% methanol as mobile phase, separating ^{3}H-testosterone and ^{3}H-DHT. Radioactivity in collected fractions was determined in a scintillation counter (WinSpektral, Wallac, Finland). Data typically represent results gained by two experiments performed in duplicate (see results section).

Data evaluation: The inhibition by 10 µM finasteride (3.7254 µg/ml) was considered 100% inhibition, the inhibitory activity of the vehicle/solvent 0% inhibition. The inhibition of the test compounds was calculated relative to that of finasteride. IC_{50} values were determined by non-linear regression (algorithm "sigmoidal dose-response"; GraphPadPrism, San Diego, USA).

2.4. Competitive AR Binding Assay

The competition of each test compound (or DHT) with [^{3}H]-DHT for binding to the rat recombinant AR was measured by detecting [^{3}H]-DHT. The basic assay conditions were taken from Freyberger and Ahr [23] and adapted to the scintillation proximity assay (SPA) format, in which beads emit light when a radio-labeled molecule is in proximity. Incubation was in assay buffer (50 mM Tris-HCl pH 7.5, 800 mM $NaCl_2$, 10 mg/ml human γ-globulin, 0.05% bovine serum albumine) at room temperature for 90 min. Then Ysi Copper His-Tag SPA beads were added and the incubation continued for another 60 min. The radioactivity-induced light was determined by a microplate reader (Microbeta, Wallac, Finland). For each test compound, two measurement series were done, each in duplicate.

Data evaluation: In order to exclude binding to non-specific sites, incubations using the radioactive ligand [^{3}H]-DHT and unlabeled DHT as the natural ligand of the AR were run simultaneously at different concentrations. Excess unlabeled DHT occupied the high-affinity specific sites and blocked the binding of [^{3}H]-DHT to the AR. As a result, the radio-labeled ligand only bound to the non-specific sites. Such radioactive binding in the presence of excess unlabeled DHT was considered non-specific binding.

The amount of [^{3}H]-DHT bound in the absence of unlabeled DHT was referred to as total binding. Specific binding was calculated as:

Total (radioactive) binding − Non-specific (radioactive) binding = Specific binding

Thus, the data of the reference compound DHT are presented as total bound radioactivity (in counts per minute, cpm). Test compound data are presented as specific radioligand binding to the receptor. The specific inhibition in % was calculated as (% specific radioligand binding mean – 100) × (−1). IC_{50} values were determined by non-linear regression (algorithm "sigmoidal dose-response"; Graph Pad Prism, San Diego, USA).

3. Results

3.1. Rat Liver 5*α*-Reductase Activity—Assay Validation

Rat liver microsomes were shown to obtain higher levels of 5-alpha-reductase compared to rat prostate microsomes [20]. Therefore, rat liver microsomes were used as source of 5*α*-R1. For 5*α*-R2, a crude liver preparation was found to be more active. 5*α*-R1 exhibits a broad pH optimum (between pH 6.0 - 8.5), whereas 5*α*-R2 shows a narrow acidic pH optimum (pH 5 - 5.5) [4]. To distinguish 5*α*-R1 from 5*α*-R2, the assays for 5*α*-R1 were performed at pH 7.0, and those for 5*α*-R2 at pH 5.0 [21]. Experiments at pH 5.0 led to a very low, almost negligible signal from the rat liver microsomal preparation (source of 5*α*-R1; data not shown).

At pH 7.0 the reference compound finasteride inhibited the activity of 5*α*-R1 derived from rat liver microsomes with an IC_{50} of 48 nM (**Figure 1**). This compares to a published IC_{50} of 21 nM [20], demonstrating test system suitability. A commercial hexanic extract of *Serenoa repens* [5] was able to inhibit 5*α*-R1 with an IC_{50} = 4.9 mg/ml and 5*α*-R2 with an IC_{50} = 1.8 mg/ml.

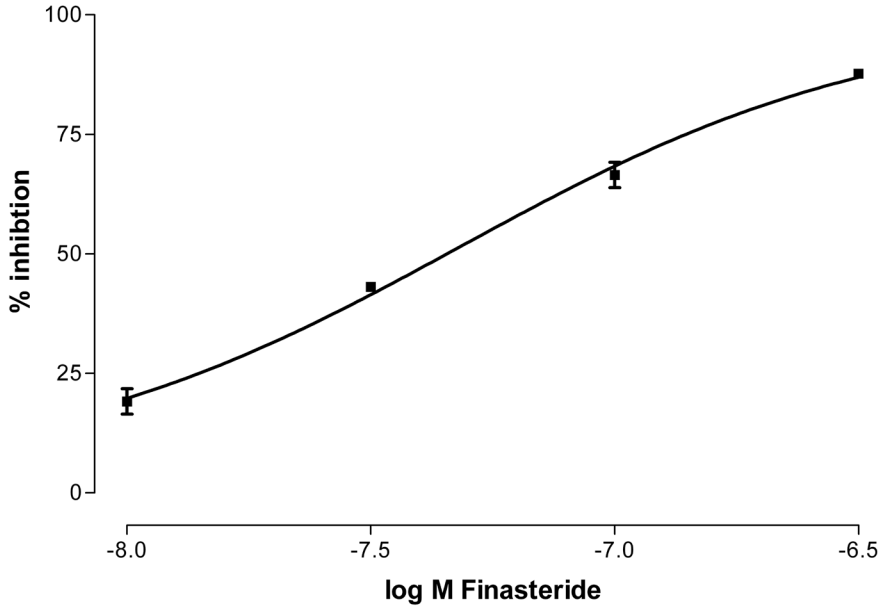

Figure 1. Inhibition of 5-alpha-reductase isoform I, derived from rat liver microsomes, at pH 7.0 by finasteride. Data represent the mean inhibition of enzyme activity (%) of one experiment performed in duplicate. An IC_{50} value of 48 nM was determined for the inhibition by finasteride.

3.2. Inhibitory Effect of the Test Compounds on Microsomal Rat Liver 5α-R1

The inhibitory potential of the test compounds on 5α-R1 was tested using our validated assay at pH 7, favoring 5α-R1 activity. Table 1 shows that pumpkin seed oil (5 mg/ml), pumpkin seed soft extract (5 mg/ml) and Δ7-sterols (0.5 mg/ml) had similar inhibitory capacities, between 70% - 95% compared to 10 µM finasteride. The results for Δ7-sterols showed a dose dependency. The calculated IC_{50} values are indicated.

3.3. Inhibitory Effect of the Test Compounds on Rat Liver 5α-R2

The inhibitory potential of the test compounds on 5α-R2 was tested using our validated assay at pH 5, favouring 5α-R2 activity. Table 2 shows that pumpkin seed oil (5 mg/ml), pumpkin seed soft extract (5 mg/ml) and Δ7-sterols (0.5 mg/ml) had similar inhibitory capacities, between 25% - 50% compared to 10 µM finasteride. The results for Δ7-sterols showed a dose dependency. The calculated IC_{50} values are indicated.

Table 1. Inhibition of microsomal rat liver 5α-R1 by the Uromedic® pumpkin-derived test compounds at pH 7.0.

Compound	Concentration	% Inhibition[a] in experiments[b]		% Inhibition[a] (mean)	IC_{50}[c]
		1	2		
Pumpkin seed oil	5 mg/ml	95.3	72.2	83.75	<5 mg/ml
Pumpkin seed soft extract	5 mg/ml	91.8	71.0	81.4	<5 mg/ml
Δ7-sterols	0.5 mg/ml	79.7	69.5	74.6	0.3 mg/ml
	0.1 mg/ml	not done	42.6	42.6	

a-c: see Table 2.

Table 2. Inhibition of rat liver 5α-R2 by the Uromedic® pumpkin-derived test compounds at pH 5.0.

Compound	Concentration	% Inhibition[a] in experiments[b]		% Inhibition[a] (mean)	IC_{50}[c]
		1	2		
Pumpkin seed oil	5 mg/ml	38.2	47.5	42.85	5.8 mg/ml
Pumpkin seed soft extract	5 mg/ml	36.4	50.1	43.25	5.8 mg/ml
Δ7-sterols	0.5 mg/ml	26.8	0.8[d]	26.8	1.0 mg/ml
	0.1 mg/ml	not done	4.9	4.9	

a: Inhibition relative to 10 µM (3.7254 µg/ml) finasteride (100% inhibition); activity with vehicle DMSO was considered 0% inhibition. b: Both experiments in duplicate; means of duplicate measurements shown; c: IC_{50} values: concentration of test compound causing 50% inhibition. d: This value was judged as outlier and not taken into consideration.

3.4. AR Binding—Assay Validation

DHT is the natural ligand of the AR. DHT competed with [^{3}H]-DHT for binding to the AR with an IC_{50} of 8.5 nM (**Figure 2**). This is in agreement with an IC_{50} of 4.2 nM described in literature [23], demonstrating test system suitability.

3.5. Binding of the Test Compounds to ARs

Overlaid mean specific binding data of pumpkin seed soft extract, pumpkin seed oil and Δ7-sterols are depicted graphically (**Figure 3(a)** and **Figure 3(b)**); numeric data is shown in Supplementary **Tables S2-S4**. All test compounds show typical concentration dependent binding. Strongest specific AR binding was observed for Δ7-sterols (mean IC_{50} = 0.2 mg/ml, corresponding to 0.48 mmol/l) and weaker binding for pumpkin seed oil (mean IC_{50} = 0.4 mg/ml) and pumpkin seed soft extract (mean IC_{50} = 1.1 mg/ml) (**Table 3**).

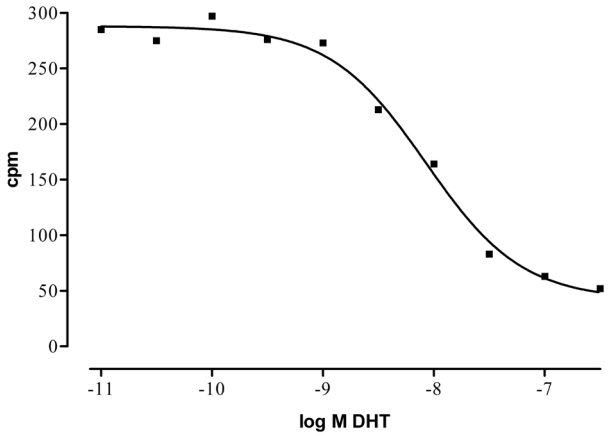

Figure 2. Concentration-dependent competition of DHT with [^{3}H]-DHT for binding to rat recombinant AR. An IC_{50} value of 8.5 nM was determined for the binding of DHT. Data represents the bound radioactivity (cpm).

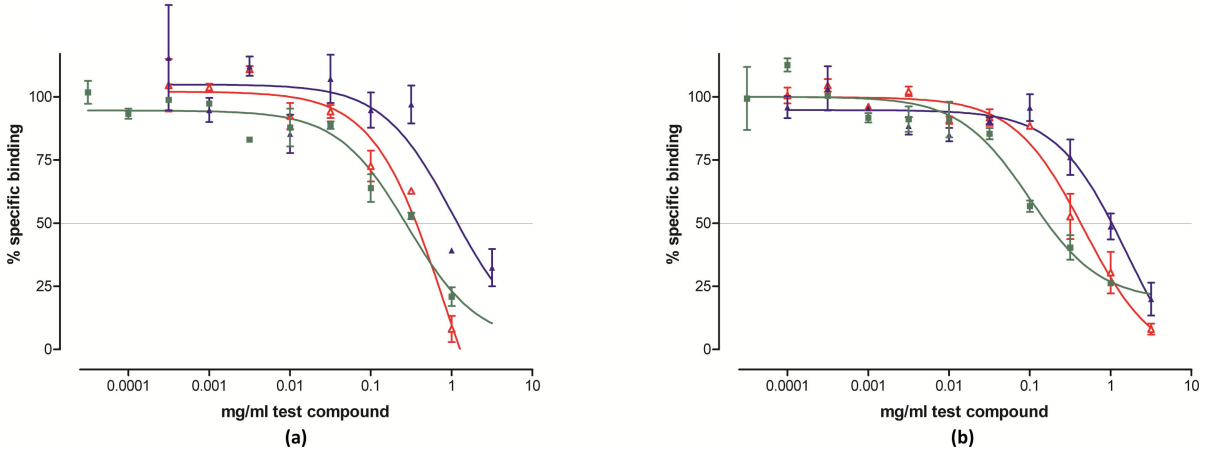

Figure 3. Competitive binding of the test compounds at the AR. The test compounds pumpkin seed soft extract (▲, blue) and pumpkin seed oil (△, red) and Δ7-sterols (■, green) competed with [^{3}H]-DHT for binding to the rat AR within two independent measurements (a and b). Data in each graph represent the mean specific binding ± standard error of the mean (SEM) of duplicate measurements. Calculated IC_{50}-values from each experiment are indicated in **Table 3**. See Supplementary **Tables S2-S4** for numeric data.

Table 3. IC_{50} values obtained within the competitive AR binding assay.

Compound	IC_{50} in mg/ml			IC_{50} in mmol/l
	1. Measurement	**2. Measurement**	**Mean**	
Pumpkin seed oil	0.36	0.43	0.4	/
Pumpkin seed soft extract	1.17	1.04	1.1	/
Δ7-sterols	0.27	0.16	0.2	0.48[a]

a: Calculated using the molecular mass of the main Δ7-sterol within pumpkin seeds: $\Delta^{7.25}$-stigmastadienol – 412 g/mol [14].

4. Discussion

4.1. Inhibition of Human 5-Alpha-Reductases

As previously published, rat liver tissue expresses 5-alpha-reductases. Microsomal liver preparations were used as source for 5*α*-R1 (isoform I). However, the same was found unsuitable for measurement of isoform II at pH 5.0, which was instead investigated in crude liver preparations. Assay validation was done for both preparations using known enzyme inhibitors. Both pumpkin seed soft extract and oil showed similar inhibitory capacities (both: IC_{50} < 5 mg/ml for 5*α*-R1 and IC_{50} ≈ 6 mg/ml for 5*α*-R2). The isolated Δ7-sterols (IC_{50} = 0.3 mg/ml for 5*α*-R1, IC_{50} = 1.0 for 5*α*-R2) were more potent than the pumpkin seed soft extract or oil, most likely since the Δ7-sterols are the main active principle within pumpkin seeds regarding anti-androgenic activity.

4.2. Androgen Receptor Binding

The test compounds inhibited the [^{3}H]-DHT binding to AR in a concentration-dependent manner (**Table 3**), with IC_{50} (Δ7-sterols) = 0.2 mg/ml), IC_{50} (pumpkin seed oil) = 0.4 mg/ml and IC_{50} (pumpkin seed soft extract) = 1.1 mg/ml. This dose-dependency indicates the capacity of all three test compounds to bind to the AR.

Among the tested substances, the Δ7-sterols had the lowest IC_{50} and thus bind most potently to the AR.

Chang and Liao classified the antagonistic effects of different cyclic hydrocarbons on the AR [24]. Accordingly, the binding of Δ7-sterols (mean IC_{50} of 0.48 mmol/l) corresponds to moderately active substances (IC_{50} range 0.1 - 2 mmol/l). In a published experiment, human cells were pre-incubated with Δ7-sterols and then treated with DHT. In a concentration-dependent manner, a reduced binding of DHT to its binding sites could be demonstrated [18]. This goes along with our findings.

4.3. Extrapolation to the *in Vivo* Situation

The inhibition of 5-alpha-reductase by Δ7-sterols and their inhibitory binding to AR are plausible due to the similar structure of these compounds compared to DHT [18] [25]. Several studies support that our *in vitro* observations also take effect *in vivo*:

Trevisan *et al.* 2012 found that orally administered Δ7 sterol (alpha-spinasterol) was well absorbed by mice [26]. Pumpkin seed or pumpkin seed oil in the diet of BPH animal models inhibited prostate growth [13] [27] [28] [29]. Effects were observed with only 40 mg pumpkin seed oil/kg body weight [28]. Furthermore, patients taking 90 mg of a Δ7 sterol mixture isolated from Uromedic® pumpkin seeds 3 - 4 days before prostatectomy had a lower DHT content in the operated prostate tissue than control patients [17]. Thus anti-androgen effects seen in our studies presumably take effect also *in vivo*, while, the safety of pumpkin seed soft extract and pumpkin seed oil has been established by human data [11] [30] [31] [32]. As a next step, the isolated Δ7 sterols (versus seed soft extract and seed oil) could be tested in BPH animal models, and finally in BPH patients.

4.4. Study Limitations

In order to get more reliable IC_{50} values, more experiments using at least 3 different concentrations of the test compounds (especially in the case of the 5-alpha-reductases), and using more independent measurements should be done. Since all our experiments were done using rat enzymes, potential differences in binding and inhibition to human orthologues cannot be excluded.

5. Conclusion

In our study, Uromedic® pumpkin seed soft extract (active ingredients of GRANUFINK® Prosta forte 500 mg), pumpkin seed oil and—more effectively—isolated Δ7-sterols inhibit 5α-reductase and competitively bind to the AR *in vitro*. It is plausible that the Δ7-sterols are the active compounds contained within the pumpkin seed soft extract and oil, regarding these activities. This is supported by the dose-dependency of the observed inhibitory effects, as well as by the lower IC_{50} values of the Δ7-sterols compared to the seed soft extract or oil. Other constituents, such as fatty acids, may also be involved. Our results are promising for a mechanistic understanding of pumpkin seed-derived preparations, and more specifically Δ7-sterols, regarding anti-androgenic effects and the inhibition of 5α-reductase.

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Supplementary

Table S1. Herbal preparations used for the *in vitro* experiments.

Herbal preparation		Pumpkin seed oil	Pumpkin seed soft extract	Δ7-sterols from pumpkin seed oil
Latin binominal name		Company-owned variety of *Cucurbita pepo* L. *convar. citrullinina GREB. var. styriaca GREB*		
Family name		Cucurbitaceae		
Common names		Uromedic® pumpkin		
Plant part		Seed		
Country of origin of herbal raw material		Hungary		
Voucher specimen numbers	Batch number of commercial Herbal Substance (corresponding to drug substance batch below)	Material 3000000293	Material 3000000299	Commercially not available
	Batch number of commercial drug substance (used for this study)	Omega Pharma batch: 511062, vendor/batch: 603827/283484	Omega Pharma batch: 531057, vendor/batch: 600516/15000533	Commercially not available
Authorized drug substance manufacturer		Estyria Naturprodukte GmbH; St. Ruprecht/Raab, Austria	Finzelberg GmbH & Co. KG; Andernach, Germany	not applicable
Further extraction		none	none	Isolation of Δ7-sterols at the Department of Pharmacy, Ludwig-Maximilians-University; Munich, Germany
Test substance used for *in vitro* experiments		Oil	Soft extract	Isolated Δ7-sterols
Type and concentration of extraction solvent used; drug-extract ratio (DER)		not applicable: pure oil without additives	Extraction solvent: ethanol 92% (m/m), DER 15 - 25:1	not applicable
Content of quantified herbal constituents/purity		100% oil, Ph. Eur.	95% - 99% native extract	not applicable, since no extract: isolated Δ7-sterols
Contained excipients		none	1-5% hydrophobic colloidal silica	none
Identification		Fatty acid profile (TLC)	Phytosterols (GC)	Δ7-sterols (GC-IT-MS)
Typical marker compounds		Phytosterols, Fatty acid profile	Phytosterols	Δ7-sterols (e.g. $\Delta^{7,25}$-stigmasterol, spinasterol, Δ^{7}-avenasterol)

GC-IT-MS, gas chromatography ion trap mass spectrometry (Muller C, Bracher F. Determination by GC-IT/MS of phytosterols in herbal medicinal products for the treatment of lower urinary tract symptoms and food products marketed in Europe. Planta Med 2015; 81: 613 - 620); TLC, thin layer chromatography; GC, gas chromatography.

Table S2. Individual data of the AR binding measurements for pumpkin seed soft extract.

	Pumpkin seed soft extract in mg/ml	% Specific radioligand binding			% Specific inhibition
		1. Value	2. Value	Mean	Mean ± SEM
1. Measurement	3.16000	25.0	39.7	32.4	67.6 ± 7.4
	1.00000	39.2	39.2	39.2	60.8 ± 0.0
	0.31600	89.5	104.4	96.9	3.1 ± 7.5
	0.10000	101.7	87.7	94.7	5.3 ± 7.0
	0.03160	116.7	97.5	107.1	−7.1 ± 9.6

Continued

	0.01000	92.9	77.7	85.3	14.7 ± 7.6
	0.00316	115.9	108.3	112.1	−12.1 ± 3.8
	0.00100	90.0	99.5	94.7	5.3 ± 4.8
	0.00032	94.6	136.3	115.4	−15.4 ± 20.8
	0.00010	*65.9**	*62.0**	/	/
2. Measurement	10.00000	26.4	13.5	19.9	80.1 ± 6.5
	3.16000	53.8	43.5	48.7	51.3 ± 5.1
	1.00000	69.0	83.1	76.1	23.9 ± 7.0
	0.31600	90.4	101.0	95.7	4.3 ± 5.3
	0.10000	91.9	89.2	90.5	9.5 ± 1.3
	0.03160	87.7	82.4	85.0	15.0 ± 2.7
	0.01000	85.0	91.9	88.5	11.5 ± 3.4
	0.00316	94.9	96.1	95.5	4.5 ± 0.6
	0.00100	112.1	94.5	103.3	−3.3 ± 8.8
	0.00032	100.3	91.5	95.9	4.1 ± 4.4

*Data excluded from further analysis, because they obviously represent measurement artifacts with an opposite value as can be expected by the corresponding dose-response-relationship.

Table S3. Individual data of the AR binding measurements for pumpkin seed oil.

	Pumpkin seed oil in mg/ml	% Specific radioligand binding			% Specific inhibition
		1. Value	2. Value	Mean	Mean ± SEM
1. Measurement	3.16000	20.9*	14.6*	/	/
	1.00000	2.9	13.3	8.1	91.9 ± 5.2
	0.31600	63.9	61.6	62.7	37.3 ± 1.2
	0.10000	78.7	66.4	72.6	27.4 ± 6.1
	0.03160	96.7	91.6	94.2	5.8 ± 2.5
	0.01000	97.5	87.2	92.4	7.6 ± 5.2
	0.00316	109.6	112.2	110.9	−10.9 ± 1.3
	0.00100	105.2	102.2	103.7	−3.7 ± 1.5
	0.00032	114.9	94.2	104.5	−4.5 ± 10.4
	0.00010	51.8*	54.8*	/	/
2. Measurement	10.00000	5.8	10.2	8.0	92.0 ± 2.2
	3.16000	22.2	38.7	30.4	69.6 ± 8.3
	1.00000	43.7	61.6	52.6	47.4 ± 9.0
	0.31600	88.5	88.5	88.5	11.5 ± 0.0
	0.10000	88.5	95.0	91.7	8.3 ± 3.3
	0.03160	92.2	89.3	90.7	9.3 ± 1.5
	0.01000	104.0	100.2	102.1	−2.1 ± 1.9
	0.00316	95.4	96.6	96.0	4.0 ± 0.6
	0.00100	107.0	102.0	104.5	−4.5 ± 2.5
	0.00032	103.6	97.4	100.5	−0.5 ± 3.1

*Data excluded from further analysis, because they obviously represent measurement artifacts with an opposite value as can be expected by the corresponding dose-response-relationship.

Table S4. Individual data of the AR binding measurements for Δ7-sterols.

	Δ7-sterols in mg/ml	% Specific radioligand binding			% Specific inhibition
		1. Value	2. Value	Mean	Mean ± SEM
1. Measurement	1.00000	17.2	24.6	20.9	79.1 ± 3.7
	0.31600	54.1	51.6	52.9	47.1 ± 1.3
	0.10000	69.3	58.3	63.8	36.2 ± 5.5
	0.03160	87.3	90.2	88.7	11.3 ± 1.5
	0.01000	95.3	80.3	87.8	12.2 ± 7.5
	0.00316	83.7	82.4	83.0	17.0 ± 0.6
	0.00100	97.8	96.8	97.3	2.7 ± 0.5
	0.00032	99.7	97.8	98.8	1.2 ± 1.0
	0.00010	91.3	95.3	93.3	6.7 ± 2.0
	0.00003	106.3	97.2	101.7	−1.7 ± 4.5
2. Measurement	1.00000	26.9	25.4	26.2	73.8 ± 0.7
	0.31600	35.5	45.2	40.3	59.7 ± 4.8
	0.10000	58.9	54.5	56.7	43.3 ± 2.2
	0.03160	83.1	87.6	85.4	14.6 ± 2.2
	0.01000	98.0	83.9	91.0	9.0 ± 7.1
	0.00316	96.2	86.1	91.1	8.9 ± 5.0
	0.00100	93.6	89.8	91.7	8.3 ± 1.9
	0.00032	99.5	101.4	100.4	−0.4 ± 0.9
	0.00010	115.1	109.9	112.5	−12.5 ± 2.6
	0.00003	86.9	111.8	99.3	0.7 ± 12.5