Pumpkin Seed and Hormonal Imbalance

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Directory

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  - Urinary Incontinence and
  - Benign Prostate Hyperplasia (BPH)
• Pumpkin Seed: Selection of Compounds
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• Technical Background, Quality and Safety
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Urinary Incontinence: **General Data**

- **definition:** inability to control urination (approx. 5 % of population affected)
- **psychological:** shame, depression, hygiene, isolation
- **patients:** men and women
- **therapy:**
  - medical treatment, physiotherapy, diapers
  - treatment of psychological and social problems
Urinary Incontinence: **Classifications**

- **stress incontinence:** weak pelvic muscle or sphincter, occurrence in context with coughing, sneezing or lifting weight

- **pressure/urge incontinence:** irritable detrusor, high frequency of contraction

- **overflow incontinence:** constriction of the urethra in the prostate gland caused by BPH
Urinary Incontinence: Dependence on age

Fig.: Medizinzeitung, 5, 2000
Urinary Incontinence: **Pathophysiology**

Stress incontinence and overflow incontinence are closely linked to hormonal deficits and shifts in hormonal balances:

**stress incontinence:**
in postmenopausal women:
- estrogen deficit: atrophy of urethra
- insufficient androgen effect: weak pelvic floor

**overflow incontinence:**
benign prostate hyperplasia in men:
- testosterone deficit
  \[ \text{5α-reductase} \]

→ aromatase
Urinary Incontinence: **Pathophysiology**

**Overflow incontinence**

- **functional**
  - weakness of bladder muscle
- **obstructive**
  - narrowing of the urethra caused by prostate growth
- **overstretching of bladder wall**
- **benign prostate hyperplasia (BPH)**
  - caused by prostate growth

*both sexes affected*

- metabolic disturbance, side effects of remedies, parkinson etc.

*men at advanced age*

- cause not clear yet, hypothesis: endocrine changes or disturbances
Urinary Incontinence: **Pathophysiology**

**Stress incontinence**

- weakening of the sphincter muscle caused by the relaxation of the pelvic floor muscles

*affected: mostly women*

- further cause in postmenopausal women: atrophy in urethral epithelium caused by hormonal changes

→

menopause influences control over function of bladder
Benign Prostate Hyperplasia: General Data

- **definition:** non malignant enlargement of fibromuscular and epithelial structures within the gland (approx. 60 % of men older 50 y)

- **symptoms:** urinary symptoms as: hesitancy, incomplete voiding of the bladder, terminal dribbling, urgency, frequency and nocturia

- **cause:** not clear

- **hypothesis:** hormonal imbalance
Benign Prostate Hyperplasia: **Classifications**

- **stage I:** obstructive and irritant symptoms

- **stage II:** begin of decompensation of the voiding mechanism, residual urine of 100-150 ml, pollakisuria (frequency↑)

- **stage III:** decompensation of the bladder: chronic and complete retention of urine or overflow incontinence, reduction of renal function, uremia.
Benign Prostate Hyperplasia: Pathophysiology

(Fig. from: Schunack, W.: BPH-Die Leiden des älteren Mannes. DAZ 1998; 138 (46): 62-63)
Benign Prostate Hyperplasia: Pathophysiology

Testosterone

5α - Reductase

Dihydro testosterone (DHT)

3α - Reductase

Androstandion

Aromatase

17β - Estradiol (E₂)
Benign Prostate Hyperplasia: Pathophysiology

Blood → Cytoplasm → Nucleus

Testosterone → 5α-Reductase → DHT

Cell membrane

Cytoplasmatic receptor

Chromatin

mRNA → Protein

Nuclear membrane

SHBG
Pumpkin Seed: Indications

According to the Commission E recommendations:

• Irritable bladder condition

• Micturition problems caused by benign prostate hyperplasia (BPH) stage I and II (relief of the symptoms, no reduction of the prostate growth)

Recommended average daily dosage:
10 g of seed or equivalent preparations
Pumpkin Seed: **Selection of Compounds**

**lipid fraction**

nutritional value as food less suited as medicinal drug

- linoleic acid 50%
- oleic acid 35%
- palmitic acid 10%
- stearic acid 5%
- delta-7-sterols 0.2 - 0.4%
- tocopherol

**fat free, polar part of pumpkin seeds:**

- proteins
- phenols (lignans) belonging to the family of **phytoestrogens**

  - secoisolariciresinol
  - enterodiol / enterolactone
Pumpkin Seed: Selection of Compounds

Lipid fraction

potential effective substance:

Spinasterol; a $\Delta^7$-sterol from *C. pepo* L.

*but:* - sterols are not absorbed in the intestines
- efficient doses are unrealistic high

⇒ no rational therapy with phytosterols
Pumpkin Seed: **Selection of Compounds**

**Polar Fraction**

potential effective substance:

Phytoestrogens (e.g. Lignans)

![Chemical structures of Secoisolariciresinol SECO and Enterodiol]

Enterodiol found in:
- urine
- feces
- prostate
- bile
- plasma
- saliva
- breast milk


*promising rational therapy*
Pumpkin Seed: **Mode of Action**

the **polar** fraction reveals the following effects:

- inhibition of peripheral and placental aromatase
- inhibition of $5\alpha$-reductase type II
- Phytoestrogens bind to estrogen receptors
- Phytoestrogens interfere with binding of testosterone, $5\alpha$-dihydrotestosterone and estradiol to SHBG

Pumpkin Seed: Active Fraction

Inhibition of 5α-reductase

Butanol fraction
Water fraction
Petrolether fraction
UVB fraction
Residue

IC$_{50}$ 1.2 mg/ml
IC$_{50}$ 24 mg/ml
IC$_{50}$ 0.85 mg/ml
Saponification
IC$_{50}$ 0.5 mg/ml

IC$_{50}$ 6 mg/ml
IC$_{50}$ 2.5 mg/ml
Pumpkin Seed: Active Fraction

Inhibition of aromatase

- Butanol fraction: 10 mg/ml 51%
- Water fraction: 10 mg/ml 46%
- Petroleum fraction: 10 mg/ml 45%
- Residue: n.d.

Saponification

- 10 mg/ml 12%
- Residue: n.d.
Pumpkin Seed: *in vitro* Inhibition of Aromatase

**Experimental design:**

- standard test to determine directly the ability of different extract fractions to inhibit aromatase from human placenta homogenate
- incubation of the enzyme with $^3$H-testosterone
- determination of radioactive $^3$H$_2$O generated from testosterone by aromatase
Pumpkin Seed: in vitro Inhibition of Aromatase

Results in vitro:

• aromatase inhibition tested in 3 series.

• inhibition with 10 mg/ml amounts to
  46.8 +/- 5.0 %
  65.4 +/- 21.4 %
  43.0 +/- 4.2 %

• IC₅₀ about 10 mg/ml
Pumpkin Seed: *in vitro* Inhibition of Aromatase

**Discussion**

- lignans assumed responsible for the inhibition of aromatase are present but bound to glycosides
- *in vitro* with 10 mg/ml extract remarkable inhibition of the aromatase achievable.
Pumpkin Seed: *in vivo* Phytoestrogenic Effect

Phytoestrogenic effect

**Assessment with the aid of the induction of the growth of rat uterus**

- application once daily in juvenile female Sprague Dawley rats (5 mg, 10 mg and 20 mg/kg)
- treatment during 4 or 8 days
- determination of the uterus weight
**Pumpkin Seed: in vivo Phytoestrogenic Effect**

**Result: experiment I**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Uterine wet weight (mg/100 g b.w.) Mean ± SEM</th>
<th>Uterine dry weight (mg/100 g b.w.) Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vehicle treated control (CMC)</strong></td>
<td>78.2 ± 8.9</td>
<td>13.6 ± 1.2</td>
</tr>
<tr>
<td><strong>85940 (mg/kg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>68.8 ± 3.2</td>
<td>11.7 ± 0.6</td>
</tr>
<tr>
<td>10</td>
<td>72.3 ± 9.6</td>
<td>12.5 ± 1.5</td>
</tr>
<tr>
<td>20</td>
<td>70.6 ± 2.8</td>
<td>12.9 ± 0.6</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Uterine wet weight (mg/100 g b.w.) Mean ± SEM</th>
<th>Uterine dry weight (mg/100 g b.w.) Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Vehicle treated control (CMC)</strong></td>
<td>108.8 ± 22.0</td>
<td>17.5 ± 3.1</td>
</tr>
<tr>
<td><strong>85940 (mg/kg)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>128.5 ± 32.1</td>
<td>18.5 ± 4.0</td>
</tr>
<tr>
<td>10</td>
<td>129.5 ± 34.9</td>
<td>21.1 ± 4.9</td>
</tr>
<tr>
<td>20</td>
<td>182.6 ± 65.7</td>
<td>23.6 ± 6.4</td>
</tr>
</tbody>
</table>
Pumpkin Seed: in vivo Phytoestrogenic Effect

Result: experiment I

- 4 days of treatment reveals no effect
- 8 days of treatment shows an increase of the dry weight of uterus of 34.9%

<table>
<thead>
<tr>
<th></th>
<th>4 days</th>
<th>8 days</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>control</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>5 mg/kg</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>10 mg/kg</td>
<td>3</td>
</tr>
<tr>
<td>4</td>
<td>20 mg/kg</td>
<td>4</td>
</tr>
</tbody>
</table>

Dry uterus weight mg/100 g b.w.
Pumpkin Seed: in vivo Phytoestrogenic Effect

Result: experiment II

8 days treatment relative values

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Uterine wet weight (mg/100 g b.w.) Mean ± SEM</th>
<th>Uterine dry weight (mg/100 g b.w.) Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle treated control (CMC 0.5%)</td>
<td>61.0 ± 4.4</td>
<td>11.4 ± 0.6</td>
</tr>
<tr>
<td>85940 (mg/kg) 5</td>
<td>58.0 ± 3.3</td>
<td>10.9 ± 0.5</td>
</tr>
<tr>
<td>85940 (mg/kg) 10</td>
<td>69.3 ± 6.5</td>
<td>14.4 ± 1.3 *</td>
</tr>
<tr>
<td>85940 (mg/kg) 20</td>
<td>56.3 ± 1.7</td>
<td>11.9 ± 0.5</td>
</tr>
</tbody>
</table>

8 days treatment absolute values

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Uterine wet weight (mg) Mean ± SEM</th>
<th>Uterine dry weight (mg) Mean ± SEM</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle treated control (CMC 0.5%)</td>
<td>42.4 ± 3.3</td>
<td>8.0 ± 0.5</td>
</tr>
<tr>
<td>85940 (mg/kg) 5</td>
<td>39.2 ± 2.6</td>
<td>7.3 ± 0.4</td>
</tr>
<tr>
<td>85940 (mg/kg) 10</td>
<td>42.5 ± 4.4</td>
<td>8.7 ± 0.8</td>
</tr>
<tr>
<td>85940 (mg/kg) 20</td>
<td>37.3 ± 1.3</td>
<td>7.8 ± 0.3</td>
</tr>
</tbody>
</table>
Pumpkin Seed: *in vivo* Phytoestrogenic Effect

**Discussion**

- experiment I shows dose dependent increase of the uterus weight
- no significance caused by strong deviation
- phytoestrogenic effect is possible
- no reproducibility yet (experiment II)
Pumpkin Seed: *in vitro* Inhibition of 5α-Reductase

**Experimental design:**

- standard test to determine directly the ability of different extract fractions to inhibit 5α-reductase type II in human prostate homogenate
- incubation of the enzyme with $^3$H-testosterone
- separation by HPLC and determination of radioactive testosterone and its metabolite dihydrotestosterone
- ratio of peak areas correlates quantitatively with the degree of enzyme inhibition
- different sample concentrations allow determination of IC$_{50}$ value
Pumpkin Seed: *in vitro* Inhibition of $5\alpha$-Reductase

**Results in vitro:**

- polar (lipid free) fraction of EFLA®940 inhibits the activity of $5\alpha$-Reductase type II ($IC_{50}$ 0.85 mg/ml)
Pumpkin Seed: in vivo Inhibition of 5α-Reductase

Experimental design:

• standard test to evaluate directly the potency of EFLA®940 / positive control (finasteride)

• investigation of the inhibitory influence of EFLA®940 on increase of prostate weight in vivo

• immature castrated male rats

• testosterone propionate for the stimulation of increase of prostate weight

• oral application of EFLA®940
Pumpkin Seed: *in vivo* Inhibition of $5\alpha$-Reductase

**Design:**

- All animals except in group I are castrated 3 days before start of study.
- All groups were treated during 4 consecutive days according to the following scheme:

<table>
<thead>
<tr>
<th>Groups</th>
<th>Castrated</th>
<th>Vehicle</th>
<th>Testosterone</th>
<th>Finasteride</th>
<th>EFLA® 940</th>
</tr>
</thead>
<tbody>
<tr>
<td>Vehicle-treated Control</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Castrated Vehicle-treated Control</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Testosterone prop., sc 1 mg/kg</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Finasteride, sc 1 mg/kg</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>Cucurbitae extr., po 100 mg/kg</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>-</td>
</tr>
</tbody>
</table>
Pumpkin Seed: *in vivo* Inhibition of 5α-Reductase

**Result**

<table>
<thead>
<tr>
<th>mg/100g body weight</th>
<th>Vehicle</th>
<th>Castrated</th>
<th>Testosterone</th>
<th>Finasteride</th>
<th>EFLA® 940</th>
</tr>
</thead>
<tbody>
<tr>
<td>70</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>60</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>50</td>
<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td></td>
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<td></td>
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<tr>
<td>30</td>
<td></td>
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<tr>
<td>20</td>
<td></td>
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<td></td>
</tr>
<tr>
<td>10</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0</td>
<td></td>
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<td></td>
</tr>
</tbody>
</table>

100% / P < 0.001  76% / P < 0.01  31% / P < 0.05
Pumpkin Seed: Inhibition of $5\alpha$-Reductase

Discussion

- Remarkable inhibition of $5\alpha$-reductase in vitro
- EFLA®940 is absorbed and intestinally metabolised
- EFLA®940 reaches its destination
- Significant reduction of the prostate weight increase
Urinary Incontinence: Open Clinical Trial

Aim and design of the study:

• reduction of number of nocturnal and diurnal micturitions and number of incontinence episodes

• study design:
  39 postmenopausal women / 6 weeks

• study medication:
  tablets containing 87.5 mg Cucurbitae seed extract EFLA®940 and 16.6 mg soybean germ extract (PEP)
Urinary Incontinence: Open Clinical Trial

Results: number of nocturnal micturitions

<table>
<thead>
<tr>
<th></th>
<th>pre (n=39)</th>
<th>week-1 (n=39)</th>
<th>week-2 (n=31)</th>
<th>week-4 (n=39)</th>
<th>week-6 (n=28)</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of micturitions</td>
<td>3.3</td>
<td>2.6</td>
<td>2.5</td>
<td>2.3</td>
<td>2.0</td>
</tr>
</tbody>
</table>
Urinary Incontinence: Open Clinical Trial

Results: number of diurnal micturitions

- pre (n=39) 8.0
- week-1 (n=39) 7.0
- week-2 (n=39) 6.8
- week-4 (n=39) 6.5
- week-6 (n=36) 6.7
Urinary Incontinence: Open Clinical Trial

Results: number incontinent episodes

<table>
<thead>
<tr>
<th></th>
<th>pre</th>
<th>week-1</th>
<th>week-2</th>
<th>week-4</th>
<th>week-6</th>
</tr>
</thead>
<tbody>
<tr>
<td>number of</td>
<td>7.3</td>
<td>5.5</td>
<td>4.1</td>
<td>2.2</td>
<td>1.5</td>
</tr>
<tr>
<td>incontinent</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>episodes</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>
Urinary Incontinence: **Open Clinical Trial**

**Results:** subjective improvement

![Graph showing subjective improvement percentages over time](graph.png)

<table>
<thead>
<tr>
<th>Time Period</th>
<th>Fulfilled</th>
<th>Incompletely Fulfilled</th>
<th>Not Fulfilled</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pre (n=38)</td>
<td>15.8%</td>
<td>52.6%</td>
<td>31.6%</td>
</tr>
<tr>
<td>Week-1 (n=36)</td>
<td>36.1%</td>
<td>52.8%</td>
<td>11.1%</td>
</tr>
<tr>
<td>Week-2 (n=28)</td>
<td>42.9%</td>
<td>50.0%</td>
<td>7.1%</td>
</tr>
<tr>
<td>Week-4 (n=38)</td>
<td>55.3%</td>
<td>42.1%</td>
<td>2.6%</td>
</tr>
<tr>
<td>Week-6 (n=25)</td>
<td>60.0%</td>
<td>32.0%</td>
<td>8.0%</td>
</tr>
</tbody>
</table>
Urinary Incontinence: Open Clinical Trial

Results: total ratio of improvement
subgroup of 33 individuals with 2 to 4 micturitions per night
Urinary Incontinence: Open Clinical Trial

Discussion:

• PEP reduces the number of incontinent episodes and the number of nocturnal and diurnal micturitions

• The effect increases with the progress of the treatment => hint at a causal mechanism

• EFLA®940 may possess estrogen-like effects
Pumpkin Seed: **Technical Background**

**Comparison:** extract with lipophilic phase versus EFLA®940

GC-diagrams: above: extract with lipophilic phase, below: EFLA®940
Pumpkin Seed: **Technical Background**

**Specific manufacturing procedure**

- effective substances in hydrophilic phase
  (for the inhibition of aromatase as well as for the inhibition of $5\alpha$-reductase)

- patented method for the elimination of lipophilic substances

- no rancidity in EFLA®940
Pumpkin Seed: **Safety of EFLA®940**

**experimental design**

- oral application of 5 mg, 10 mg, 20 mg, 30 mg, 100 mg and 300 mg/kg body weight of EFLA®940 a day in juvenile female SD rats

- oral application of 100 mg/kg body weight of EFLA®940 a day in immature male SD rats

- treatment for 4 consecutive days

- assessment of the mortality
Pumpkin Seed: Safety of EFLA®940

results and conclusion

• no rat died during the experiment

• EFLA®940 is well tolerated in the experiment

• Commission E recommends 500 mg of extract a day for humans. For a man weighing 70 kg this recommendation corresponds to about 7 mg/kg body weight a day.
Pumpkin Seed: Quality of EFLA®940

- raw material tested in conformity with official monographs
- standardized and validated manufacturing procedure
- transparency of patented manufacturing procedures
- quality controls during manufacture (in process controls)
Pumpkin Seed: Quality of EFLA®940

- extract high quality standard controls
- extract long-term stability
- detailed and comprehensive extract documentation (drug master file)
Pumpkin Seed: Final Summary of Effects

- Active phase of pumpkin seed extract EFLA®940 is the hydrophilic phase
- Inhibition of aromatase in vitro
- Possible phytoestrogenic effect
- Inhibition of $5\alpha$-reductase in vitro and in vivo, significant reduction of prostate growth in rat
- Decrease of nocturnal and diurnal micturitions
- Significant decrease of incontinent episodes