Pumpkin Seed and Hormonal Imbalance

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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

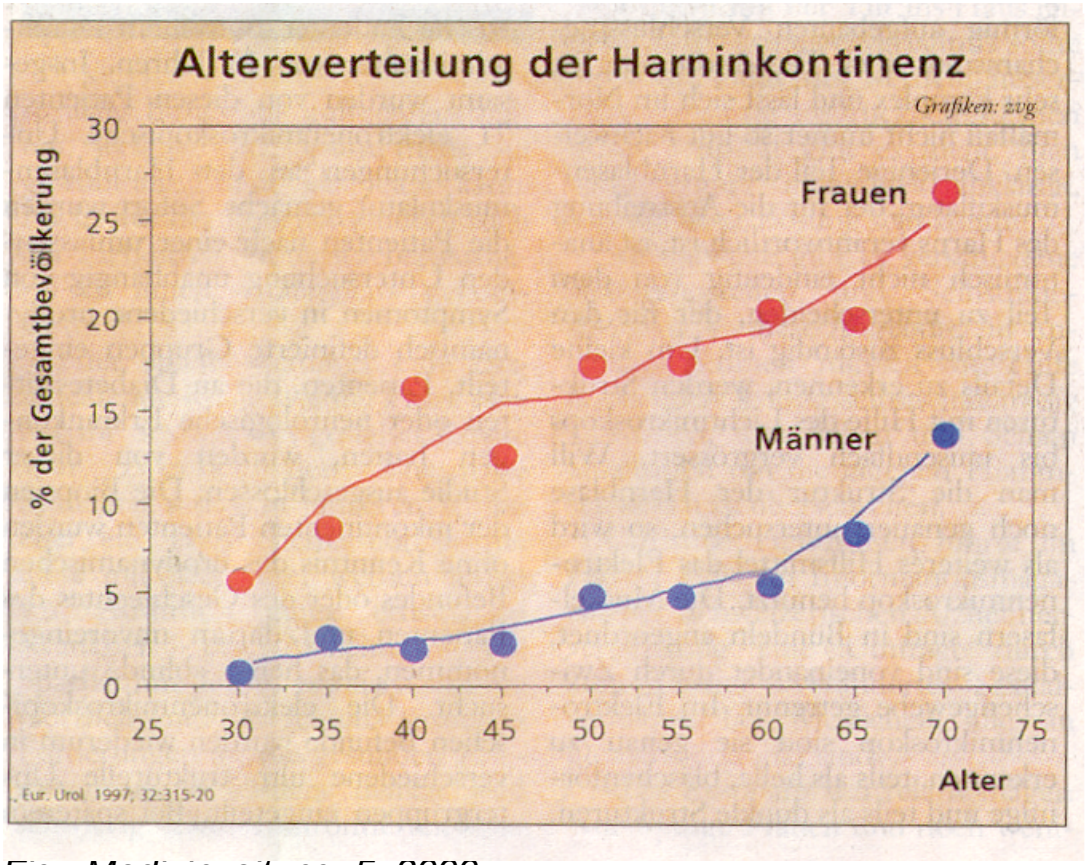

Fig.: Medizinzeitung, 5, 2000

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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

→ **aromatase**

overflow incontinence:

benign prostate hyperplasia in men:

- testosterone deficit

→ **5α-reductase**

Urinary Incontinence: Pathophysiology

Overflow incontinence

functional	obstructive
weakness of bladder muscle	narrowing of the urethra caused by prostate growth
overstretching of bladder wall	**benign prostate hyperplasia (BPH)**
both sexes affected	***men at advanced age***
metabolic disturbance, side effects of remedies, parkinson etc.	cause not clear yet, hypothesis: endocrine changes or disturbances

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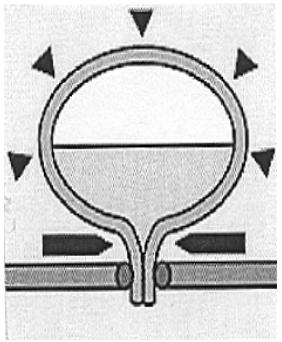
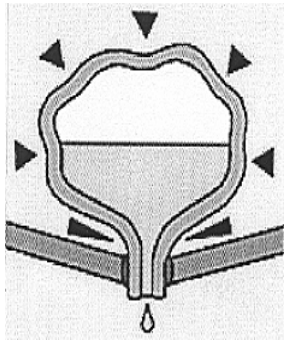
Urinary Incontinence: Pathophysiology

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

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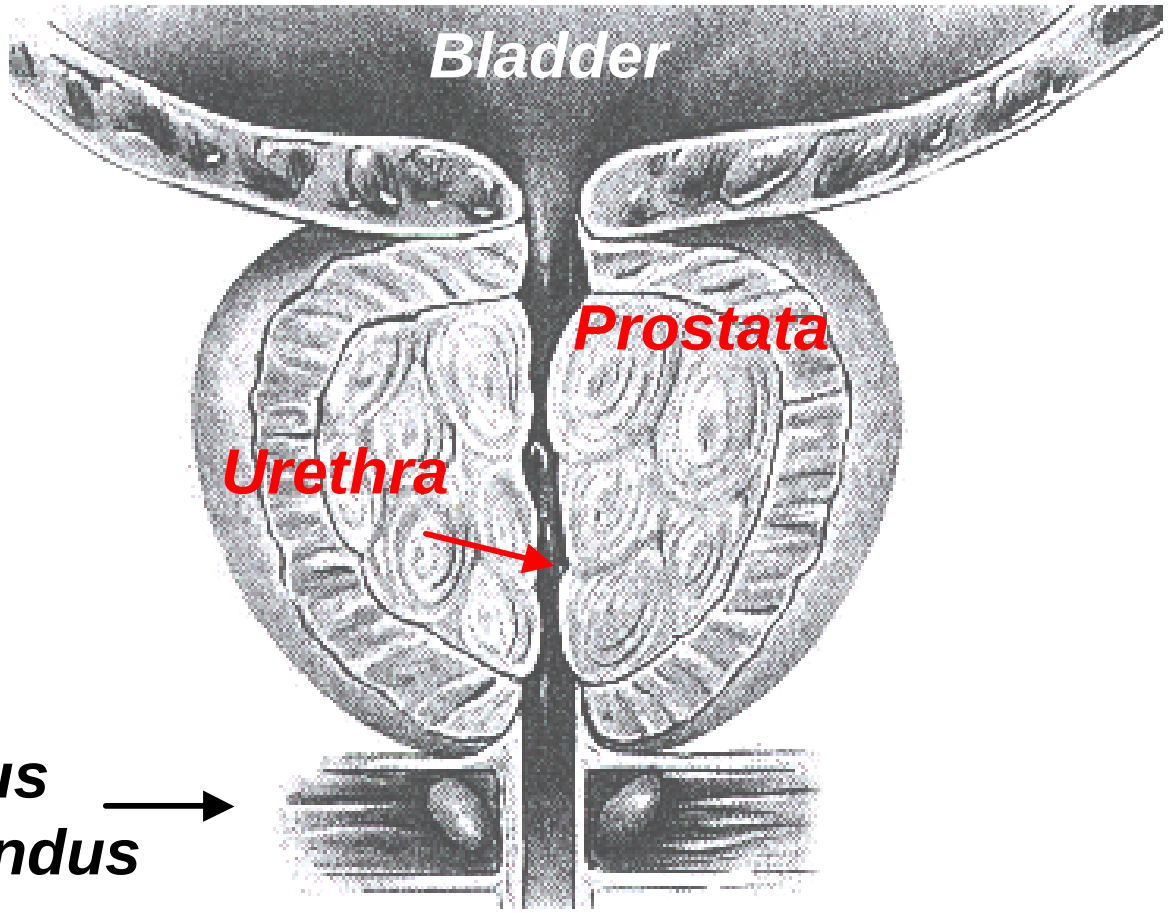
Benign Prostate Hyperplasia: General Data

- 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



M. transversus perinei profundus →

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

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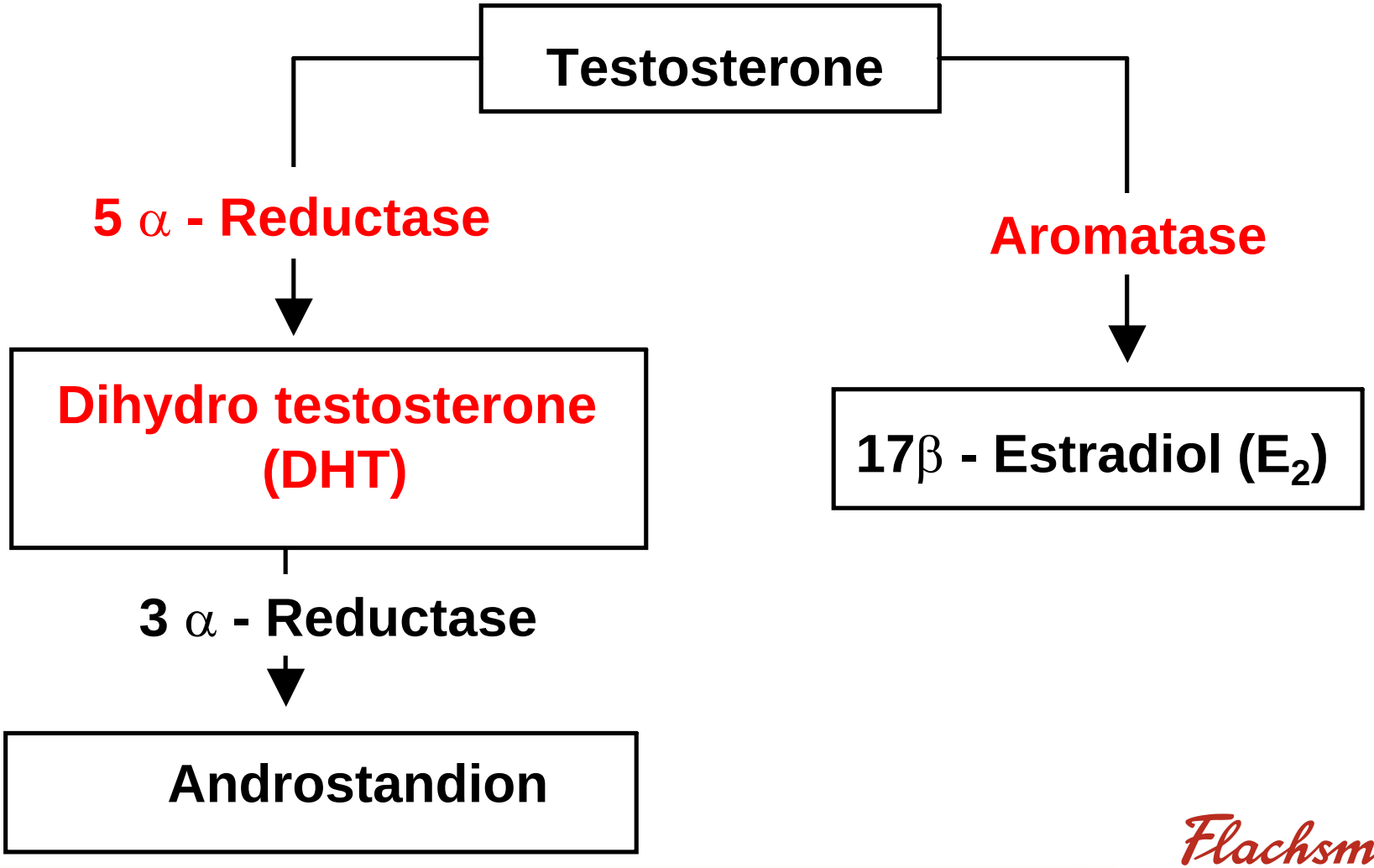
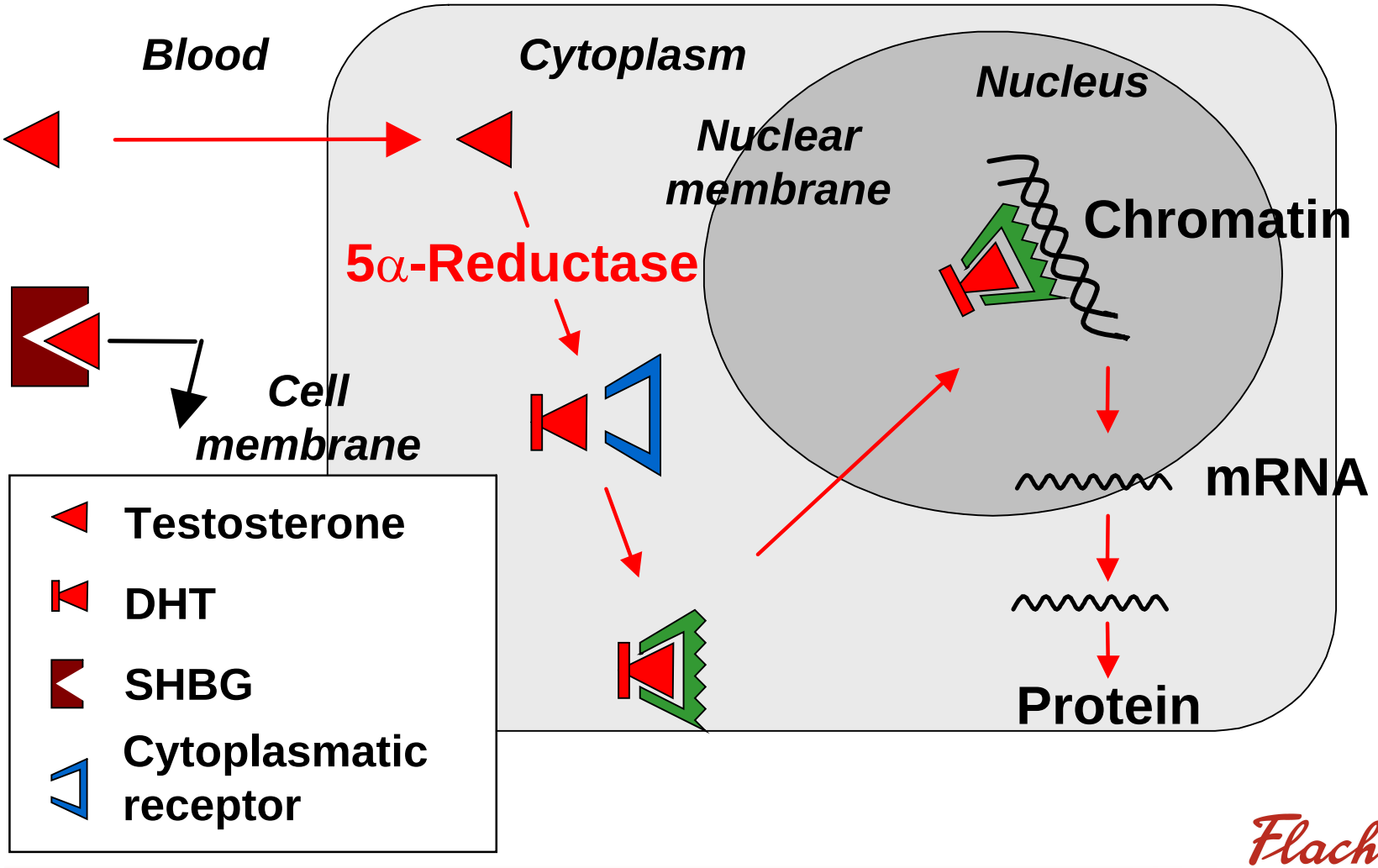
Benign Prostate Hyperplasia: Pathophysiology



Benign Prostate Hyperplasia: Pathophysiology



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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

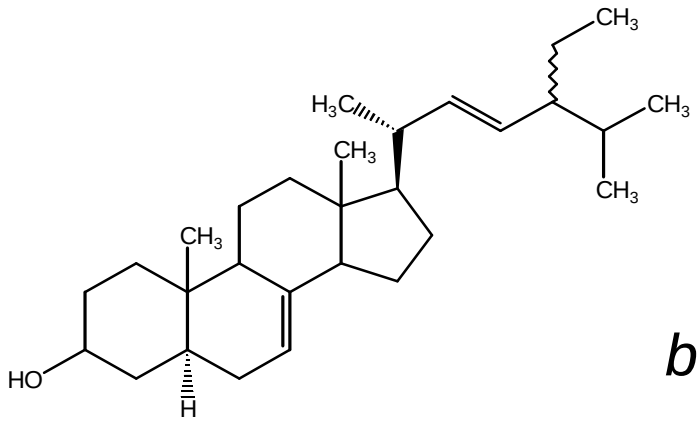
↓

enterodiol / enterolactone

Pumpkin Seed: Selection of Compounds

Lipid fraction

potential effective substance:



Spinasterol;
a Δ^7 -sterol from *C. pepo L.*

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

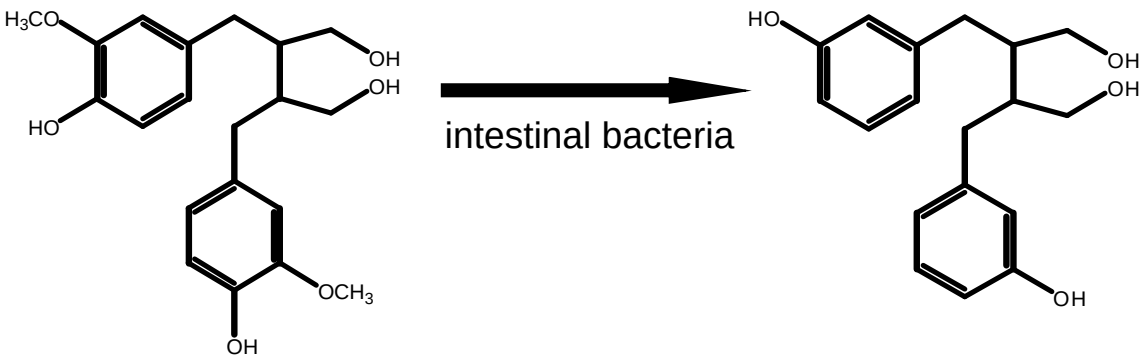
→ no rational therapy with phytosterols

Pumpkin Seed: Selection of Compounds

Polar Fraction

potential effective substance:

Phytoestrogens (e.g. Lignans)



Secoisolariciresinol
SECO

Enterodiol

Enterodiol found in:

- urine
- feces
- prostate
- bile
- plasma
- saliva
- breast milk

Murkies A, (1998) *Aust Fam Physician*, 27 (Suppl. 1), 47-51

→ ***promising rational therapy***

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Pumpkin Seed: Mode of Action

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

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

Schottner M, et al. (1998) *J Nat Prod.* 61(1): 119-21

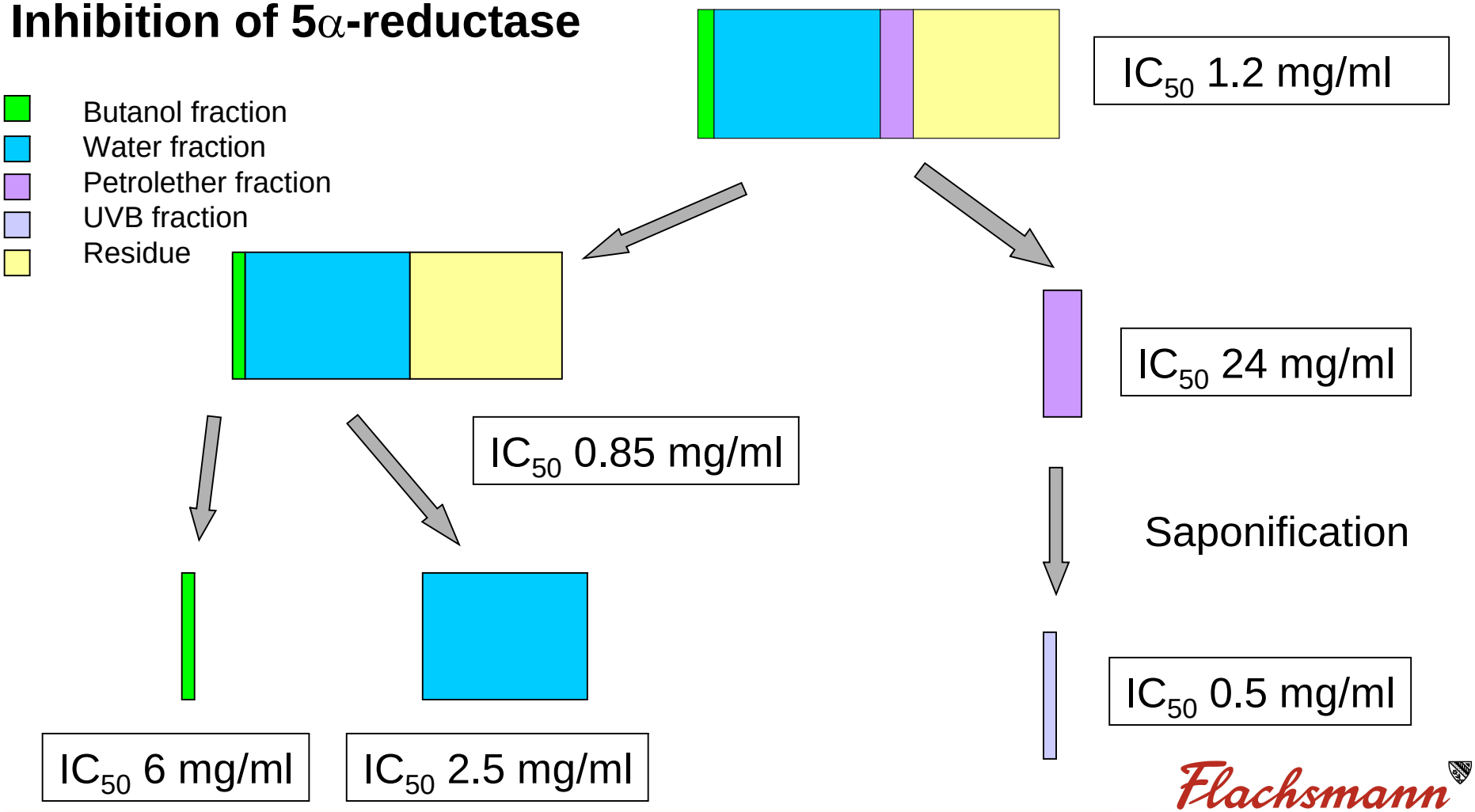
Schottner M, et al. (1997) *Z Naturforsch* [C] 52(11-12): 834-43.

Schottner M, et al. (1997) *Planta Med.* 63(6): 529-32.

Martin ME, et al. (1996) *Life Sci.* 58(5): 429-36.

Pumpkin Seed: Active Fraction

Inhibition of 5α-reductase



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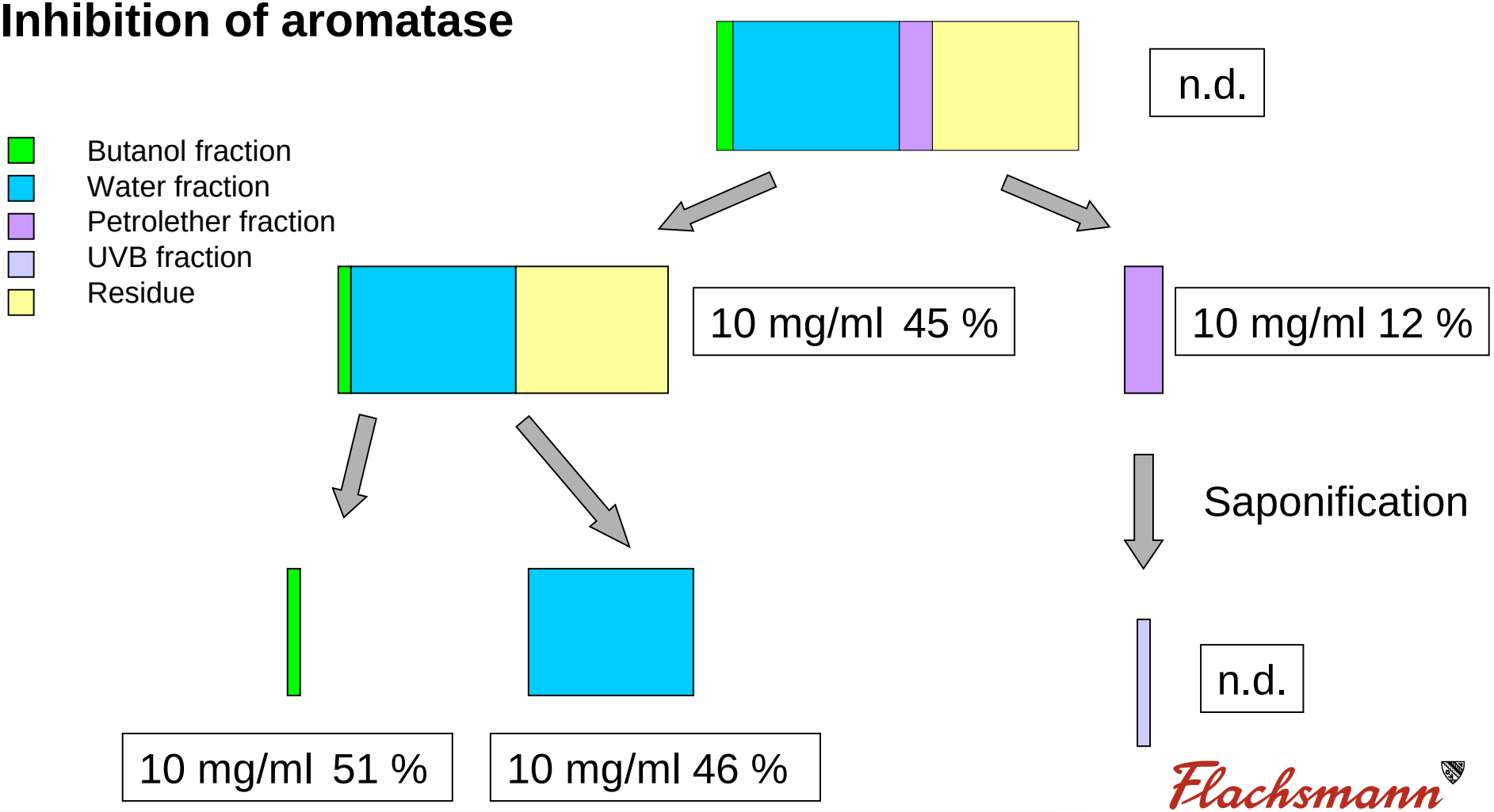
Pumpkin Seed: Active Fraction

Inhibition of aromatase



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Pumpkin Seed: in vitro Inhibition of Aromatase

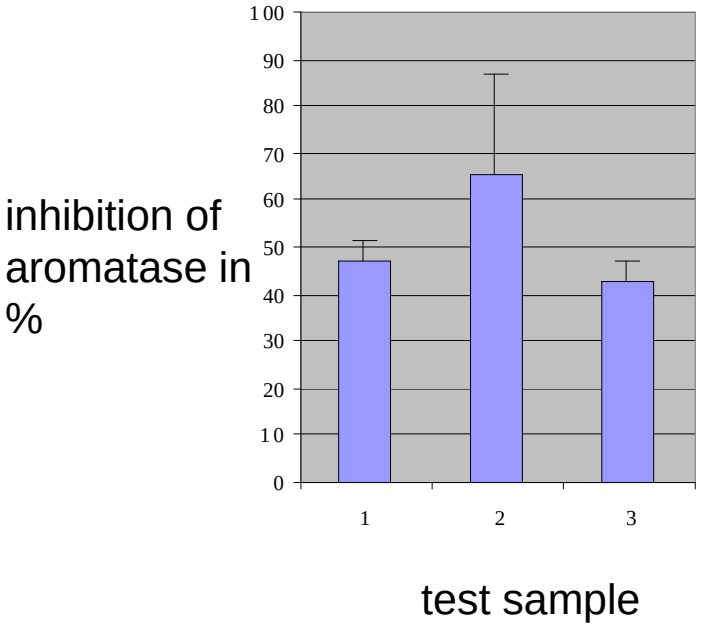
Experimental design:

- 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 $^{3}H_2O$ 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 %
- IC_{50} about 10 mg/ml



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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

4 days application

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

8 days application

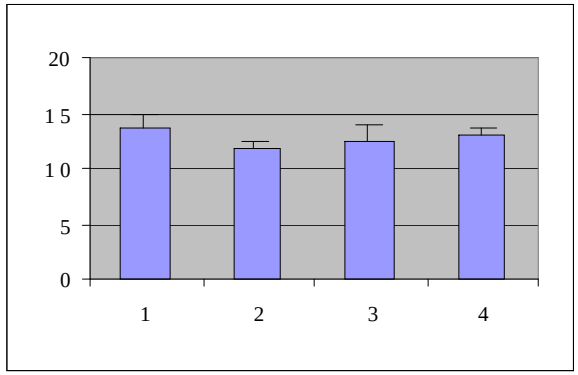
Treatment		Uterine wet weight (mg/100 g b.w.) Mean ± SEM	Uterine dry weight (mg/100 g b.w.) Mean ± SEM
Vehicle treated control (CMC)		108.8 ± 22.0	17.5 ± 3.1
85940 (mg/kg)	5	128.5 ± 32.1	18.5 ± 4.0
	10	129.5 ± 34.9	21.1 ± 4.9
	20	182.6 ± 65.7	23.6 ± 6.4

Pumpkin Seed: in vivo Phytoestrogenic Effect

Result: experiment I

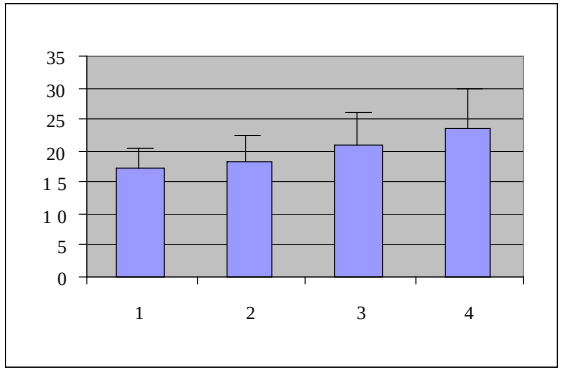
- 4 days of treatment reveals no effect
- 8 days of treatment shows an increase of the dry weight of uterus of 34.9 %

4 days



1: control
2: 5 mg/kg
3: 10 mg/kg
4: 20 mg/kg

8 days



dry uterus weight mg/100 g b.w.

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Pumpkin Seed: in vivo Phytoestrogenic Effect

Result: experiment II

8 days treatment relative values

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

8 days treatment absolute values

Treatment		Uterine wet weight (mg) Mean ± SEM	Uterine dry weight (mg) Mean ± SEM
Vehicle treated control (CMC 0.5%)		42.4 ± 3.3	8.0 ± 0.5
85940 (mg/kg)	5	39.2 ± 2.6	7.3 ± 0.4
	10	42.5 ± 4.4	8.7 ± 0.8
	20	37.3 ± 1.3	7.8 ± 0.3

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 5α-Reductase

Results in vitro:

- polar (lipid free) fraction of EFLA®940 inhibits the activity of 5α-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 5α-Reductase

Design:

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

Groups	Treatment				
	Castrated	Vehicle	Testosterone	Finasteride	EFLA® 940
Vehicle-treated Control	-	+	-	-	-
Castrated Vehicle-treated Control	+	+	-	-	-
Testosterone prop., sc 1 mg/kg	+	+	+	-	-
Finasteride, sc 1 mg/kg	+	+	+	+	-
Cucurbitae extr., po 100 mg/kg	+	+	+	-	+

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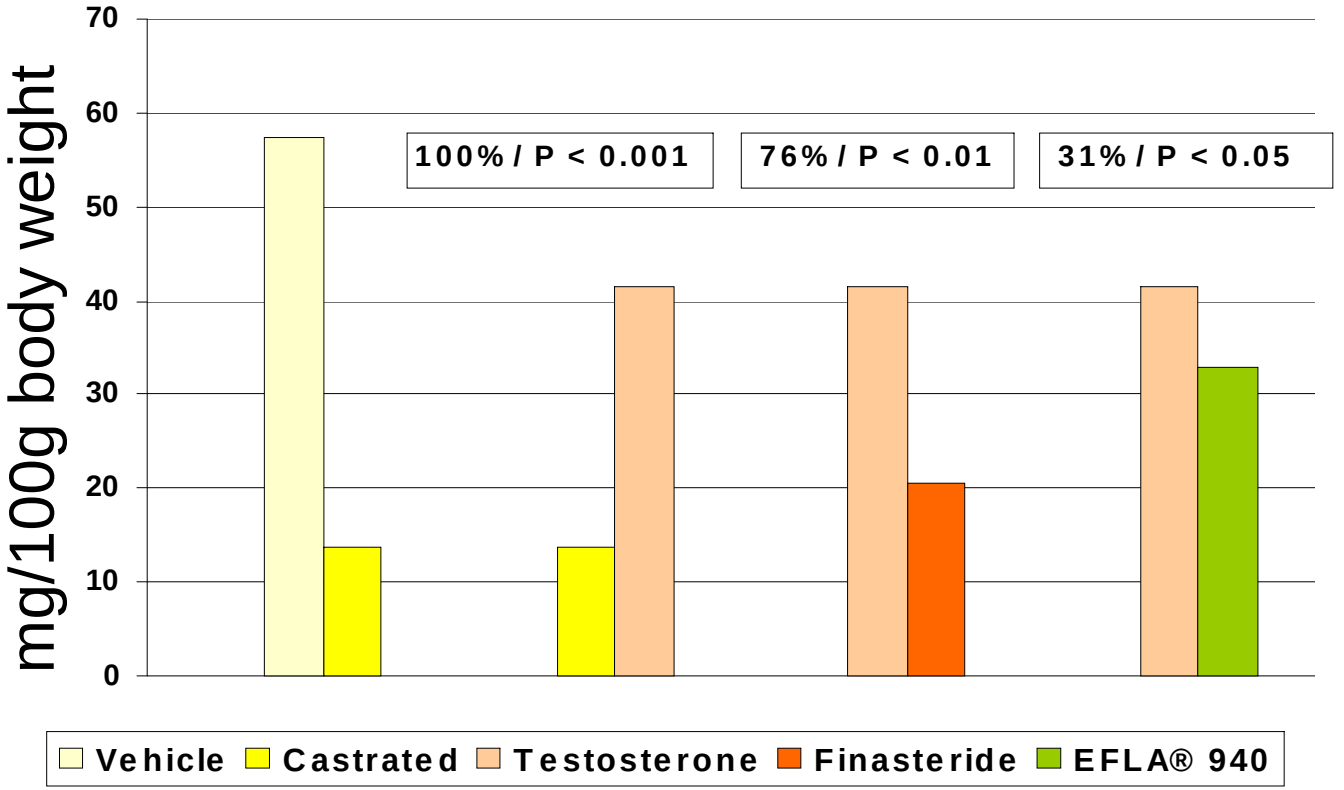
Pumpkin Seed: in vivo Inhibition of 5α-Reductase

Result



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Pumpkin Seed: Inhibition of 5α-Reductase

Discussion

- Remarkable inhibition of 5α-reductase in vitro
- EFLA®940 is absorbed and intestinally metabolised
- EFLA®940 reaches its destination
- Significant reduction of the prostate weight increase

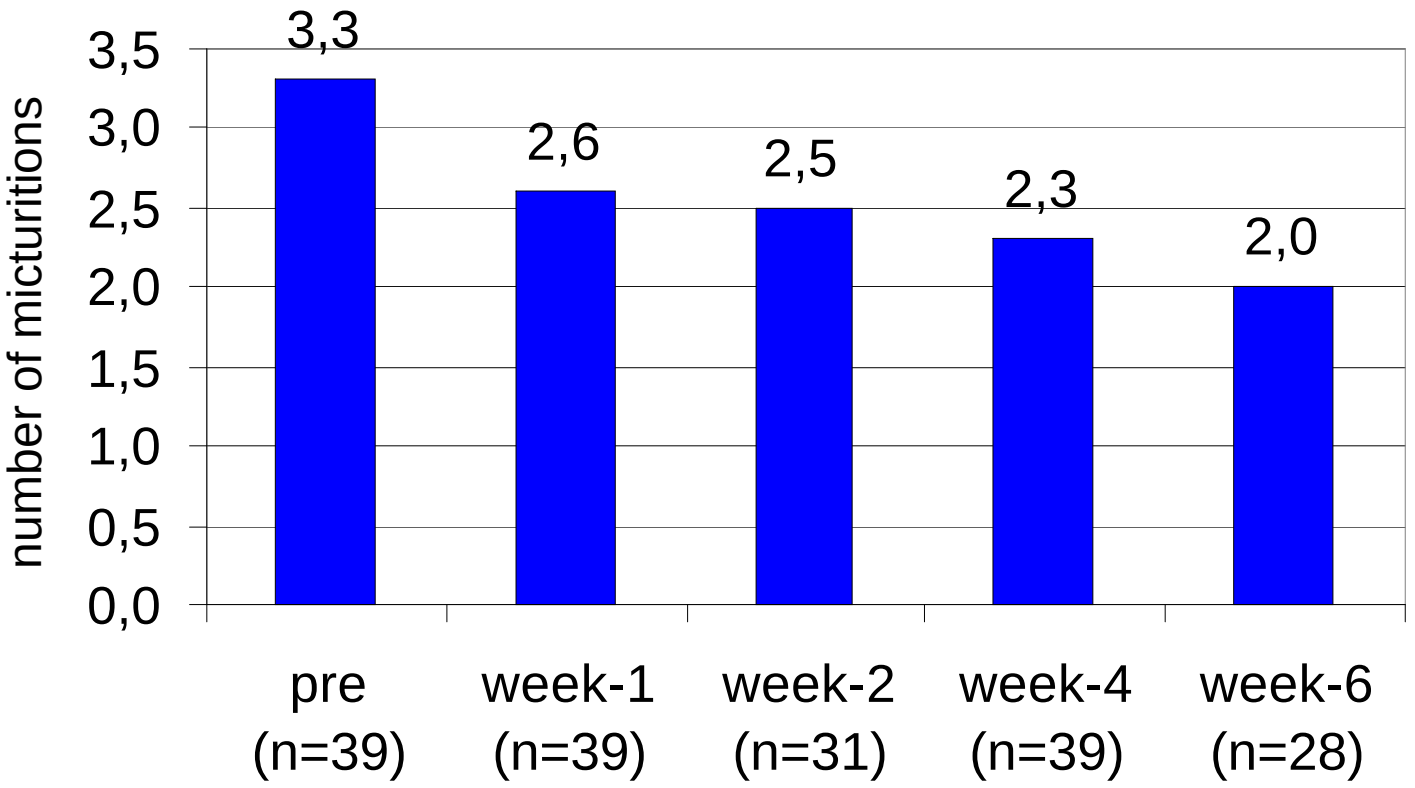
Urinary Incontinence: Open Clinical Trial

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)

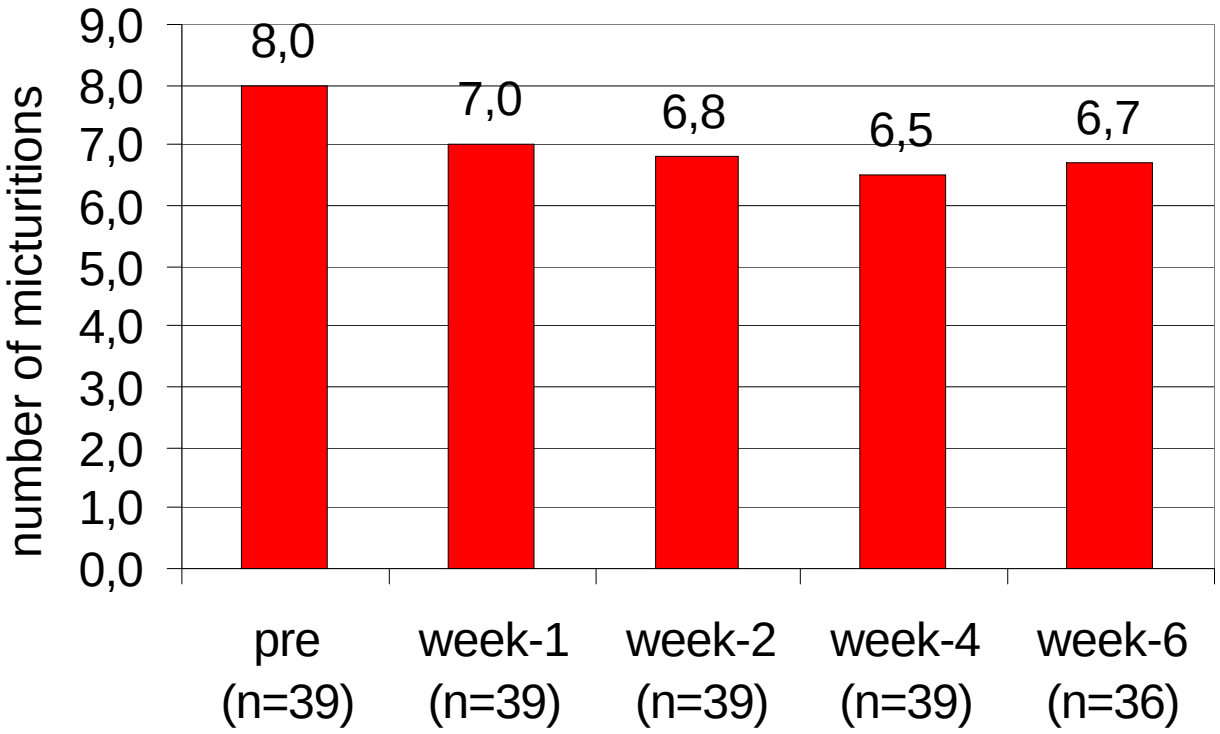
Urinary Incontinence: Open Clinical Trial

Results: number of nocturnal micturitions



Urinary Incontinence: Open Clinical Trial

Results: number of diurnal micturitions



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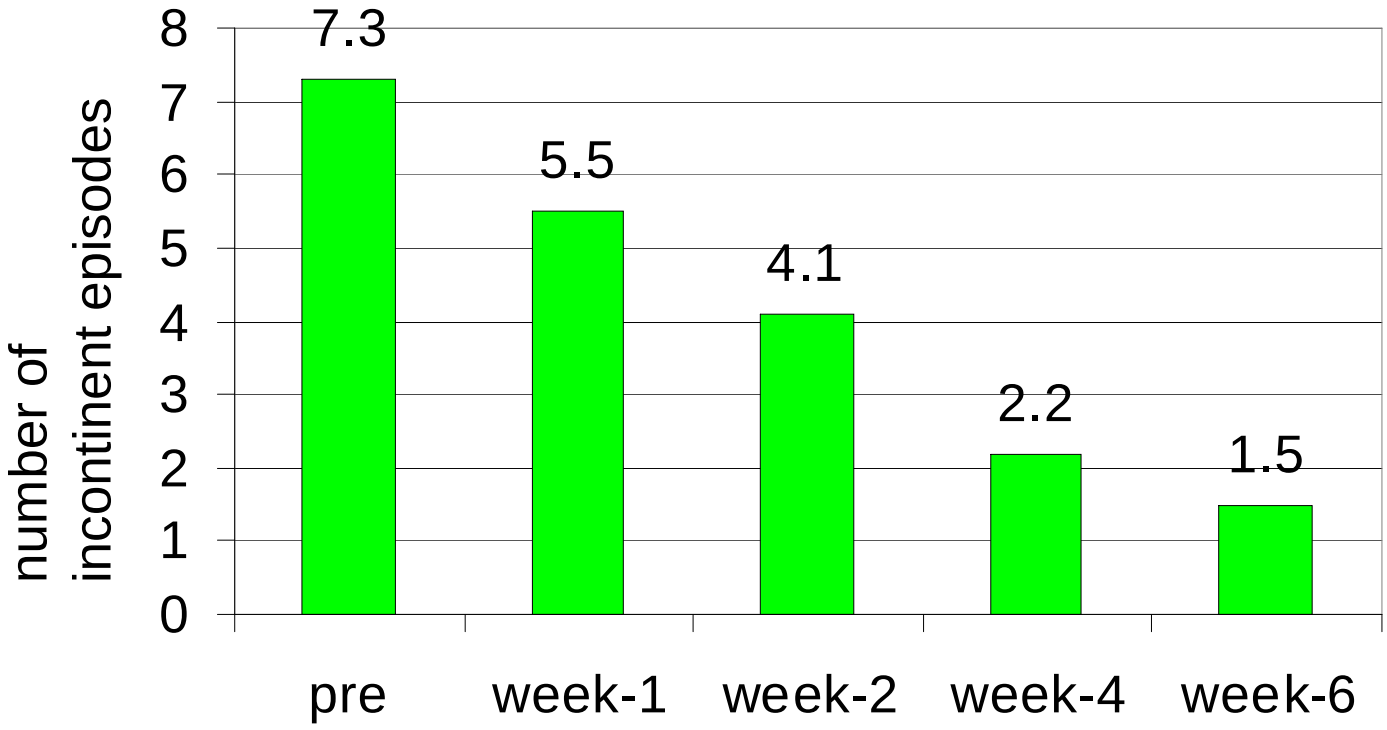
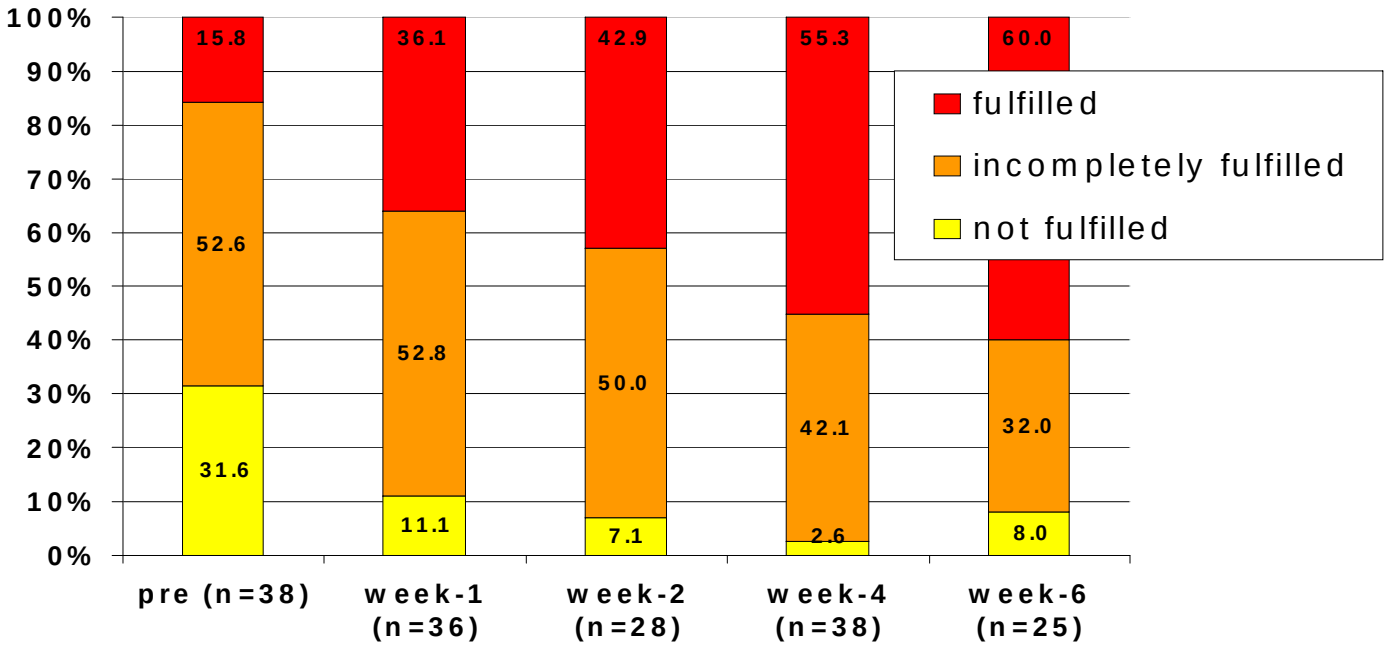
Urinary Incontinence: Open Clinical Trial

Results: **number incontinent episodes**



Urinary Incontinence: Open Clinical Trial

Results: subjective improvement



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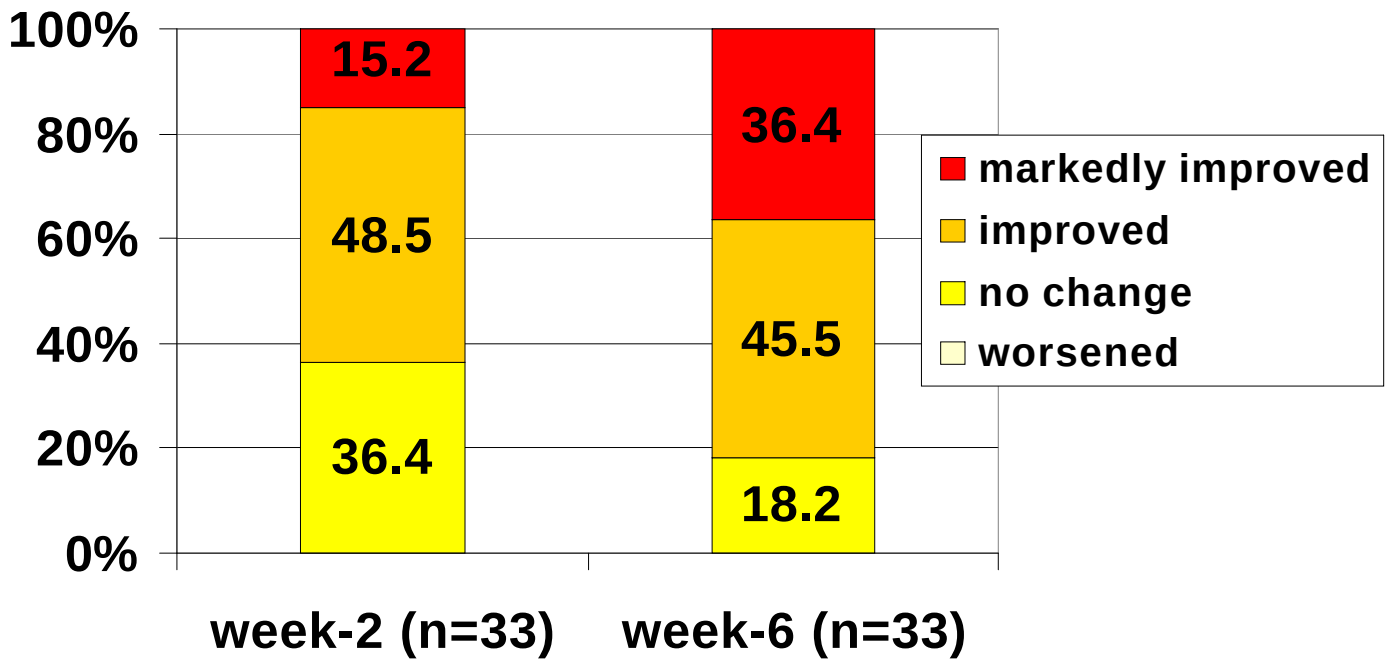
Urinary Incontinence: Open Clinical Trial

Results: **total ratio of improvement**

subgroup of 33 individuals with 2 to 4 micturitions per night



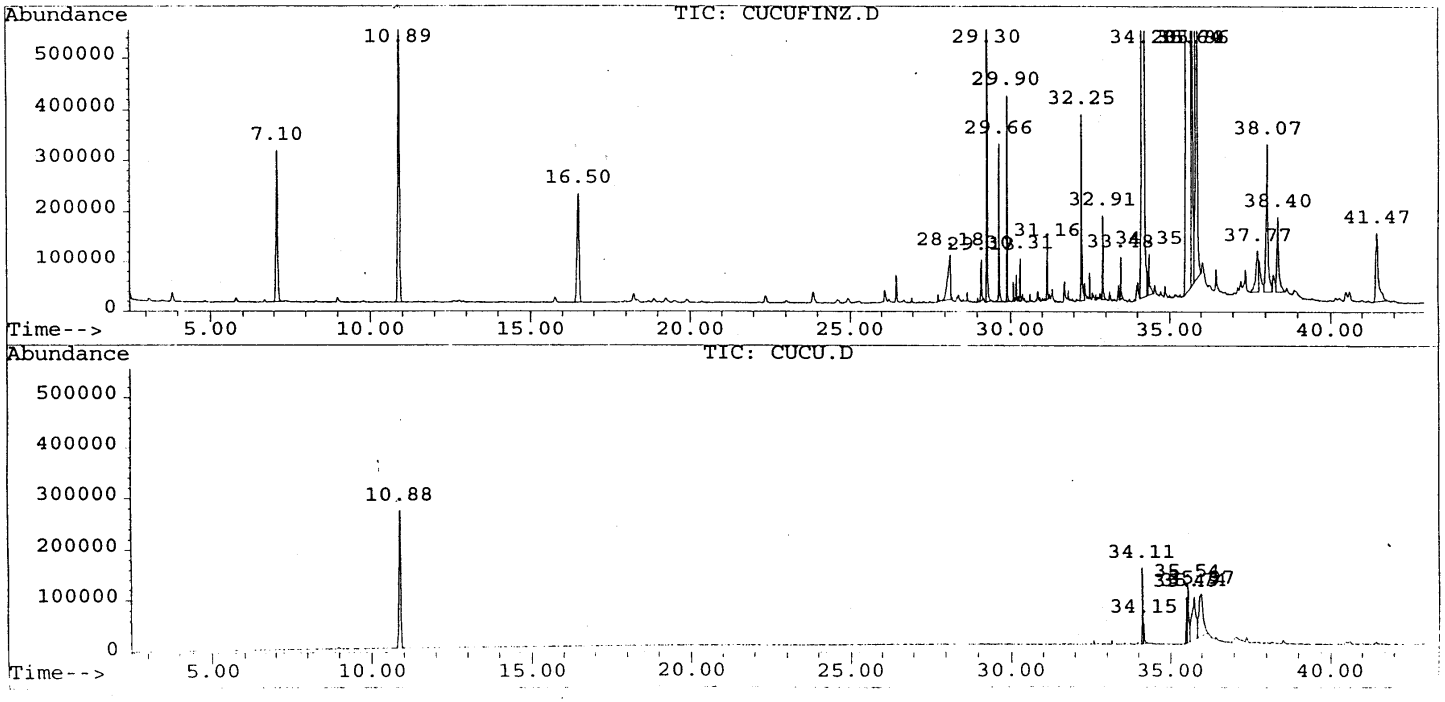
Urinary Incontinence: Open Clinical Trial

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

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Pumpkin Seed: Technical Background

Specific manufacturing procedure

- effective substances in hydrophilic phase
  (for the inhibition of aromatase as well as for the inhibition of 5α-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 5α-reductase in vitro and in vivo, significant reduction of prostate growth in rat
- Decrease of nocturnal and diurnal micturitions
- Significant decrease of incontinent episodes