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Translation from the original

Open Clinical Study of Effects of Pumpkin Seed Extract/ Soybean Germ Extract Mixture-containing Processed Food on Nocturia

Abstract

An open clinical study was conducted to investigate the efficacy and safety of a processed food containing a mixture of pumpkin seed *(Cucurbita pepo* L) extract and soybean germ extract (abbreviated as PEP, hereinafter) when given to elderly female patients with nocturia. The following results were obtained:

(1) PEP decreased the frequency of urination during both the night and daytime and frequency of incontinence with statistical significance, and also improved patients' sleep - related satisfaction.

(2) The frequency of urination incontinence was significantly decreased compared with before dosing PEP.

(3) There were 17 adverse events (43.6%) observed with 14 subjects (35.9%) in the total 39 subjects. Two subjects showed the increase of testosterone level in which the' causality of PEP was excluded with one subject and unknown with another. One of the two subjects recovered to the initial testosterone level on the 68m day after the end of administration, while another recovered on the 143m day. There were neither abnormal changes nor ether adverse events in which the causality of PEP was suspected. All the findings indicated PEP hag a feature of high safety.

In conclusion, PEP has demonstrated a highly favourable effect on various symptoms including nocturia in postmenopausal women as well as a high safety and tolerance.

I. Introduction

Urinary frequency becomes more common as the age advances, and nearly a half of elderly persons older than 60 years are known to suffer from nocturia, that is two or more episodes of micturition during the night. Nocturia is caused by a variety of factors such as prostate hypertrophy, uterine/ovarian disease, prostatitis, cystitis, diabetes insipidus, diabetes mellitus and neurogenic factors (particularly, cerebrovascular disorders, insomnia and stress). Urinary frequency and incontinence during the night have been indicated not only to induce insomnia and marked deterioration of patients' QOL, hut also to adversely affect the vital prognosis[1)]. As therapy for nocturia, various modalities such as pelvic floor muscle exercise, drug therapy, surgical procedures and ethers are available. Nevertheless, only a small proportion of the affected population visit hospitals, because most patients are likely to consider their symptoms as an inevitable complication of aging.

PEP is a health food product of SANA Co., Ltd., composed of triangle-shaped tablets containing pumpkin seed (Cucurbita pepo L. of Cucurbitales, Cucurbitaceae) extract and soybean germ extract. Pumpkin seed extract hag been granted approval by the German Federal Ministry für Health (published on B. Anz. No. 223 dated Nov. 3O, 1985 and revised B Anz. No.11 dated Jan. 17, 1991) as a therapeutic agent with the indications of irritable bladder (urinary frequency, pressure, incontinence, and sensation of residual urine) and early-stage prostate hypertrophy as described in Commission E Monograph, and is known to be effective in improving symptoms such as oliguria, sensation of residual urine, necessity to go to the toilet frequently during the night, and insomnia due to frequent micturition episodes at night[1),2)]. Soybean germ extract contains phytoestrogen of isoflavones[3)] and is potentially effective in ameliorating symptoms of geriatric disorders associated with estrogen deficiency such as osteoporosis, climacteric disorders (psychoneurological symptoms like headache, insomnia, depressive mood, etc.), atrophy of urogenital organs (including senile vaginitis, urinary incontinence, etc.) and cardiovascular disorders (including atherosclerosis, hypertension, etc.)[4)].

The design of the present study on the efficacy and safety of PEP in the treatment of nocturia was as follows.

II. Methods

1. Study subjects

The study subjects enrolled in the present study included “postmenopausal female patients (aged 55 to 79 years) who reported two or more micturition episodes during the night excluding an episode at leaving bed in the morning", who were outpatients of Medical Corporation Shinsenkai-Daiichi Hospital, Medical Corporation Juntenkai-Houshasen-Daiichi-Hospital and Medical Corporation Ohnishi Clinic. Before the study, written informed consent was obtained from the patients after providing with information on PEP.

Exclusion criteria included patients with (1) a fasting blood sugar level of 131 mg/dL or higher; (2) symptoms such as difficulty of urination, urinary retention, etc. indicative of definite obstruction of the lower urinary tract and urinary frequency due to cystitis; (3) severe complications disease such as heart, liver, kidney or hematologic disease; (4) regular consumption of a large amount of alcohol; (5) treatment with agents für urinary frequency within three months prior to the study; (6) known allergy to soybean or pumpkin; and (7) those who were judged as inappropriate at the investigator's discretion due to other reasons.

2. Study food

The study food used was a processed food product containing a mixture of pumpkin seed extract and soybean germ extract (PEP: SANA Co., Ltd.) Pumpkin seed extract (EFLA®940: Production No. 3012501) was procured from Emil Flachsmann AG, Switzerland, and soybean germ extract was procured from Tokiwa Phytochemical Co., Ltd. Six PEP tablets (1.5 g) contained 525 mg pumpkin seed extract and 100 mg soybean germ extract.

3. Study duration and dosage schedule

The duration of the study was 7 weeks in total, consisting of one week für observation and six. weeks für study food dosing. In weeks 1 and 2 of dosing, 10 tablets of PEP were given a day (corresponding to 875 mg pumpkin seed extract and 167 mg soybean germ extract), and in weeks 3, 4, 5 and6 of dosing, 6 tablets were given a day (corresponding to 525 mg of pumpkin seed extract and 100 mg soybean germ extract), each divided into two doses, in the morning and evening, together with a sufficient amount of co1d water or moderately warmed water.

4. Disallowed medication / allowable concomitant meditation

In principle, medication with agents that .might affect the assessment results of the study food including parasympatholytics and antispasmodics was prohibited. However, concomitant use of anticholinergics, tranquilizers, α-blockers, Ca-antagonists and antidepressants was allowed in unavoidable cases without changing the type and posology of concomitant medication(s) throughout the study period.

5. Assessment items and methods

(1) Subjective symptoms and objective findings

In the dosing period, the study subjects were asked to evaluate daily each of the items listed in Table 1 by themselves, and to make a record in a study diary, and the investigator conducted an interview to determine any objective findings each on the day and after 2 and 6 weeks of dosing.

Table 1 Diary items

- Subjective symptoms

[Frequency of urination during the night]

(number of episodes of going to the toilet during the period from the previous bedtime to the time of getting up in the morning)

[Frequency of urination during the daytime]

(number of episodes of going to the toilet during the period from getting up in the morning to bedtime)

[Frequency of urinary incontinence during the daytime]

(number of episodes of involuntary leakage of urine during the period trom getting up in the morning to bedtime)

[Degree of satisfaction]

(fulfilment following sleeping)

1. Fulfilled 2. Incompletely fulfilled 3. Not fulfilled

[Any findings to be reported]

(anything without restraint)

(2) Efficacy

Efficacy was rated as one of four grades "Markedly improved", "Improved", "No change" and "Worsened", by the investigator based on his (her) global judgment of subjective symptoms (by each item in the study diary) and objective findings each after 2 and 6 weeks of dosing.

(3) Other examinations

Vital sign recording (body weight, blood pressure, pulse rate), laboratory tests and hormone determinations (estrone, estradiol, progesterone, testosterone) were performed before and after 2 and 6 weeks of dosing. The laboratory tests performed included hematologic tests (WBC, RBC, hemoglobin, hematocrit and platelet count), blood biochemical tests (total bilirubin, BUN, creatinine, GOT, GPT, ALP and y-GTP) and urinalysis (qualitative tests of protein, glucose and urobilinogen).

(4) Adverse events

Adverse events in relation to subjective symptoms, objective findings and various laboratory test results were closely scrutinized of various factors including newly appearing symptom(s) (test item(s) with abnormal value), date of anger, degree, action, outcome, relation to the study food, etc. Abnormal laboratory test results were assessed by the investigator according to the "Severity Classification Criteria for Adverse Drug Reactions (former MHW Guidelines)" as cited in Table 2, and any change with a shift in grade toward higher severity was defined as an adverse event.

< Table 2 >

Table2: Severity Classification Criteria tor Adverse Drug Reactions
(N= the upper limited value of at each clinicals)

	Grade 1	Grade 2	Grade 3
Leucocyte (count/μl)	< 4000	3000	2000
Erythrocyte (count/μl)	$< 35 \times 10^6$	30×10^6	25×10^5
Hgb (g/dl)	11	9.5	8
Platelet (count/μl)	1×10^6	7.5×0^3	50×10^3
GOT, GPT	N x 1.25	N x 2.5	N x 5
ALP	N x 1.25	N x 2.5	N x 5
γ-GTP	N x 1.25	-	-
Total bilirubin (mg/dl)	1.6	3.0	10
BUN (mg/dl)	N	25	40
Creatinine (mg/dl)	N	2	4

III. Results

1. Study subjects

Age-related inclusion criteria were stipulated in the study protocol to include ages from 55 to 79 years, while informed consent was actually obtained from 42 women aged 52 to 86 years based on discretion that this difference in age ranges would not be a matter of concern from the viewpoint of patient characteristics, since the subjects were all in a postmenopausal state. Among these 42 subjects, one was excluded from the study because the blood sugar level exceeded the inclusion criterion in the pre-study screening examination, and two withdrew their consent to participate in the study voluntarily after registering for entry. The subject No.N-04 showed an abnormal high testosterone level of 227.4 ng/dL before dosing (female standard range: 6~86 ng/dL), but other aspects remained eligible with clinical data. As a result, 39 study subjects were eligible for efficacy and safety assessment, their characteristics being shown in Table 3. Mean age was 68.4 years old.

< Table 3 >

Table 3: Characteristics being of the subjects

Characteristics being (n=39)		Number of the subjects (%)
Age	52 - 59	7 (17.9)
	60 - 64	6 (15.4)
	65 - 69	9 (23.1)
	70 - 74	9 (23.1)
	75 - 79	4 (10.3)
	80 - 86	4 (10.3)
	Means ±SD	684+ 7.9
Frequency of urination during the night	2 times a day	14 (35.9)
	3 times a day	11 (28.2)
	4 times a day	8 (20.5)
	5 times a day	4 (10.3)
	6 times a day	2 (5.1)
	Means ±SD	3.4 ± 1.7 times a day
Past history	No	36 (92.3)
	Yes	3 (7.7)
Complication	No	23 (59.0)
	Yes	16 (41.0)
Concomitant medication	No	24 (61.5)
	Yes	15 (38.5)

2. Study results

1) Efficacy

(1) Records in study diary

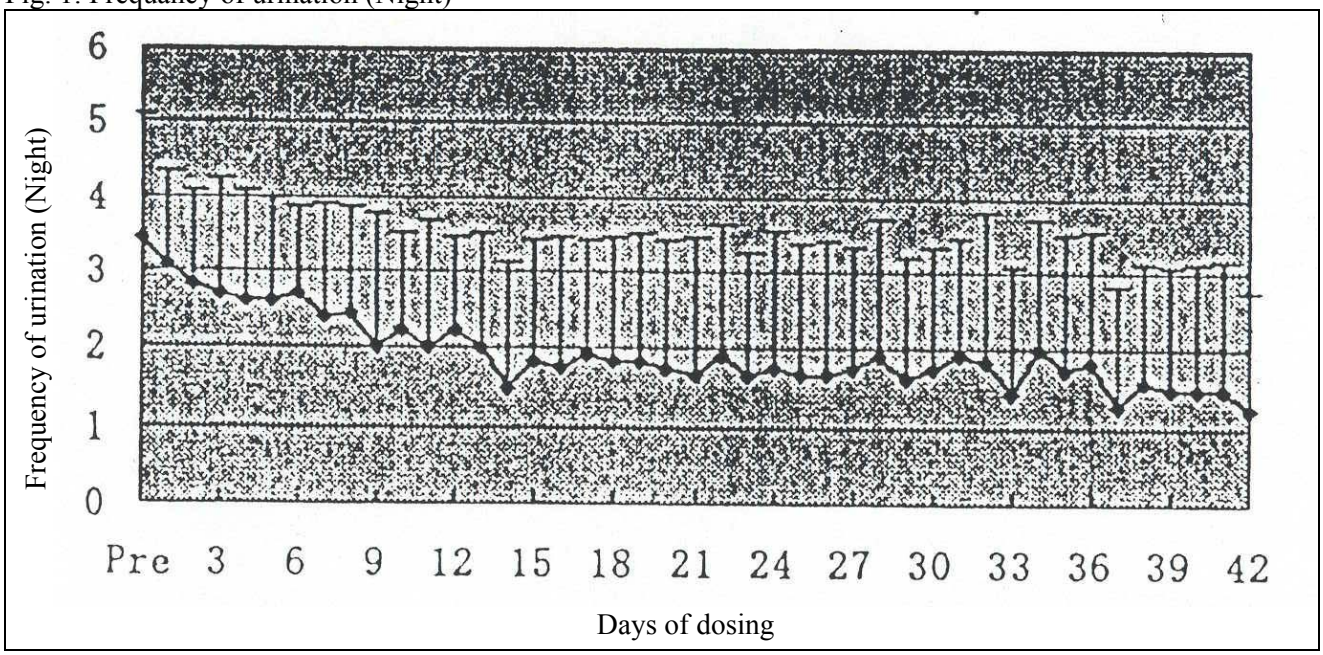
Figures 1 to 3 depict the time-course of changes in frequency of urination during the night, frequency of urination during the daytime, and subject satisfaction in the 39 efficacy assessment-eligible subjects. Subjective satisfaction was rated according to a scoring system of "l (fulfilled)", "2 (incompletely fulfilled)" and "3 (not fulfilled)", and items left blank due to the subject's unintentional omission were handled as missing values. Data analysis was performed by comparison of the median before (pre) and after dosing using paired *t-test* to determine statistical significance (Table 4). Effects on the frequency of urinary incontinence were examined using the data obtained from 16 particular subjects who experienced episodes of incontinence during the observation period, by comparing total episodes a week, during weeks 1, 2, 4 and 6 of dosing with total episodes in the observation period, using paired *t-test* to determine statistical significance (Table 5).

The following results were obtained: The frequency of urination during the night and daytime was already

markedly improved ($p<0.01$) at the week-l assessment, followed by continuing improvement at the week-2 assessment and no further additional improvement at the week-4 assessment and thereafter. The degree of satisfaction was improved ($p<0.05$) at the week-l assessment, followed by marked improvement ($p<0.01$) at the week-2 assessment. The frequency of urinary incontinence was improved ($p<0.05$) at the week-2 assessment, followed by marked improvement ($p<0.01$) at the week-4 assessment.

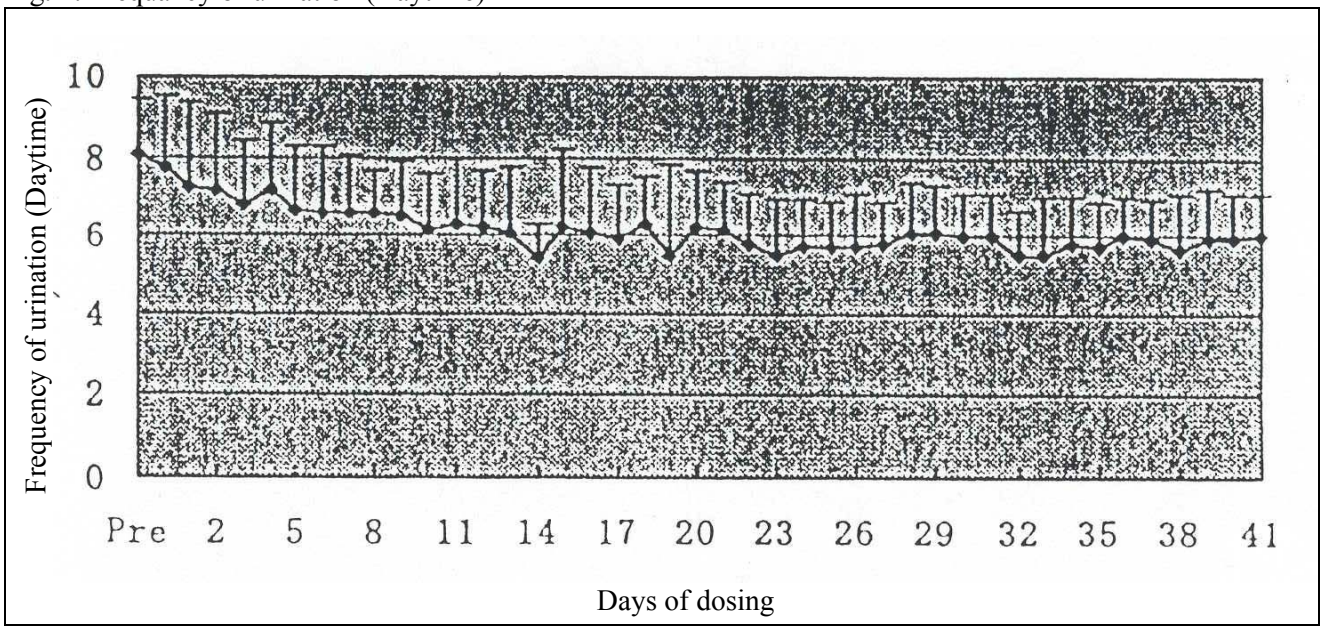
<Fig.l >

Fig. 1: Frequancy of urination (Night)

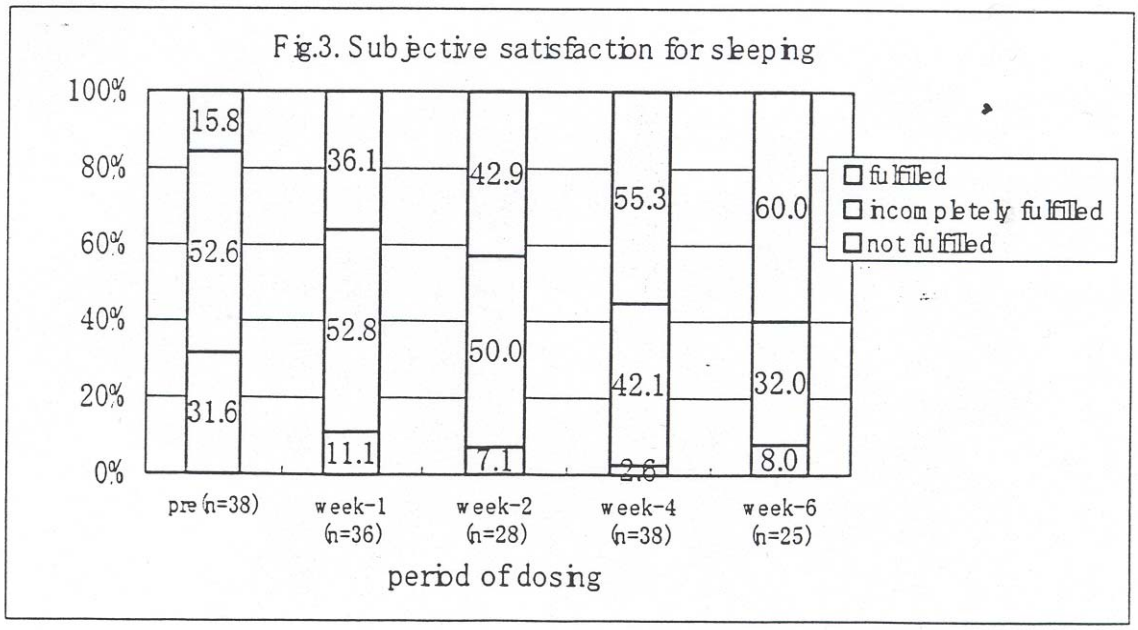


<Fig.2 >

Fig. 2: Frequancy of urination (Daytime)



< Fig. 3 >



< Table 4 >

Table 4: Subjective symptoms score: Frequency of urination during the night and the daytime, and subjective satisfaction.

	Means ± SD				
	Before dosing	Week 1	Week 2	Week 4	Week 6
Frequency of urination during the night (n)	3.3 ± 1.6 (39)	2.6 ± 1.5** (39)	2.5 ± 22** (31)	2.3 ± 1.9** (39)	2.0 ± 2.3** (28)
Frequency of urination during the day (n)	8.0 ± 2.6 (39)	7.0 ± 2.5** (39)	6.8 ± 2.9** (39)	6.5 ± 2.0** (39)	6.7 ± 2.3** (36)
Subjective satisfaction (n)	2.1 ± 0.6 (38)	1.8 ± 0.6* (36)	1.6 ±0.6** (28)	1.5 ± 0.6** (25)	1.5 ± 0.7** (25)

Mean ± S.D: ** $p < 0.01$, * $p < 0.05$

< Table 5 >

Table 5: Subjective symptoms score: Frequency of urination during the night and the daytime, and subjective satisfaction.

	Means ± SD				
	observation period (-7 – -1 days)	1st Week (0 – 6 days)	2nd Week (7 – 13 days)	4th Week (28 – 34 days)	6th Week (35 – 41 days)
Frequency of the urinary incontinence (n=16)	7.3 ± 8.3	5.5 ± 3.4	4.1 ± 3.5*	2.2 ± 2.2**	1.5 ± 2.7**

Mean ± S.D: ** $p < 0.01$, * $p < 0.05$

(2) Hormone determinations

Table 6 shows the results of hormone determinations. Statistical analysis was performed using paired *t*-test for comparison of the measurements before and after each of the dosing periods. There were no statistically significant changes in the levels of estrone, estradiol and progesterone.

The mean value of testosterone at the week-6 measurement (42.61±111.62 ng/mL) was higher than those obtained before dosing and at week-2 measurement, and this higher value was found to be ascribable to higher values in 2 of the 39 subjects. Testosterone levels in these two subjects were found to be higher than the mean value for normal men even at the pre-study measurement, the background of these higher values not being clear except for some possible involvement of high physiological sensitivity to testosterone of these subjects or effects of the study food.

After excluding these two subjects, testosterone level at week-2 and week-6 was 20.71±11.90 and 17.82±7.96 ng/mL, respectively, and the changes were found to be statistically insignificant by paired *t*-test.

Table 6: Results of hormone determination before and after each of the dosing periods.

Analysis items			Period of evaluation		
			Before dosing (n=39)	Week 2 (n=38)	Week 6 (n=39)
Hormones concentration in blood	Estrone (pg/mL)	Means ± SD (Max-Min)	17.0 ± 3.51 (28.2-15.0)	17.23 ± 3.39 (26.1-15.0)	16.78 ± 5.39 (46.0-15.0)
		paired *t*-test	-	p=0.79	p=0.75
	Estradiol (pg/mL)	Means ± SD (Max-Min)	9.86 ± 4.88 (28.6-8.0)	11.62 ± 10.17 (57.8-8.0)	11.78 ± 13.97 (90.3-8.0)
		paired *t*-test	-	p=0.30	p=0.24
	Progesterone (ng/mL)	Means ± SD (Max-Min)	0.26 ± 0.14 (0.6-0.1)	0.23 ±0.14 (0.6-0.1)	0.25 ± 0.15 (0.8-0.1)
		paired *t*-test	-	p=0.35	p=0.92
	Testosterone (ng/mL)	Means ± SD (Max-Min)	27.54 ± 34.48 (227.4-5.0)	38.58 ± 87.89 (536.9-5.0)	42.61 ±111.62 (619.1-5.9)
		paired *t*-test	-	p=0.29	p=0.13

Measurements below the determination limit (Estrone: 15 pg/mL, Estradiol: 8 pg/mL, Progesterone: 0.1 ng/mL)

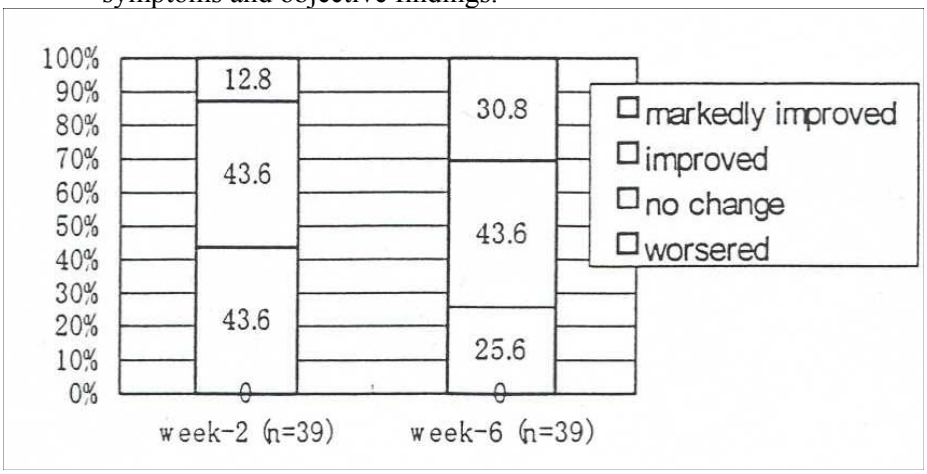
(3) Global improvement rate

Figure 4 depicts the global improvement rate based on global judgment of subjective symptoms and objective findings in the 39 subjects eligible for efficacy assessment. At the week-2 assessment, 12.8% and 59.0% of subjects were rated as "Markedly improved" and "Improved" or better, respectively, and at the week-6 assessment, 30.8% and 74.4% of me subjects were rated as "Markedly improved" and "Improved" or better,

respectively. The subject No.N-04 with high testosterone level before dosing was rated " Improved " at the week-2 and " Markedly improved" at the week-6. The subject No.D-20 with a remarkable increase of testosterone level during the test period was rated " No change" at the week-2 and "Improved" at the week-6. Figure 5 depicts the same global improvement rate in a particular subgroup of 33 subjects in whom me frequency of urination during the night was 2 to 4 episodes/day during the observation period. 5 subjects (15.2%) and 21 subjects (63.6%) were rated as "Markedly improved" and "Improved" or better, respectively, at the week-2 assessment, and 12 subjects (36.4%) and 27 subjects (81.8%) were rated as "Markedly improved" and "Improved" or better, respectively, at the week-6 assessment.

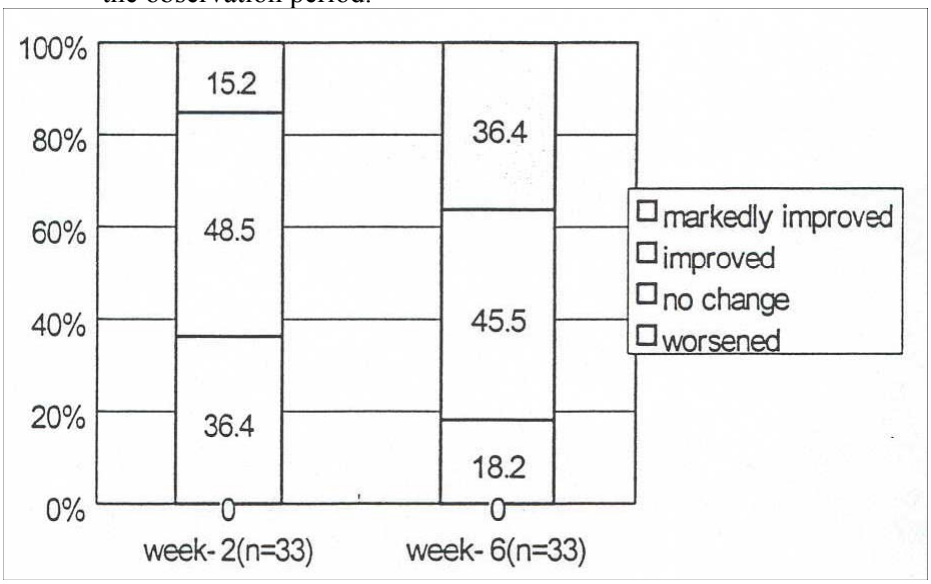
<Fig.4 >

Fig. 4: The global improvement rate based on global judgment of subjective symptoms and objective findings.



<Fig.5 >

Fig. 5: Global improvement rate in a particular subgroup of 33 subjects the frequency of urination during the night was 2 to 4 episodes/day during the observation period.



2) Safety

(1) Vital signs and laboratory test results

As shown in Table 7, there were no changes of clinical concern in mean values of vital signs and laboratory test results in the 39 subjects.

(2) Adverse events

Abnormal changes in laboratory test results and ether determinations are shown in Table 8, and adverse events in relation to subjective symptoms and objective findings are shown in Table 9.

Among the assessed 39subjects, the causality of PEP was not denied of the increase of testosterone level with the subject No. D-20 who showed an increase of estron and estradiol levels as well. In this case the causality was first considered to be ascribable to instability of sex hormone levels and/or disorder of adrenal function. However it was not identified and concluded unknown in the outcome of the present study.

In relation to other adverse events, causality of the study food was ruled out.

Table 7: Vital signs and laboratory test results before and after each of the dosing periods (n=39).

		Standard value		Period of evaluation		
				Before dosing	Week 2	Week 6
Vital sign	Weight	- (kg)	Means ± SD (Max-Min)	51.8 ± 11.0 (85-34)	51.9 ± 11.0 (85-35)	51.8 ± 10.8 (89-34)
	Systolic blood pressure	90-140 (mmHg)	Means ± SD (Max-Min)	136.7 ± 21.3 (196-95)	137.7 ± 23.1 (190-98)	130.4 ± 19.8 (164-82)
	Diastolic blood pressure	~ 90 (mmHg)	Means ± SD (Max-Min)	74.9 ± 12.7 (100-50)	75.5 ± 13.16 (102-42)	72.1 ± 13.7 (100-50)
	Pulse	50-110 (/min)	Means ± SD (Max-Min)	74.4 ± 10.7 (100-60)	73.3 ± 10.4 (100-54)	73.4 ± 9.7 (96-60)
Blood	Leucocyte	40-80 (count/μl)	Means ± SD (Max-Min)	67.4 ± 18.6 (123-34)	64.9 ± 17.3 (106-38)	68.5 ± 21.7 (141-43)
	Erythrocyte	370-490 (count/μl)	Means ± SD (Max-Min)	427.6 ± 36.2 (521-357)	421.8 ± 38.43 (532-335)	423.5 ± 42.6 (510-309)
	Hgb	10.7-15.0 (g/dl)	Means ± SD (Max-Min)	13.41 ± 1.20 (15.8-9.6)	13.17 ± 1.27 (15.5-9.6)	13.21 ± 1.33 (15.9-9.1)
	Hematocrit	34.4-44.0 (%)	Means ± SD (Max-Min)	39.82 ± 3.47 (47.6-30.2)	39.43 ± 3.69 (46.2-29.1)	39.51 ± 3.94 (45.8-28.7)
	Platelet	15-40 (count/μl)	Means ± SD (Max-Min)	22.51 ± 5.75 (40.3-12.7)	22.09 ± 6.23 (43.2-14.7)	22.48 ± 6.73 (39.0-12.3)
Biochemical	GOT	10-35 (IU/L)	Means ± SD (Max-Min)	23.4 ± 5.8 (42-16)	22.8 ± 5.4 (45-16)	21.9 ± 4.6 (34-14)
	GPT	5-38 (IU/L)	Means ± SD (Max-Min)	20.0 ± 10.4 (67-9)	19.1 ± 10.7 (68-9)	18.2 ± 8.4 (47-9)
	ALP	70-250 (IU/L)	Means ± SD (Max-Min)	180.2 ± 60.2 (366-82)	177.1 ± 57.8 (308-94)	173.5 ± 55.0 (296-174)
	γ-GTP	4-63 (IU/L)	Means ± SD (Max-Min)	22.3 ± 14.6 (62-7)	22.1 ± 14.9 (64-5)	23.0 ± 18.1 (96-5)
	Total bilirubin	0.2-1.2 (mg/dl)	Means ± SD (Max-Min)	0.65 ± 0.24 (1.2-0.2)	0.64 ± 0.25 (1.4-0.3)	0.67 ± 0.22 (1.1-0.2)
	BUN	8.0-20.0 (mg/dl)	Means ± SD (Max-Min)	16.03 ± 3.73 (25.2-11.2)	15.33 ± 3.82 (26.5-8.7)	15.25 ± 3.49 (22.3-8.2)
	Creatinine	0.5-1.1 (mg/dl)	Means ± SD (Max-Min)	0.64 ± 0.14 (1.0-0.4)	0.62 ± 0.11 (1.0-0.5)	0.61 ± 0.12 (0.9-0.4)
Urin	Protein	- ~ ±	+	1	0	2
			±	6	6	3
			-	32	33	34
	Urine sugar	- ~ ±	+	0	0	0
			±	0	0	0
			-	39	39	39
	Urobilinogen	- ~ ±	+	2	0	0
			±	37	39	39
			-	0	0	0

< Table 8 >

D-06	62	Erythrocyte count	37-49 ($x10^6$/μL)	35.7*	34.4* mild	36.1	recovery during dosing	No
D-08	59	systolic blood pressure	90 –140 mmHg	144*	160	82* mild	need no follow- up study	No
D-20	52	testosterone	6 - 86 ng/dL	46.2	183.6*	383.3* middle	recovery to 27.1 ng/dL after 143 days of study	unclear
D-21	74	Erythrocyte count	37 – 49 ($x10^6$/μL)	36.6*	35.1*	33.0* mild	need no follow- up study	No
		Hgb	10.7 - 15.0 g/dL	9.6*	9.6*	9.1* mild	need no follow- up study	No
		Protein	- ~ ±	±	±	+* mild	need no follow- up study	No
D-22	71	Erythrocyte count	4-8 ($x10^6$/μL)	4.6	3.8* mild	4.4	recovery during dosing	No
D-26	69	BUN	8.0 -20.0 (mg/dL)	15.2	21.3* mild	14.4*	recovery during dosing	No
		Protein	- ~ ±	±	±	+* mild	recovery ± after 29 days of indication	No
D-28	71	γ-GTP	4-63 (IU/L)	62	64	96* mild	need no follow- up study	No
D-29	79	BUN	8.0 -20.0 (mg/dL)	22.3*	26.5* mild	18.4	recovery during dosing	No
N-03	84	Erythrocyte count	37-49 ($x10^6$/μL)	37.1	33.5*	30.9* mild	need no follow- up study	No
N-04	59	Testosterone	6-86 (ng/dL)	227.4*	536.9*	619.1* middle	recovery to 101ng/dL after 68 days of indication	No

< Table 9 >

Table 9: Adverse events based on subjective symptoms and objective findings.

Subject No.	age	symptoms	time of occurrence	grade of symptom	Medical treatment	exitus	relevancy
D-12	74	cold syndrome	after 3 days	mild	No	Disappear after 2 days	No
D-16	80	cold syndrome	after 11 days	mild	treatment with general medicine	recover after 2 days	No
D-24	57	Abdominal distension	the day starting to	mild	No	Disappear bifore starting to dose	No
D-34	61	diarrhea	after 38 days	mild	No	Disappear at the day having diarrhea	No

IV. Discussion

The results of the present study revealed that the frequency of urination during the night and daytime, degree of satisfaction following sleeping, and number of episodes of urinary incontinence were decreased with statistical significance after six weeks of intake of the processed food product containing a mixture of pumpkin seed extract and soybean germ extract (PEP) compared with be fore its intake. These favourable effects were reflected in high improvement rates as shown by global improvement rating of "Improved" or better in 59.0% (23/39) and 74.4% of subjects (29/39) at the week-2 and week-6 assessment, respectively. In addition, in the 33 subjects with 2 to 4 episodes of nocturia per night, the improvement rate was ever higher, as shown by a rating of "Improved" or better in 81.8% of subjects (27/33) at the week-6 assessment. This high efficacy is considered to be ascribable to anti-inflammatory, antimicrobial and bladder tissue-reinforcement effects;) of pumpkin seed extract, and affinity to SHBG (sex hormone binding globulin), and 5a-reductase- and aromatase-inhibitory effects of lignan, one component of pumpkin seed extract, as well as estrogen-like effects3) of soybean germ extract, resulting not only in reducing the frequency of urination bur also in ameliorating climacteric disorder-associated symptoms such as psychoneurological symptoms including insomnia and urinary incontinence[4)]. Other pathologic conditions causing urinary frequency and urinary incontinence, however, include central nervous system disorders associated with cerebral infarction or cerebral thrombosis, spinal cord injury and aging-associated spondylosis deformans. The finding obtained in the present study that the study food could achieve higher improvement rates in the subjects with urinary frequency suffering from relatively fewer episodes of urination is considered to be due to the general circumstances that patients with intractable neurogenic bladder are not incorporated in the population of patients with a few episodes of urination. Neurogenic bladder is known to present same problems in that 10 to 20% of patients do not respond positively to medication with anticholinergic agents, and attention should be paid to their possible adverse drug reactions such as urinary disturbance, mouth dryness, constipation, etc. as well as to concomitant diseases and medications, and thus, introduction of the study food into practical use would be highly valuable.

The adverse events were observed of 17 cases with 14 subjects (Table 8). There was no considerable fluctuation in the change in vital signs and laboratory test results. In hormone determinations, levels were not significantly different before and after dosing, allowing us to exclude the involvement of hormone, esp. female sex hormone, in the outcomes of the present study. The abnormal increase of testosterone level was observed with the subject No. D-20 whose global improvement was however rated "Improved" at the week 6 assessment. In this case the causality of PEP was not identified and concluded unknown in the outcomes of the present study.

In relation to other abnormal changes and adverse events, causality of PEP was ruled out, indicating PEP has a feature of high safety and tolerance.

In conclusion, the study food, containing a mixture of pumpkin seed extract and soybean germ extract, has demonstrated that it is a promising health food endowed with favourable effects on various symptoms including mild nocturia in postmenopausal women.

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Yanagisawa, Ei et al (2003)

Study of Effectiveness of Mixed Processed Food Containing Cucurbita Pepo Seed Extract and Soybean Seed Extract on Stress Urinary Incontinence in Women

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Translated from Japanese

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Abstract

An effectiveness test was conducted for fifty female patients (aged between 35 and 84) with overactive bladder and stress urinary incontinence for six weeks to investigate the improvements in conditions and safety of PEP, a mixed processed food containing Cucurbita pepo seed extract and soybean seed extract, (hereinafter referred to as “PEP”). The results revealed by the test are as follows:

1) In forty-eight cases, the number of stress urinary incontinence was significantly reduced compared to the state before the administration.

2) In twenty-eight cases (56.0%) of fifty cases, there were 30 adverse events (56.0%), where the doubtful relevance with the intake of PEP were shown in four cases (8.0%) with 6 adverse events. Considering that the adverse events were mild and also quickly disappeared, PEP was regarded as highly safe.

From the above, it is suggested that PEP is highly effective and safe to improve the conditions of the stress urinary incontinence.

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Clinical study of mixed processed foods containing of pumpkin seed extract and soybean germ extract on stress urinary incontinence (SUI) in women

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Key words: pumpkin seed, soybean germ, SUI, women, clinical study

Introduction

Irritable bladder syndrome has been reported to affect 20% to 40% of moderately aged women, where 70% to 80% of those women suffer from stress urinary incontinence[1)].

The stress incontinence is a urinary disease that includes involuntary loss of urine during physical exertion with increased intra-abdominal pressure due to coughing and sneezing. It is caused by obesity, aging, delivery of births, etc.

The preservation treatments for stress urinary incontinence include pelvic floor stimulation aiming at strengthening and toning weak pelvic floor muscles, via medications such as α- and β-receptor stimulant or estrogen, and electromagnetic stimulation which pulses electromagnetic waves through an electrode to vagina, anus, perineum and so on [2)]. However, most patients give up receiving therapeutic treatments for the syndrome because they take it for granted as a natural process of aging mechanism and thus there are not many patients who actually receive outpatient treatments.

PEP is a triangular tablet sold by Tervis Co., Ltd. and a supplementary food for nutrition which contains Cucurbita pepo seed extract and soybean seed extract. On one hand, Cucurbita pepo seed was approved as an efficacious treatment for irritable bladder syndrome (such as frequent urination, urgent urination, urinary incontinence, and constant urination feeling) and benign prostatic hypertrophy at the early stage in the Guideline of Plant-Derived Medicines made by the German Ministry of Health (released on November 30, 1985), and it was reported to be effective in the treatment for syndromes such as reduced volume of urine, constant feeling of urination, frequent urination at night, which may disturb sound sleep.[3)] On the other hand, soybean seed extract contains phytoestrogen[4)] which is derived from less active ingredient isoflavone, and it is proven to relieve geriatric diseases[5)] such as cardiovascular diseases (arteriosclerosis, hypertension, etc.), involution of urogenital organs (geriatric vaginitis, urinary incontinence, etc.) psychiatric syndromes (headache, insomnia, depression and other menopause disorders), and osteoporosis which is caused due to lack of estrogen.

In Japan so far, it has been reported that the intake of PEP significantly reduces the number of urinations per day as well as per night [6)]. In this study, PEP will be examined and reviewed to identify any improvement and safety against stress urinary incontinence as one of the methods of evaluating the Effectiveness test of PEP.

I. Evaluation Method

1. Subject

We selected female outpatients suffering from stress incontinence, except pregnant women, who had visited Hakwaikai Ichinoseki Hospital in Ichinoseki Iwateken and Satoh Ladies' Clinic in Maebashi Gunmaken from January to May 2003 and explained to them about the test method and PEP prior to their participation in the test and obtained their written consent.

Since it is common to conduct pelvic floor stimulation or other treatments at least for consecutive twelve months until the effect is revealed, we decided to exclude patients who had continuously received non-medication therapies such as pelvic floor exercise or electromagnetic stimulation, and failed to complete 12 weeks from the onset of the test on the date from the acquisition of the consent because it was considered that they would affect the evaluation of effectiveness of PEP. In addition, we excluded those patients who had complication such as serious diseases from heart, liver, stomach and blood, and who had an allergy to soybeans or pumpkins in the aspect of safety as well as patients who had previously received surgical therapies because of stress urinary incontinence, and those a doctor in charge considers inappropriate for other reasons.

2. Ingredients

We used a mixed process food called 'PEP' (manufactured by Tervis Co., Ltd.) that contains Cucurbita Pepo seed extract and soybean seed extract. The extract of Cucurbita Pepo seed (EFLA 940) was prepared by Swiss firm Emil Flaschsmann and the extract of soybean seed (ISOMAX-30) was manufactured by Tokiwa Phytochemical Co., Ltd.

PEP contains 875mg of Cucurbita Pepo seed extract and 167mg of soybean seed extract per 10 tablets (2.5g). It also contains 525mg of Cucurbita Pepo seed extract and 100mg of soybean seed extract per 6 tablets (1.5g).

3. Period of Test and Administration

The duration of the test included one week of observation period (hereinafter referred to as the "Step 1") and six weeks of administration period. The total period to be expected is 7 weeks. The patient took 5 tablets every time and twice (morning and night) per day from the first week to the second week of the administration period (referred to as the "Step 2") and 3 tablets every time and twice per day from the third to the sixth week (referred to as the "Step 3") with plenty of water

or warm water.

4. Combination Prohibited Drugs / Combination Limited Drugs

In principle, estrogen and α- and β-receptor stimulant, which are considered to be effective in the effectiveness evaluation of the test food and are used in the treatment for stress incontinence, are prohibited to be combined with the food. If there is no choice, in principle, but to combine α- and β-receptor inhibitor, anti-cholinergics, anti-anxiety medication, sleeping pill, herb medicine (*Bojungikgitang, Galgeuntang,*and *Dangguijakyaksan*) during the test period without changing medicines, usage and dosage, the combination was allowed for only a very limited period of time.

Table 1: Items to be filled in a daily report by a patient

【Food Administration Time】 day and night
【Number of Urinations per Day】 Number of urinations from rising to sleeping
【Satisfaction】 Satisfaction grade based on urinary incontinence, etc. 1. Satisfied 2. Quite satisfied 3. Quite disappointed 4. Disappointed
【Subjective Symptoms】 Comments by subjects

5. Test Items and Test Method

1) Subjective Symptoms and Objective Symptoms

During the test period, patients were requested to keep daily record with respect to the items in Table 1 and doctors asked patients detailed questions about their conditions on the date of outset of intake (after the completion of Step 1), two weeks from the date (after the completion of Step 2), and six weeks from the date (after the completion of Step 3) on the basis of the daily report.

2) Improvement

Comprehensively judging from the daily report recorded by patients and the detailed information asked by doctors, we classified the improvement levels into four phases: “remarkably improved”, “improved”, “unchanged” and “aggravated” after the completion of Step 2 and Step 3.

3) Clinical Trial

We conducted biochemical profile (AST, ALT, ALP, DLH, γ-GTP, BUN, creatinine, TG, T-Cho, HDL and LDL) and urine test (protein, sugar, urobilinogen) at the time of outset of Step 1 and after the completion of Step 2 and Step 3 in the clinical trial.

4) Adverse Event

We investigated the adverse events shown in subjective symptoms, objective symptoms and the clinical trial with respect to detailed symptoms (including abnormal changes of clinical test value), time of occurrence, rating (seriousness, critical condition), treatment, disease progress and the relevance with the test food. The adverse events of clinical test values were judged by the doctor in charge with reference to “Standard on Judgment of Abnormality of Clinical Test Value” in Table 2. If the doctor finds any abnormal changes, they were determined as adverse events.

Table 2: Standard on Judgment of Abnormality of Clinical Test Value
(The proposal of the Japanese Society for Chemotherapy partially applied to the Standard.)

【Biochemical Profile】

〔AST(GOT), ALT(GPT), ALP, LDH, γ-GTP, BUN, Creatinine, TG, T-Cho, LDL〕

□ Within the limit → Off the limit
If the value exceeds the upper limit by 1.2 times, it is considered as an adverse event.

□ Off the limit → Off the limit
If the value exceeds the upper limit by 2 times, it is considered as an adverse event.

〔HDL〕

□ Within the limit → Off the limit
If the above value is 0.8 times less than the lowest limit, it is considered as an adverse event.

□ Off the limit → Off the limit
If the above value is 0.5 times less than the lowest limit, it is considered as an adverse event.

【Urine Test】

1. Urine protein: Adverse event in case of having changes of two or more steps in (-)→(+)
2. Urine sugar: Adverse event in case of having changes of two or more steps
3. Urobilinogen: Adverse event in case of changes of one or more step

II. Result

1. Subject

Fifty two patients aged between 35 and 84 agreed to give case reports on the test. There was a case record that a combination of estradiol and testosterone (prohibited from combination)

was used once a month, but the combination had been administered without a change in usage or dosage for one year and it did not give any effect in the stress incontinence. Therefore, the subject was admitted to participate in the test as being judged that it would give no impact on the evaluation of effectiveness of PEP. In addition, two patients failed to administer PEP because one patient withdrew the consent for the reason that it was impossible to keep daily report and the other patients could not keep daily report due to the worse complication during the observation period. As for one patient, who failed to visit hospital as scheduled due to a flu (one case), we suspended the test because the patient was not able to cooperate in the test. After the completion of the test, it was found that one case breached the criteria for exceptional exclusion.

From the above information, the test cases of effectiveness were set to total of 48 cases and the test cases of safety were set to 50 cases in the medication of PEP. Of total 48 cases, patients with hay fever reached six cases. There were no significant factors in medical history of the other patients. Table 3 showed the background factors of 52 cases. The mean value of their age was 53.5.

Table 3: Background Factors of Patients

Background Factor (n=52)		Cases (%)
Age	35□39	3(5.8)
	40□49	18(34.6)
	50□59	17(32.7)
	60□69	9(17.3)
	Mean SD	53.5 10.3
Age when stress incontinence is developed*	23□29	3(6.5)
	30□39	8(17.4)
	40□49	19(41.3)
	50□59	11(23.9)
	60□69	4(8.7)
	Mean SD	48.0 11.0
Allergy to pumpkin	None	52(100.0)
	Yes	0(0)
Surgical treatment	None	52(100.0)
	Yes	0(0)
Complication	None	18(34.6)
	Yes	34(65.4)
Combined therapy	None	51(98.1)
	Yes	1(1.9)

Menopause	None Yes	22(42.3) 30(57.7)

* 6 Cases were unidentified.

2. Test Result

1) Effectiveness

□ Intake rate of test food

The PEP intake rate of patients is shown in Table 4.

Table 4: Intake Rate of Test Food (n=48)

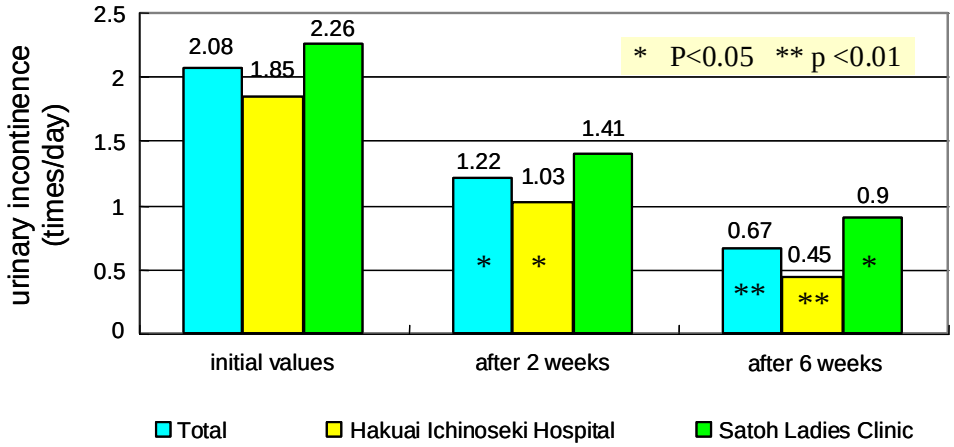
	Step 2	Step 3
Total	94.3%	95.6%
Hakwaikai ichinoseki Hospital	94.4%	94.7%
Satoh Ladies' Clinic	94.1%	96.5%

□ Number of incontinence episodes per day

The transition of the number of incontinence per day in Step 1, Step 2 and Step 3 of 48 cases in the test of effectiveness in Figure 1. After comparing the mean values of the number of incontinence of Step 1 with the average values after intake (Step 2 and Step 3), we conducted a paired sample t-test. As a result, the number of incontinence per day was significantly reduced in Step 3 ($p<0.01$) (Table 5).

Three cases of six patients with hay fever showed an improvement in the number of urinary incontinence. Two other cases showed the reduction in the number of urinary incontinence in Step 2 but there was no significant change in Step 3. In case of the other case, the number of incontinence reached 2.71 times per day in Step 1, but increased to 6.21 times per day due to the aggravated hay fever during the period when pollen flied in Step 2. The number of incontinence reached 7.57 times per day in Step 3. Even three cases, which did not show any decrease in frequent incontinence, showed the reduction in the amount of urine.

【Figure 1】

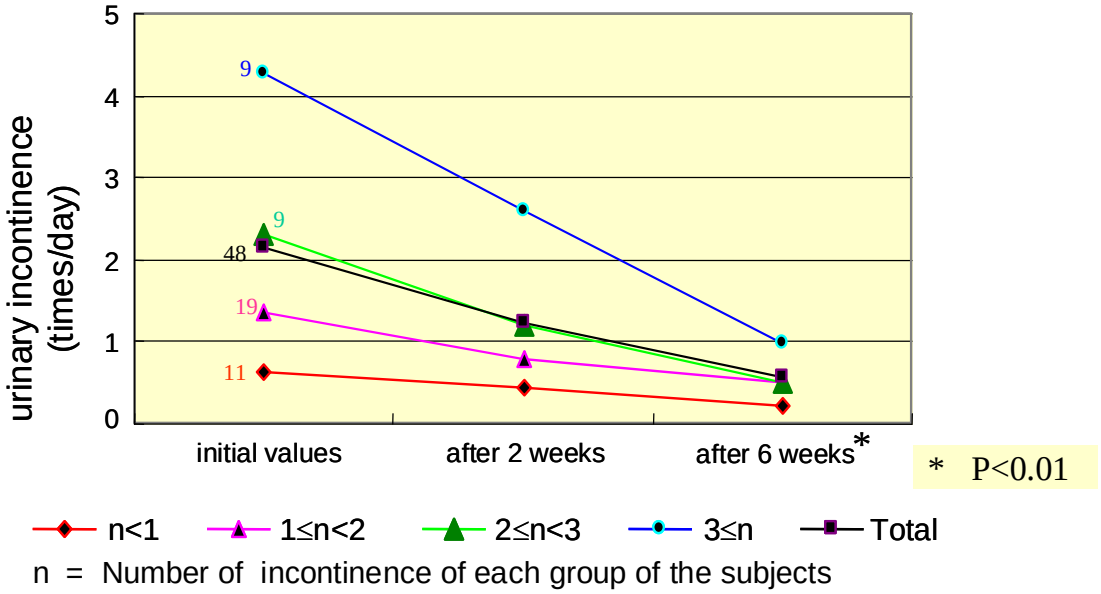


In comparison of the cases in the number of urinary incontinence in Step 1, irrespective of the frequency of incontinence during the observation period, it showed significant reduction after six weeks from the food intake (Figure 2: $p<0.01$). In particular, the patients who had the large number of incontinence showed the significant reduction.

Table 5: Number of Urinary Incontinence per Day (n=48)

Number of urinary incontinence per day (Times/Day)	Mean SD		
	Step 1	Step 2	Step 3
Total (Max□Min)	2.08 2.0 (12□0)	1.22 1.3* (6.21□0)	0.67 1.2** (7.57□0)
Hakwaikai ichinoseki Hospital	1.85 1.6	1.03 1.2*	0.45 0.5**
Satoh Ladies' Clinic	2.26 2.4	1.41 1.4	0.90 1.6*

【Figure 2】



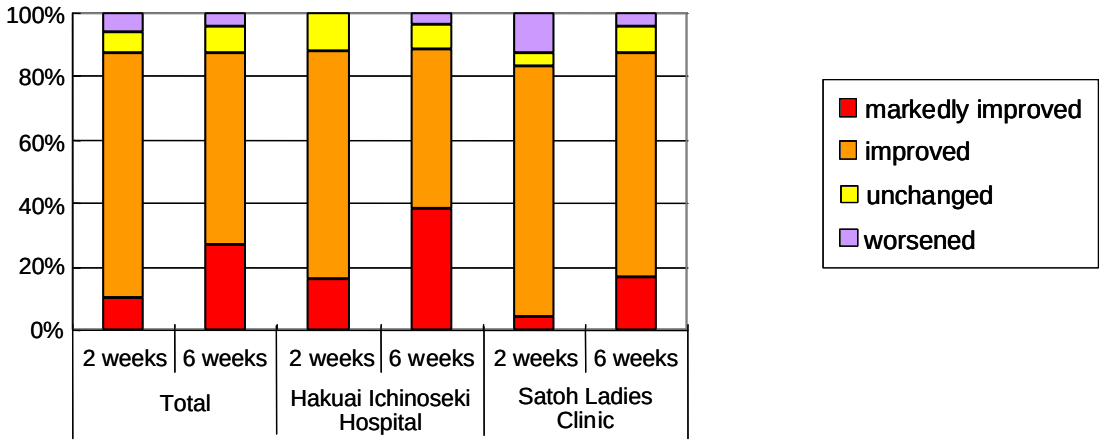
n = Number of incontinence of each group of the subjects

□ Improvement

Figure 3 showed overall improvement as judged comprehensively by doctors in the subjective symptoms from 48 patients in the Effectiveness test. Both of the medical institutions showed similarly significant results as follows: “remarkably improved” cases reached five (10.4%) and “improved” cases were 42 patients (87.5%) in Step 2 and “remarkably improved” cases reached 13 patients (27.1%) and “improved” cases were 42 patients (87.5%) in Step 3.

Of the cases which were shown “unchanged” or “aggravated” in the improvement, most patients showed an increase in frequent urinary incontinence by coughing or sneezing due to diseases such as aggravated hay fever as complication or flu. Yet we confirmed the reduction of the amount of urine from the daily reports of most patients and the number of incontinence was reduced when the patients recovered from hay fever or a flu.

【Figure 3】



2) Safety

□ Clinical test value

Table 6 showed the transition of clinical test values. There was no transition of 50 cases in the safety test which might trigger problems in a clinical trial.

Table 6: Transition of Clinical Test Value (n=50)

Test Item		Unit		Test Period		
				Outset of Observation	2 weeks after food intake	6 weeks after food intake
Biochemical Profile	AST	IU/L	Mean SD (Max□Min)	22.0 6.6 (49□12)	23.0 7.6 (48□13)	21.6 6.2 (43□12)
	ALT	IU/L	Mean SD (Max□Min)	21.9 14.7 (96□6)	22.1 14.6 (87□6)	20.0 11.8 (67□6)
	ALP	IU/L	Mean SD (Max□Min)	220.8 69.1 (402□118)	221.7 72.4 (457□123)	215.8 68.7 (406□107)
	LDH	IU/L	Mean SD (Max□Min)	185.9 27.6 (243□128)	189.4 29.2 (252□125)	190.0 31.3 (267□129)
	γ-GTP	IU/L	Mean SD (Max□Min)	21.0 9.6 (49□8)	20.6 10.8 (62□8)	20.4 12.5 (71□8)
	BUN	mg/dL	Mean SD (Max□Min)	14.0 3.6 (22.1□7.3)	13.2 2.7 (19.0□7.0)	13.6 3.0 (21.7□7.0)
	Creatinine	mg/dL	Mean SD (Max□Min)	0.62 0.12 (0.9□0.4)	0.61 0.11 (0.8□0.4)	0.57 0.10 (0.8□0.4)
	TG	mg/dL	Mean SD (Max□Min)	149.4 110.5 (613□42)	134.3 94.3 (550□38)	122.6 73.4 (358□42)
	T-Cho	mg/dL	Mean SD (Max□Min)	209.8 34.1 (315□134)	205.1 31.1 (321□135)	207.7 34.1 (355□146)
	HDL	mg/dL	Mean SD (Max□Min)	60.7 15.6 (110□34)	60.8 15.5 (112□34)	62.9 15.5 (107□39)
	LDL	mg/dL	Mean SD (Max□Min)	121.3 29.8 (203□66)	116.6 27.4 (215□63)	119.8 28.7 (246□59)
Urine Test	Urine Protein		+ + + + -	1 1 6 42	0 2 8 40	0 3 4 42
	Urine Sugar		+ + + + + + + + + +	0 0 1 1 1 47	0 2 0 1 0 47	1 0 1 1 0 46
	Urobilinogen		+ + -	0 50 0	0 50 0	1 48 0

□ Adverse Event

Table 7 describes the adverse events of clinical test and Table 8 shows the adverse

events shown in subjective and objective symptoms. There were 39 adverse events from 28 cases (8.0%) from total of 50 cases, of which 6 events (8.0%) from four cases were related to the relevance of PEP. Conducting urine sugar (++□+++) tracking survey of Patient No. A-27, we found that the sugar was recovered to the limit value. It was considered that the patient would possibly have the disease of sugar metabolism but we could not find the cause from this test alone. In addition, the urine protein (+) of patient No. B-21 had no problem in the test for stomach function and in a clinical trial. Accordingly, it was judged that the tracking survey is not required. Since we could not specify the relevance with PEP in all causes of the two cases through this test, the relevance was judged as “possible”.

When the tracking survey of TG level of patient No. A-18 (358 mg/dl) was conducted, the level was detected at 231 mg/dl. The changed level of TG was thought to be influenced by meals because the TG level is sharply influenced by food and also the range of fluctuation was wide. Provided that the blood-gathering was conducted with the same condition as usual, we could not find the cause of the high level of TG and this made us judge “possible factor”.

Since we could specify the causes with respect to the adverse events of clinical test irrelevant to PEP, the tracking survey was judged to be unnecessary.

The uneasiness of the stomach which occurred three times two days after the intake of Patient No. B-6 had been frequently shown when the patient had overeaten. The symptom did not occur in Step 3. Since the patient had no good stomach, it was appropriate to make the patient administer PEP three pills each time. We could not deny the possibility that the uneasiness was caused by the administration of five pills each time but it was judged as “possible” because we could not find the relevance with PEP through the test. Besides, the relevance with the test food was denied in 30 adverse events of 22 cases (44.0%).

Table 7: Adverse Events in Clinical Trial

No. of Patients	Age	Test Item	Limit Value	Test Value			Remark	Relevance
				Before intake	2 weeks	6 weeks		
A-3	59	Urine Sugar	-	+ - *	+++* Moderate	+++* Moderate	Unnecessary to track	None
A-6	69	LDH (IU/L)	106□211	208	222* Mild	267* Mild	Unnecessary to track	None
A-18	40	TG (mg/dL)	50□150	186*	150	358* Mild	231 Tracking (14 days after intake)	Possible
A-27	53	Urine Sugar	-	+ *	++□+++* Mild	++* Mild	- Tracking (10 days after intake)	Possible
B-7	61	TG (mg/dL)	30□149	114	188* Mild	79	Unnecessary to track	None
B-21	81	Urine Protein	-	-	+ *	+ *	Unnecessary to track	Possible

*: Deviation of limit value

Table 8: Adverse Event in Subjective/Objective Symptoms

No. of Patient	Age	Symptom	Occurrence	Seriousness	Treatment	Disease Progress	Relevance
A-1	52	Flu	8th day from intake	Mild	Antibiotics	Disappeared after 3 days	None
A-2	36	Flu	9th day	Mild	Cold medicine	Disappeared after 6 days	None
A-4	54	Thigh rash Constipation	9th day / 10th day	Mild Mild	Non treatment Non treatment	Pleasant after intake Disappeared after 2 days	None None
A-9	84	Flu	18th day	Mild	Cold medicine	Disappeared after 3 days	None
A-16	59	Abdominal disease	15th day	Mild	Non treatment	Disappeared after one day	None
A-20	52	Face/leg trauma	22nd day	Mild	Antibiotics	Pleasant after intake	None
A-21	42	Flu	10th day	Mild	Antibiotics	Disappeared after 30 days	None
A-22	46	Toothache	15th day	Mild	Sedative/ Antipyretics	Disappeared after 2 days	None
A-24	46	Sore throat	11th day	Mild	Antibiotics	Disappeared after 2 days	None
A-25	45	Flu Insomnia	13th day 20th day	Mild Mild	Cold medicine Hypnotics	Disappeared after 3 days Disappeared on the day	None None
A-27	53	Sour stomach	6th day	Mild	Digestive	Disappeared after 2 days	None
A-28	49	Flu	31st day	Mild	Cold medicine	Disappeared the next day	None
B-3	57	Diarrhea Diarrhea	2nd day 12th day	Mild Mild	Non treatment Non treatment	Disappeared on the day Disappeared on the day	None None
B-6	55	Stomach Uneasiness Stomach Uneasiness Stomach Uneasiness	2nd day 6th day 14th day	Mild Mild s Mild	Digestive Digestive Digestive	Disappeared on the day Disappeared on the day Disappeared on the day	Possible Possible Possible
B-7	61	Flu	18th day	Mild	Cold medicine	Disappeared after 3 days	None
					Medicine for		

III. Conclusion

This test revealed that the mixed processed food (PEP) containing Cucurbita pepo seed and soybean seed extract significantly reduces the number of incontinence nearly 6 weeks after its intake and improves the conditions of the disease overall. In addition, the patients showed the high improvement up to 87.5 percent (42 cases/48 cases) after the completion of the intake in consideration with the level 'improved" or better conditions. In comparison of effectiveness by the frequency of incontinence before the intake of PEP, PEP significantly improved all the incontinence and showed excellent efficacy even in cases with comparatively serious incontinence.

Despite the sharp decrease in quality of life of patients with urinary incontinence, few patients are still treated by doctors because the disease is rarely considered as a condition which may give them a life-threatening danger and even patients may not perceive that urinary incontinence is a disease indeed. Some drugs such as α- and β-receptor stimulant or estrogen are used to treat stress incontinence but it is necessary to make precautions on side effects and possible complications, combinatory medications. It is true that pelvic floor stimulation exercise is usually used to treat mild stress incontinence. To obtain satisfactory results and effects, patients, however, the therapy should be conducted several times per day on a regular basis for a period of from two to three months.

Notwithstanding the short period of test which lasted for six weeks, we found that PEP intake with two pills per day significantly reduced the number of incontinence. Furthermore, considering that there were cases that showed the significant reduction in the amount of incontinence a day without the improvement in the number of incontinence, we believe that PEP will largely contribute on the improved quality of life of patients.

Twenty-eight out of fifty cases showed 39 adverse events (Tables 7 and 8) but they did not bring significant change in the transition of clinical test values. Patient No. B-6 expressed uneasiness of the stomach as subjective symptom which occurred two days after the intake of PEP and had been frequently experienced when the patient had overeaten. However, we could not find any relevance with PEP from this test alone and we thus judged it would be possible that they may be relevant. Through the test, we failed to ascertain the cause of urine sugar (++□+++) and urine protein (+), the relevance of which is unclear with PEP. With respect of other adverse events, the relevance with PEP was denied. The Guideline of Plant-Derived Medicines made by the German Ministry of Health did not disclose any incompatibilities, adverse effects or any interactions with any other drugs thus showing the superior safety of PEP.

In conclusion, the supplementary food (PEP) containing Cucurbita pepo seed and

soybean seed extract is shown highly effective in improving the conditions of stress urinary incontinence.

Please allow me to show my deepest thanks to Tervis president Dachizaki Masaru for their contributions to the test food ‘PEP’ and the opportunity for conducting the test.

Terado T et al. (2004)

Clinical Study of mixed processed foods containing pumpkin seed extract and soybean germ extract on pollikiuria in night in elderly men

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English Translation

Clinical study of mixed processed foods containing Pumpkin seed extract and soybean germ extract on pollakiuria in night in elder men

Terado T[1)], Sogabe H[2)], and Saito K[3)]

Abstract

To confirm the efficacy and safety of the mixed processed foods (PEP®) containing of Pumpkin seed extract EFLA®940 and soybean germ extract on pollakiuria in night, a clinical study was performed by 6-week intake using 54 males (age 65 – 88) with pollakiuria in night symptoms. The results showed the following:

1) The frequency of urination in the night was significantly reduced in all 45 cases included in the efficacy evaluation, as compared to before intake.
2) Out of 51 cases included in the safety evaluation, adverse events were observed in 33 incidences in 22 cases (43.1%). Those for four incidences in 3 cases (5.9%) were judged 'could be related to PEP® intake'. Since all of them were mild, followed by disappearance of or recovery from symptoms later, the results suggest that PEP® is highly safe.

Thus, PEP® effectively improves symptoms of pollakiuria in night and PEP® is used safely.

Introduction

PEP®, a granular product in triangular shape, is sold by Tervis Co., Ltd. It is a nutrition-supplemental food containing a water-soluble extract of Pumpkin seed EFLA®940 in the squash group of the melon family and soybean germ extract. The edible Pumpkin seed are approved as an effective therapeutic against hyperactive bladder (pollakiuria, urgency, incontinence, and residual) and early stages of prostatic hypertrophy in the guideline for plant therapeutics from the Ministry of Health in Germany (published on November 30, 1985). They are reported to be effective against symptoms of smaller volume of urination, residual feeling, pollakiuria, and insufficient sleep due to pollakiuria in the night[2)3)]. Soybean germ extract contains isoflavone-derived phytoestrogen[4)] and is known to relieve aging symptoms such as osteoporosis due to estrogen insufficiency, psychological/sympathetic nerve symptoms (menopausal syndromes such as headache, insomnia, and depression), urinary symptoms (prostatic symptoms and erectile dysfunction), and cardiovascular symptoms (arteriosclerosis and hypertrophy).

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Clinical study of mixed processed foods containing of Pumpkin seed extract EFLA®940 and soybean germ extract on pollakiuria in night in elder men

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Key words: pumpkin seed, soybean germ, pollakiuria in night, elderly men, clinical study

Pollakiuria in night is defined as 'the condition in which one is awakened by an urge to urinate during sleep in the night' {The Word Standards by International Continence Society (ICS), 2002}. According to the 'epidemiological study on urination' performed by the Japanese Urination Function Society in 2002, pollakiuria in night occurs with about an 80% frequency in one's 60s and more than 90% in people over the age 80.

Pathology of pollakiuria in night are classified in the two main groups: (1) lower-urinary tract-associated (urinary disease) and (2) lower-urinary tract-non-associated (non-urinary disease).

Urinary diseases include prostatic hypertrophy, prostatitis, neuropathic bladder, and hyperactive bladder which cause a decrease of organic and functional bladder volume resulting from bladder involution and residual urine.

On the other hand, non-urinary diseases include cardiac and renal dysfunction, excessive water intake, age-associated decrease of diuretic hormone secretion, and sleep disorder. Sleep disorder reduces the threshold for urination sensation, leading to reduction in functional bladder volume and pollakiuria in night. In elderly men, these complex symptoms make diagnosis and treatment difficult.

It has been reported in efficacy studies that PEP® is significant effect on pollakiuria in night and abdominal pressure-associated incontinence in women[5) 6)]. The present study examines the efficacy and safety of PEP® on pollakiuria in night in men.

I. Methods

1. Subjects

Between January and June 2004, the subjects were men older than 65 years of age who woke up more than two times for urination in the night and who were selected from among the out-patients at Shinseikai-Daiichi Hospital in Imabari City in Ehime Prefecture, Ohnishi Clinic in Ochi-Gun in Ehime Prefecture, and Saito Internal Medicine & Urology Clinic in Maebashi City in Gunma Prefecture. Prior to the study, written consent was obtained from each subject after explaining about the test method and PEP®. The following patients were excluded from the study: (1) Ones with serious complications involving the heart, liver, kidney or blood, (2) ones with allergies to soybeans and Pumpkin, (3) ones who received surgical therapy for prostatic hypertrophy in the past year, (4) ones who had had hormone drugs for prostatic hypertrophy in the past month, (5) ones with urinary tract stent or urinary tract catheter, (6) ones with urinary tract disorder from urinary calculus or pollakiuria from acute inflammation in the urinary tract such as, for example, cystitis, (7) ones who may have taken health food known to have effects on pollakiuria in night during the study period, (8) ones who have drunk excessive alcohol, (9) ones with a fasting blood level higher than 140 mg/dL glucose, and (10) ones who are judged 'improper' by the physician-in-charge for other reasons.

2. Food tested

The test sample was the mixed processed food (PEP®, Tervis Co., Ltd.) containing of water-soluble extract EFLA®940 of edible Pumpkin seed and soybean germ. Ten tablets of PEP® (2.5 g) contained 875 mg of water-soluble extract EFLA®940 of edible Pumpkin seed and 167 mg of soybean germ extract, and six tablets of PEP® (1.5 g) contained 525 mg of water-soluble extract EFLA®940 of edible Pumpkin seed and 100 mg of soybean germ extract.

3. Study period and intake method

The study period was one week of observation (Step 1) followed by six weeks of intake, in a total of 7 weeks. In the first and second weeks of intake (Step 2), five tablets were taken with cool water or warm water twice a day in the morning and evening. In the following third week through the sixth week (Step 3), three tablets were taken twice a day.

4. Drugs prohibited or restricted from concurrent use

Hormone drugs (anti-androgens and estrogen drugs) which are used as therapeutics for prostatic hypertrophy and may influence the efficacy evaluation of the test food were prohibited from concurrent use. The following drugs were allowed without changes in drug type, method of use, and

Table 1. Entries in subject's diary

[Intake compliance for test food]	
Morning/evening	
[Subjective symptoms]	
• Urination frequency during the day:	The number of times you went to bathroom between the times you woke up and went to bed.
• Urination frequency during the night:	The number of times you woke up to go to bathroom through the night.
• Satisfaction with sleep:	Satisfaction level about sleep after consideration of urination in the night. 1. Satisfied 2. Slightly satisfied 3. Slightly unsatisfied 4. Unsatisfied
• Comments:	Describe any changes noticed in yourself.

Table 2. Evaluation standards for abnormality in laboratory analyses
(Partially quoted from the standards by the Japanese Chemotherapy Society)

[Blood biochemistry]
AST (GOT), ALT (GPT), ALP, LDH, γ-GTP, BUN, creatinine
[1] Standard range to outside of standard range
Abnormal changes are those which are more than 1.2 times the upper limit of standard range.
[2] Outside of standard range to outside of standard range
Abnormal changes are those which are more than 2 times the previous level.
[Urinalysis]
1. Urinary protein: Changes of more than 2 grades from (-) to (+)
2. Urinary glucose: Changes by more than 2 grades
3. Urobilinogen: Changes by more than 1 grade

dosage: α-receptor blockers, plant extracts/Chinese medicines, amino acid drugs and α, β receptor stimulators, anticholinergics, anti-anxiety drugs, and antidepressants.

5. Evaluation items and methods

1) Subjective and objective symptoms

During the study period, subjects recorded each day intake compliance and subjective symptoms (**Table 1**). Based on the records, the physician-in-charge questioned subjects on the day of intake start (after Step 1 completion), after intake for two weeks (after Step 2 completion) and after intake for six weeks (after Step 3 completion) and made evaluations on subjective symptoms.

2) Improvement of subjective symptoms

Comprehensively judging based on responses to questioning and records made by subjects (Table1), the physician-in-charge evaluated improvements of subjective symptoms by the four grades of 'significantly improved', 'improved', 'unchanged', and 'aggravated'.

3) Laboratory analysis

Laboratory analyses were performed at the time of Step 1 start and after completion of Steps 2 and 3, including blood biochemistry (AST, ALT, ALP, LDH, γ-GTP, BUN, and creatinine) and urinalysis (qualitative protein, qualitative glucose, and qualitative urobilinogen).

4) Adverse events

Symptoms (including abnormal changes in laboratory analyses), time of occurrence, grade (severity and seriousness), treatment, outcome, and relatedness to the test food were examined for all adverse events.

Adverse events included all subjective and objective symptoms identified in subject's records which were not beneficial to subjects who took the test food.

For the evaluation of abnormal changes in laboratory analyses, the physician-in-charge determined them to be 'adverse events' in reference to 'Evaluation standards for abnormality of laboratory analyses' (a part of the Japanese Chemotherapy Society Notification: **Table 2**).

Table 3. Background factors of subjects (n=51)

Background factor			Number of cases (%)
Age (years)		65 - 69	27 (52.9)
		70 - 74	14 (27.5)
		75 - 79	8 (15.7)
		80 - 84	1 (2.0)
		85 - 88	1 (2.0)
		Mean±SD	70.4±4.8
Age of onset of pollakiuria in night (years)		53 - 59	3 (5.9)
		60 - 69	31 (60.8)
		70 - 79	8 (15.7)
		80 - 81	1 (2.0)
		Unknown	8 (15.7)
		Mean±SD	65.4±5.3
Period with pollakiuria in night (years)		<1	2 (3.9)
		1 - 4	23 (45.1)
		5 - 9	11 (21.6)
		10 - 14	6 (11.8)
		15 - 19	1 (2.0)
		Unknown	8 (15.7)
		Mean±SD	5.3±3.8
The number of urination in the night at Step 1, (times/night)		1.7 - 1.9	2 (3.9)
		2.0 - 2.9	28 (54.9)
		3.0 - 3.9	10 (19.6)
		4.0 - 4.9	8 (15.7)
		5.0 - 9.3	3 (5.9)
		Mean±SD	3.09±1.30
Presence or absence of soybean or Pepo Pumpkin allergy		No	51 (100.0)
		Yes	0 (0.0)
Complications	Presence/ absence	No	22 (43.1)
		Yes	29 (56.9)
	Disease name[*2]	Hypertension	15
		Prostatic hypertrophy[*1]	7
		Insomnia	4
		Hyperuricemia	3
		Arrhythmia	3
		Angina pectoris	3
		Constipation	3
		Gastritis	3
		Gonarthrosis	2
		Obliterating arteriosclerosis	2
		Hyperlipidemia	2
		Pollakiuria	1
		Old myocardial infarction	1
		Chronic bronchitis	1

Background factor			Number of cases (%)
Complications	Disease name[*2]	Hypotension	1
		Thoraco-abdominal aneurysm	1
		Cerebral infarction	1
		Parkinson's disease	1
		Direct hernia	1
		Right upper arm nerve pain	1
		Lumbar slipping	1
		Rib fracture	1
		Gout	1
		Osteolumbago	1
		Cerebral infarction after-effect	1
		Atrial fibrillation	1
		Supraventricular premature beat	1
		Osteomyelopathy	1
		Neurogenic bladder	1
		Alcoholic hepatopathy	1
		Gastric ulcer	1
		Anxiety neurosis	1
Disease history	Presence/ absence	No	25 (49.0)
		Yes	26 (51.0)
	Disease name[*2]	Appendicitis	3
		Cholecystitis	3
		Prostatic hypertrophy	2
		Direct hernia	2
		Lung cancer	2
		Duodenal ulcer	2
		Angina pectoris	1
		Cerebral thrombosis	1
		Colon cancer	1
		Rectal polyp	1
		Bladder tumor	1
		Left wrist fracture	1
		Acute myocardial infarction	1
		Maxillary empyema	1
		Pulmonary Aspergillosis	1
		Dysentery	1
		Herniated disc	1
		Pulmonary tuberculosis	1
		Drug-associated hepatopathy	1
		Cataract	1
		Pleurisy	1
		Abdominal arterial aneurysm	1
		Acute pancreatitis	1
		Unknown	1
Presence or absence of concurrent use of drugs for pollakiuria		No	43 (84.3)
		Yes	8 (15.7)

*1: Name of diagnosed diseases recorded in clinical records. *2: Include multiple answers.

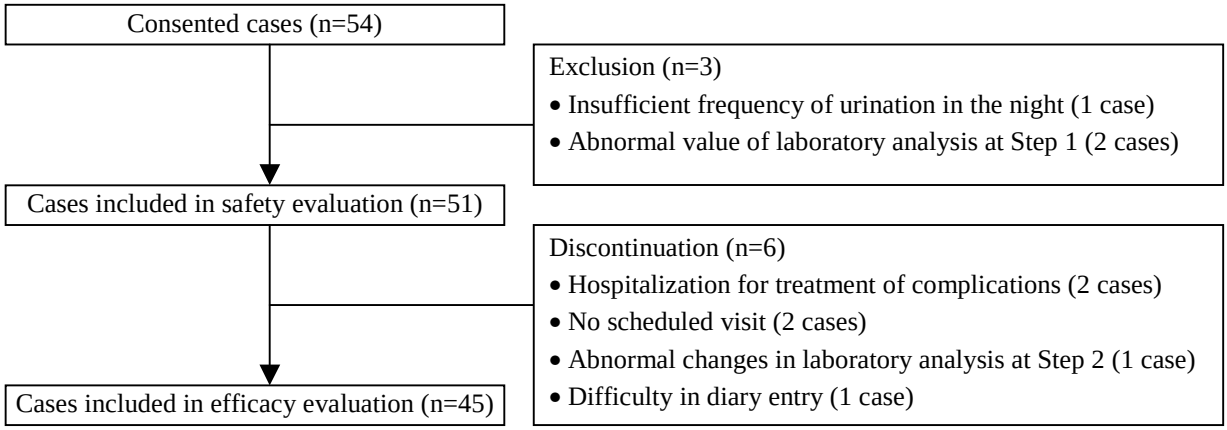

Fig. 1. The number of cases

II. Results

1. Subjects

Consents were obtained from a total of 54 subjects between ages 65 and 88. However, three cases were excluded from the study before the start of PEP® intake, including one subject who did not meet the selection standard for pollakiuria in night and two subjects with highly abnormal values (grade 3) by the laboratory analysis performed at the start of Step 1. Therefore, the safety was evaluated in a total of 51 cases who took PEP®. **Table 3** shows the background factors of these subjects.

In addition, the study was discontinued in the following six cases; two cases who were hospitalized for the treatment of complications during the study period, two cases who could not make scheduled visits to hospital for personal reasons, one case with a Grade 3 abnormality in laboratory analyses performed at Step 2 completion, and one case who had somebody else make record entries due to a difficulty of recording in diary by himself. Thus, the efficacy was evaluated in a total of 45 cases (**Fig. 1**).

Among the 45 cases included in the efficacy evaluation, seven subjects had complications of prostatic hypertrophy, and four subjects had a prostate condition apparently associated with hypertrophy. In addition, there were one subject each with complications of pollakiuria and neurogenic bladder and four subjects with complications of insomnia. No particular condition was identified in the backgrounds of the other cases.

Compliance for intake of the test food was examined from the records in the diaries made by the subjects themselves. **Table 4** shows an excellent compliance in intake for all subjects, showing no interference for evaluations.

Table 4. Compliance with schedule for test food intake (n=45)

Intake compliance	Step 2	Step 3
Total mean (%)	99.01±3.00	99.04±3.02

2. Study results

1) Efficacy

[1] Night and daytime urination frequency

Fig. 2 shows the changes in total frequency (%) after Step 2 and Step 3, in comparison to total urination frequencies in the day and night at Step 1, which was taken as 100%. The urination frequency in the night appeared to decrease, showing about a 40% decrease after intake for 6 weeks.

On the other hand, no significant change in urination frequency in the daytime was observed after intake for 6 weeks.

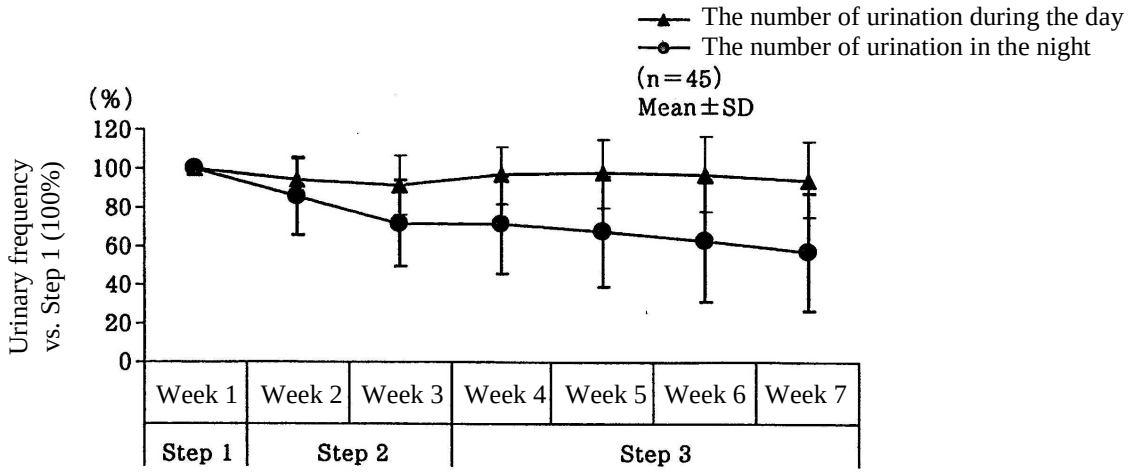

Fig. 2. Change in urination frequency (1) – Average weekly urination frequency

Table 5. Number of urinations in the night (times/night)

	Mean±SD		
	Step 1	Step 2	Step 3
Total (n=45) (highest-lowest)	3.04±1.30* (9.3 - 1.7)**	2.46±1.35* (9.4 - 1.0)	2.08±1.65** (10.2 - 0.2)
Group A (n=8) (concurrent use of urination drugs)	3.45±2.50 (9.3 - 2.0)	3.50±2.52 (9.4 - 1.7)	3.45±3.01 (10.2 - 1.0)
Group B (n=37) (no concurrent use of urination drugs)	2.95±0.89†‡ (5.9 - 1.7)	2.23±0.84† (4.4 - 1.0)	1.79±1.02‡ (5.5 - 0.2)

*, **, †, ‡ : p<0.01

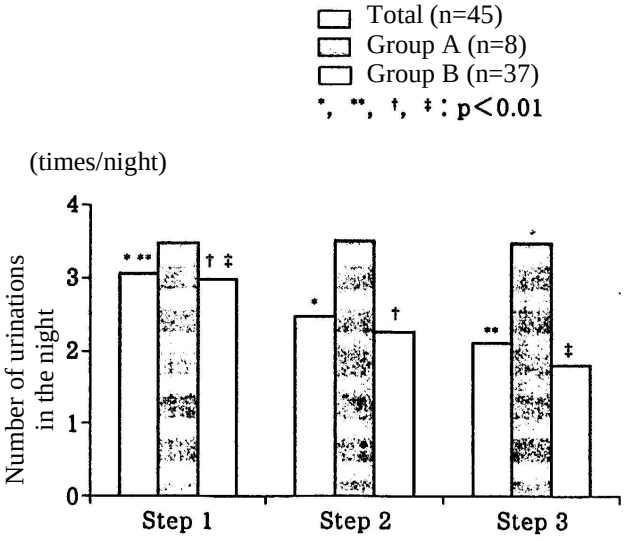

Fig. 3. Change in urination frequency (2)
Average nocturnal urination frequency in the night

The 45 cases included in the efficacy evaluation were divided into the two groups; one with concurrent use of therapeutic drugs for pollakiuria (Group A) and another without (Group B). **Table 5** and **Fig. 3** show mean values of urination frequency in the night at each step.

Corresponding t test was performed between the mean values at Step 1 and after intake (Step 2 and 3). A significant decrease was observed in the frequencies for the total and Group B ($p<0.01$). On the other hand, there was no significant change in Group A.

[2] Sleep satisfaction level

The sleep satisfaction level was scored by 2 points for 'satisfied', 1 point for 'slightly satisfied', -1 point for 'slightly unsatisfied', and -2 points for 'unsatisfied'. The mean value was calculated for each Step (**Table 6**). The sleep satisfaction level was analyzed in the two groups similarly as described above, one with concurrent use of therapeutic drugs for pollakiuria (group A) and another without (Group B), among 45 cases included in the efficacy evaluation. Corresponding t test was performed between Step 1 and after intake (Step 2 and 3).

Table 6. Sleep satisfaction (points)

	Mean±SD		
	Step 1	Step 2	Step 3
Total (n=45) (highest-lowest)	0.08±1.17* (2.0 - -2.0)**	0.73±0.97* (2.0 - -1.2)	0.90±0.93** (2.0 - -1.3)
Group A (n=8) (concurrent use of urination drugs)	0.00±1.34 (2.0 - -2.0)	0.04±1.13 (1.9 - -1.2)	0.24±1.13 (1.6 - -1.3)
Group B (n=37) (no concurrent use of urination drugs)	0.10±1.15†‡ (2.0 - -2.0)	0.88±0.88† (2.0 - -1.0)	1.04±0.83‡ (2.0 - -1.0)

*, **, †, ‡ : $p<0.01$

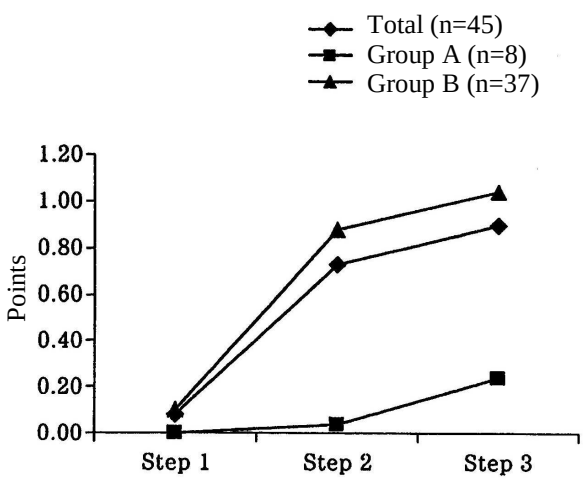

Fig. 4. Change in sleep satisfaction level

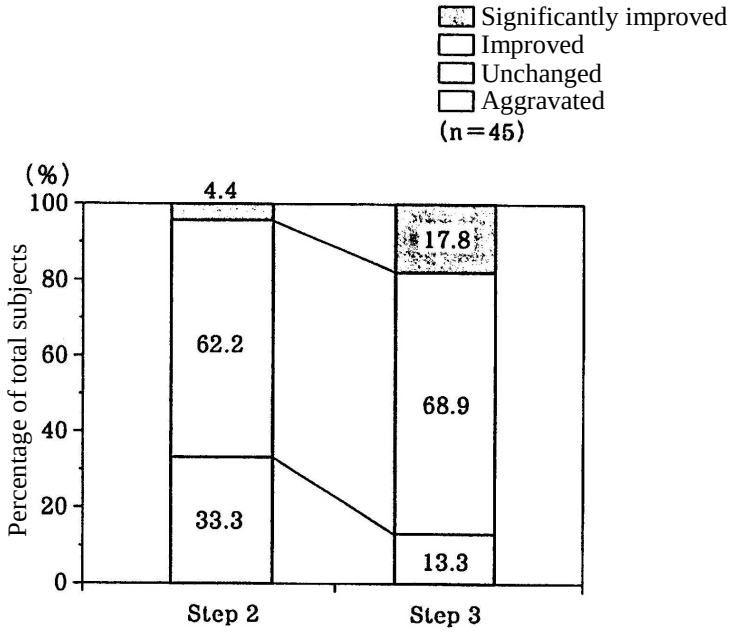

Fig. 5. Changes in subjective symptoms improvement

As shown in **Fig. 4**, a significant improvement was observed ($p<0.01$) in the total and Group B. In particular, when Step 1 and Steps 2 are compared, almost no change is observed in Group A, whereas there was a significant improvement in Group B.

[3] Improvement level of subjective symptoms

Fig. 5 shows the improvement levels of subjective symptoms which were comprehensively evaluated by the physician-in-charge by questioning and diary entries of 45 cases included in the efficacy evaluation. At Step 2, 2 cases (4.4%) were 'significantly improved' and 28 cases (62.2%) were 'improved'. At Step 3, 8 cases (17.8%) were 'significantly improved' and 31 cases (68.9%) were 'improved'. There was no 'aggravated' case in both periods.

2) Safety

Table 7 shows adverse events observed in subjective symptoms and objective observation. Abnormal changes in laboratory analyses are shown in **Table 8**. Adverse events were observed on 33 incidences in 22 out of 51 cases (43.1%). Among them, the relationship to PEP® could not be excluded on 4 incidences in 3 cases (5.9%). Other 29 incidences in 19 cases (37.3%) were judged to be not related to the test food.

Table 7. Adverse events identified by subjective symptoms and objective observation

Subject identification code	Age (years)	Symptom	Time of occurrence	Severity[*1]	Treatment/ procedure	Outcome	Relationship to test food[*2]
A-01	67	Diarrhea	Intake day 2	Mild	Yes	Disappeared on the day of appearance	No
A-04	66	Thoracic aortic aneurysm	Intake day 3	Severe	Yes	Improved	No
A-06	75	Common cold	Intake day 14	Mild	Yes	Disappeared on the next day	No
A-08	68	Abdominal distension	Intake day 4	Mild	No	Disappeared on the next day	Probably related
		Abdominal distension	Intake day 35	Mild	Yes	Disappeared after 5 days	Probably related
A-11	68	Cerebral infarction	Intake day 25	Severe	Yes	Improved	No
A-16	72	Itchiness	Intake day 10	Mild	No	Disappeared after 5 days	Probably related
C-01	74	Common cold	Intake day 23	Mild	No	Disappeared on the day of appearance	No
C-02	67	Common cold	Intake day 11	Mild	Yes	Disappeared on the day of appearance	No
C-05	66	Diarrhea	Intake day 16	Mild	No	Disappeared on the day of appearance	No
C-06	66	Constipation	Intake day 7	Mild	No	Disappeared after 2 days	No
C-10	70	Flu	Before intake	Mild	Yes	Disappeared after 9 days	No
C-11	70	Diarrhea	Intake day 7	Mild	No	Disappeared on the next day	No
C-12	65	Common cold	Intake day 8	Mild	No	Disappeared after 2 days	No
C-18	66	Common cold	Intake day 28	Mild	Yes	Disappeared after 2 days	No
		Gastric pain	Intake day 30	Mild	Yes	Disappeared on the day of appearance	No
		Diarrhea	Intake day 30	Mild	Yes	Disappeared on the day of appearance	No
		Abdominal distension	Intake day 1	Mild	Yes	Disappeared after 18 days	Probably related
C-19	66	Common cold	Intake day 1	Mild	No	Disappeared on the day of appearance	No
		Palpitation	Intake day 10	Mild	No	Disappeared on the next day	No
		Irregular pulse	Intake day 10	Mild	No	Disappeared on the next day	No
C-28	71	Common cold	Intake day 4	Mild	Yes	Disappeared on the day of appearance	No
		Toothache	Intake day 5	Mild	Yes	Disappeared after 3 days	No
		Flare in both forearms	Intake day 27	Mild	No	Disappeared after 11 days	No
C-30	81	Constipation	Intake day 3	Mild	No	Disappeared on the next day	No

*1: Severity: Physician-in-charge determines in reference to 'Severity Classification Standards of Drug Side Effects' (The Ministry of Welfare and Health Guideline).
Mild (Grade 1) / Moderate (Grade 2) / Severe (Grade 3)

*2: Causal relationship:
None: (There are clear evidences that other factors besides the test food are responsible or that it cannot be caused by test food from its pharmacological effects)
Probably related: (The causal relationship to test food cannot be excluded.)
Related: (Other factors, besides test food, cannot at all be responsible.)

[1] Subjective symptoms and objective observations

As shown in Table 7, abdominal distension developed sporadically 4 days after intake in case A-08. Similarly in case C-18, abdominal distension occurred 1 day after intake. In both cases, the relationship to PEP® could not be excluded, and the causal relationship was judged 'probably related'.

Itchiness developed 10 days after intake in case A-16. Although it had been observed previously in this subject, a relationship to PEP® could not be excluded, and it was judged 'probably related'.

[2] Laboratory analyses

Among the abnormal changes in laboratory analysis results shown in Table 8, a follow-up survey was performed on cases with abnormal changes at the time of study completion (or discontinuation). From the results, a relationship to PEP® was excluded in all these cases.

Table 9 shows the changes of laboratory values over time. The number of cases is shown for each category of urinalysis results. There was no case with clinically significant changes from the mean value of the cases included in the evaluation.

Table 8. Abnormal changes of laboratory values

Subject identification code	Age (years)	Analysis item	Standard range	Test values			Notes	Causal relationship
				Step 1	Step 2	Step 3		
A-02	75	AST (IU/L) ALT (IU/L) γ-GTP (IU/L)	8 - 38 4 - 44 4 - 63	31 34 406*	66* 61* 404*	52* 47* 591*	77* By follow-up (after 49 days) 58* By follow-up (after 49 days) 626* By follow-up (after 49 days)	None None None
A-05	67	Urinary glucose	–	–	–	⫴*	– By follow-up (after 40 days)	None
A-18	76	AST (IU/L)	8 - 38	18	24	58*	17 By follow-up (after 34 days)	None
B-02	78	Urinary glucose	–	–	⧺*	–	Transient change	None
C-02	67	BUN (mg/dL)	8.0 - 22.0	18.5	16.7	27.1*	19.9 By follow-up (after 8 days)	None
C-13	71	Urinary protein	–	–	⫴*		⧺* By follow-up (after 7 days)	None

*: Outsides the standard range

Table 9. Changes in laboratory values

Analysis item		Unit		Time of performance		
				Step 1 (n=51)	Step 2 (n=49)	Step 3 (n=45)
Blood biochemistry	AST	IU/L	Mean±SD (Max - Min)	23.1±7.0 (36 - 8)	23.2±8.5 (66 - 11)	24.1±8.5 (58 - 11)
	ALT	IU/L	Mean±SD (Max - Min)	22.5±9.3 (58 - 12)	22.3±10.1 (62 - 11)	21.0±9.2 (47 - 9)
	ALP	IU/L	Mean±SD (Max - Min)	214.9±78.5 (452 - 73)	215.7±79.7 (490 - 96)	222.7±80.9 (445 - 74)
	LDH	IU/L	Mean±SD (Max - Min)	248.0±93.5 (471 - 139)	243.3±99.1 (499 - 135)	235.5±97.3 (447 - 135)
	γ-GTP	IU/L	Mean±SD (Max - Min)	48.3±61.6 (406 - 10)	47.8±60.8 (404 - 10)	54.2±89.7 (591 - 10)
	BUN	mg/dL	Mean±SD (Max - Min)	16.0±4.6 (27.3 - 8.4)	16.3±4.2 (29.1 - 9.1)	16.3±4.0 (27.1 - 10.3)
	Creatinine	mg/dL	Mean±SD (Max - Min)	0.877±0.205 (1.40 - 0.40)	0.860±0.194 (1.34 - 0.40)	0.840±0.154 (1.31 - 0.50)
Urinalysis	Protein (qualitative)		⫴ ⧺ + ± –	0 2 2 8 39	1 1 1 9 37	0 0 1 4 40
	Glucose (qualitative)		⫴ ⧺ ± –	1 0 0 50	0 1 2 46	1 0 0 44
	Urobilinogen (qualitative)		+ ±	1 50	3 46	1 44

III. Discussions

The present study shows that the processed food (PEP®) containing of a mixture of edible Pumpkin seed EFLA®940 and soybean germ extracts significantly decreased urination frequency in the night and elevated sleep satisfaction by its intake twice a day for 6 weeks. In addition, an excellent efficacy was shown by the improvement level of subjective symptoms after completion of intake in which 86.7% of subjects (39/45 cases) evaluated 'significantly improved' or 'improved'.

No significant changes were observed in either urination frequency in the night or sleep satisfaction in Group A (the group which continued to use drugs for pollakiuria during intake: n=8). On the other hand, sleep satisfaction improved as urination frequency in the night decreased in Group B (the group without concurrent use of drugs: n=35), showing a correlation between urination frequency in the night and sleep satisfaction.

Since four out of the six cases whose improvement level of subjective symptoms was 'unchanged' at the end of Step 3 were in Group A, PEP® is not effective in the cases with complications of prostatic hypertrophy and neurogenic bladder. However, it is very interesting that there were four out of seven subjects with complications of prostatic hypertrophy whose improvement level of subjective symptoms was 'improved' at Step 3, suggesting improvement of the symptoms in the early stage of prostatic hypertrophy including urination dysfunction such as urinary tract blockage and urination urgency. Many types of drugs were used for pollakiuria in 8 cases, but there were no differences in improvement level between drugs.

A high percentage of people have symptoms of pollakiuria in the night. However, most of them do not seek medical attention. In fact, the subjects in the present study had symptoms of pollakiuria in night for relatively long periods, an average of 5.3±3.8 years, but only 15.7% of the total subjects used drugs for pollakiuria. Thus, it is clear that few people attempt to receive treatment even in the presence of symptoms.

Among elderly persons over the age 65, the risk of falling is 2.15 fold higher for those whose urination frequency in the night is not less than three, as compared to those with urination frequency in the night of less than one[1)]. Thus, pollakiuria in night influences the QOL significantly. In the present study, there were comments that subjective sleep condition was improved not only for the subjects themselves, but also for family members and care-takers who attend to the subject's need for urination. Thus, improvement of QOL can be expected from the use of PEP® in patients as well as people involved in their lives.

Adverse events were observed on 33 incidences in 22 out of 51 cases (Tables 7 and 8). There were no clinically significant changes in laboratory analyses. In subjective symptoms and objective observations, itchiness was observed in one case. Since its cause was not clear, the relationship to PEP® was judged 'unknown'. Two cases complained of abdominal distension whose relationship to PEP® could not be excluded. Similar adverse events were reported in the study of PEP® in abdominal pressure-associated urination incontinence[6)]. In consideration of the report on side effects in the digestive systems by plant extract drugs such as Eviprostat, the causal relationship was judged 'probably related'. The tablet of PEP® is relatively large, and three to five tablets are taken at once, needing a significant volume of water upon swallowing. The large volume of water intake could have caused the abdominal distension. The relationship to PEP® was excluded for other adverse events. Since prohibition of concurrent use, side effects, and the interaction with other drugs are not reported on Pumpkin seed in the guideline for plant therapeutics by the Ministry of Health of Germany, PEP® is highly safe.

A large number of therapeutic drugs for pollakiuria including α, β receptor stimulants and estrogen drugs are available for treatment for pollakiuria in night, but cautions are required upon their use due to side effects, complications and restricted drugs for concurrent use. On the other hand, there was no development of adverse events which were judged 'related', even when subjects with various backgrounds took PEP®. In addition, there was no case in which subjective symptoms were 'aggravated'.

Thus, the nutrition supplemental food PEP® can be safely used regardless of complications and concurrent use of drugs due to the components in edible Pumpkin seed EFLA®940 and soybean germ extracts. PEP® is a useful supplement as a first step for improvement of QOL for those who suffer from urination condition without seeking therapeutics.

Acknowledgement

Thanks are due to the CEO of Tervis Co., Ltd. Mr. Masaru Tatsuzaki for supplying PEP® and giving us opportunity to perform this study.

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Effects of Pumpkin Seed Extract on urinary bladder function in anesthesized rats

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Translated from Japanese

Effects of Pumpkin seed extract on urinary bladder function in anesthetized rats

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Summary

To confirm the clinical efficacy of 'Pepo Pumpkin seed: PEP®' (PEP®), the effects of its components, Pumpkin seed EFLA®940 and soybean germ extracts, were examined by cystometrogram using rats anesthetized with pentobarbital. Pumpkin seed water-soluble extract induced a significant decrease of excretion frequency and a significant increase of the excretion delay index, as measured by cystometrogram. In the experiments using different lots, the excretion frequency per one minute was 1.62±0.38 times and 0.58±0.14 times, respectively, before and after administration of 250 mg/kg of Lot No. 3038141 (n=3) ($p<0.05$). The excretion frequency was 1.51±0.20 times after solvent administration, with no difference from that before administration. With the same concentration of Lot No. 3036525 (n=4), it was 2.61±0.66 times and 1.13±0.27 times, respectively, before and after administration ($p<0.001$) whereas it was 2.41±0.43 times after solvent administration, with no difference from that before administration. When the excretion delay index before administration was taken as one, it was 1.06±0.15 after solvent administration vs. 2.96±1.19 after administration of Lot No. 3038141 (n=3) ($p<0.05$). After administration of Lot No. 3036525 (n=4), it was 2.33±0.35 vs. 1.08±0.19 by solvent administration ($p<0.001$).

On the other hand, the excretion frequency and excretion delay index both showed no significant difference before and after administration of 250 mg/kg of soybean germ extract.

These results suggest that Pumpkin seed extract EFLA®940 decreases in-bladder pressure and increases the maximal bladder capacity. Pumpkin seed water-soluble extract therefore appears to mediate the clinical efficacy of PEP®.

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Effects of Pumpkin seed extract EFLA®940 on urinary bladder function in anesthetized rats

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Key words: pumpkin seed, pollakiuria, systometrogram, urinary bladder function, anesthetized rat

Introduction

Due to increasingly aging society, the number of patients with urination dysfunction has been increasing in Japan as in the United States and Europe, and it is estimated to be 4 to 5 millions, including those who do not receive medical treatments. The cause of urination dysfunction is not clearly known, although various causes are known to contribute. Particularly, older persons regard it as an aging symptom, rather than a disease, and leave it untreated. Although frequent urination and urinary incontinence are not symptoms which threaten life, they significantly reduce the QOL of patients and contribute to psychological and physical burden. In the aged, men present with frequent urination and urinary incontinence associated with prostatic hypertrophy, while most of women present with abdominal pressure-associated symptoms and frequent urination.

Drugs for urination dysfunction sold in Japan include anticholinergics, bladder smooth muscle-directed drugs, Chinese medicines, and health foods. For long-term usage of these therapeutics, cautions are required due to side effects with severe symptoms from drugs and drug interactions with Chinese medicines. On the other hand, health foods are not associated with side effects, but they lack biochemical, pharmacological or clinical data to support their clinical efficacy.

A variety of health foods which are used for frequent urination and urinary incontinence are mostly processed food made from Pumpkin seeds or Saw palmetto. Whole seeds or oil extract of Pumpkin seeds have been used for benign prostatic hypertrophy-associated frequent urination and urinary incontinence for many years in Germany, and there are many reports describing their clinical efficacy[1)]. Active components present in seeds are known to be sterols, lignans, isoflavons and unsaturated fatty acids, but the mechanism of their actions are not known.

Processed food PEP® which is a mixture of Pumpkin seed water-soluble extract and soybean germ extract is health food used for frequent urination and urinary incontinence due to the properties both of Pumpkin seeds and soybean germ. Among health foods sold in Japan, it is the only one for which efficacy has been confirmed by clinical studies. Sogabe et al. reported the efficacy of night-time frequent urination in older women[2)]. The efficacy of abdominal pressure-associated urinary incontinence in older women was reported by Yanagisawa et al.[3)]. Terado et al.[4)] reported the efficacy of frequent urination in the night in older men. All these results showed an excellent clinical efficacy.

In the present study, the results of these clinical studies were tested by measurement of in-bladder pressure (cystometrogram) in rats. The effects on bladder function were examined using water-soluble extract of Pumpkin seeds EFLA®940 or soybean germ extract, both of which were used in PEP® (Tervis Co., Ltd.).

I. Experimental Methods

1. Test preparations

Water-soluble extracts of Pepo Pumpkin seeds were the preparations (EFLA®940: Lot No. 3038141 and 3036525) made by Frutarom Switzerland Ltd. (Wadenswil, Switzerland). Soybean germ extracts (ISOMAX-30: Lot No. 77900303) were manufactured by TOKIWA Phytochemical Co., Ltd. (Chiba, Japan).

The solvent preparation was 1% dimethyl sulfoxide (DMSO, Wako Pure-Chemical Industry Co., Ltd., Tokyo) diluted in sterile physiological saline (Ohtsuka Pharmaceutical Co., Ltd., Tokyo). Test preparations were dissolved in 1% DMSO at specified concentrations and sterilized by passing through 0.45 μm filter. Sterile preparations were stored in refrigerator until measurements.

2. Experimental animals

All animals were obtained from Nippon SLC Co., Ltd. (Shizuoka, Japan) and preconditioned for more than one week before use. Std:Wister male rats (Specific Pathogen Free-SPF grade) were older than 12 weeks and weighed not less than 300 g. Three to four animals were used for each group. Rats were kept in metal cage (Natsume Manufacture Co., Ltd.) in the isolated animal facility with 23±2°C temperature, 55±10% humidity and 12-hour lighting (7:00-19:00). Rats were fed ad lib with Mouse/Rats Solid Diet MF (Oriental Yeast Industry, Tokyo) and chlorine-disinfected, UV-sterilized water.

3. In-bladder pressure measurement[5)]

An incision was made in the mid-abdomen of rats anesthetized deeply with 0.5% sodium pentobarbital. Through a small incision made on the bladder cervix, a polyethylene tube (PE50) was inserted and tied. The other end of the tube was connected, through a three-way cock, to injectors attached to a 10-ml syringe and a transducer for in-bladder pressure measurement apparatus. Prior to administration of solvent and test preparations, sterile physiological saline was injected in the bladder at the flow rate of 0.04 ml/min to confirm stable urination reflex. In-bladder pressure was monitored on polygraph. Solvent and then test drug preparations were administered through a cannula inserted in the common cervical vein.

4. Group composition

Ten rats were assigned in the following three groups. Group name, number of animals and administration dose of test sample are as follows.

Group A: 3 animals, EFLA®940 Pumpkin seed water-soluble extract (Lot No. 3038141) 250 mg/kg

Group B: 4 animals, EFLA®940 Pumpkin seed water-soluble extract (Lot No. 3036525) 250 mg/kg

Group C: 3 animals, soybean germ extract (Lot No. 77900303) 250 mg/kg

5. Statistical analysis

All data were shown as mean±SD (standard deviation). Analysis of variance (ANOVA) and post-fox test by Fisher's PLSD

method were performed. The significant level was set at $p<0.05$.

II. Results

Fig.1 shows the effect on in-bladder pressure (cystometrogram). Cystometrogram was obtained for 5 to 8 minutes when in-bladder pressure became stable while sterile physiological saline was injected at the flow rate of 0.04 ml/min {when the maximal in-bladder pressure/the maximal peak of curve was obtained, and urination-associated, transient urination involution (pressure reduction) was observed}, at the time of solvent administration (1% DMSO), and after administration of test preparation. These curves were obtained from each separate animal.

After the start of sterile physiological saline injection, recording was initiated when stable urination was obtained. The number of urination frequency per 1 min was observed before administration, after solvent administration and after sample administration, in a total of three times (Urination Frequency, Table 1). Table 2 shows the urination delay indexes (-fold change) after solvent and sample administrations, when the urination frequency before administration was taken as one. As shown in Fig.1, in-bladder pressure patterns are similar before and after solvent administration in all animals of Group A, B, and C. On the other hand, a significant delay of in-bladder pressure curve was observed after administration of test samples (Pumpkin seed water-soluble extract) in Group A and B. With soybean germ extract in Group C, a delay of in-bladder pressure curve was not observed, showing a similar pattern to those before administration and after solvent administration.

With the administration of Pumpkin seed extract EFLA®940 Lot No. 3038141 at 250 mg/kg, there was no difference in urination frequency per one minute before and after solvent administration (1.62±0.38 times vs. 1.51±0.20 times), whereas it decreased to 0.58±0.14 times after extract administration. The urination delay index was 2.96±1.19 after extract administration, showing clearly a delay as compared to 1.06±0.15 with solvent administration. After extract administration, the statistical significance was $p<0.05$ for both urination frequency and delay index, as compared to before administration. It was $p<0.005$ for urination frequency and there was no statistical significance for urination delay index, as compared to those after solvent administration. Similarly with the different lot (Lot No. 3036525) at 250 mg/kg, there was no difference in urination frequency before and after solvent administration (2.61±0.66 times vs. 2.41±0.43 times) and the urination delay index was 1.08±0.19 after solvent administration, whereas it decreased to 1.13±0.27 times after extract administration, with the urination delay index of 2.33±0.35. The reproducibility between lots was confirmed from the analysis of statistical significance for the Lot No. 3036525; urination frequency: $p<0.001$ and urination delay index: $p<0.001$ vs. before administration, urination

frequency: $p<0.001$ and urination delay index: $p<0.001$ vs. after solvent administration.

On the other hand, with soybean germ extract at 250 mg/kg, urination frequency was 1.61±0.33 times and 1.51±0.30 times, respectively, before and after solvent administration, with a urination delay index of 1.07±0.07, whereas urination frequency was 1.51±0.13 times after extract administration, with a urination delay index of 1.09±0.31 times. Thus, there was no statistical significance in both urination frequency and delay index (-fold change).

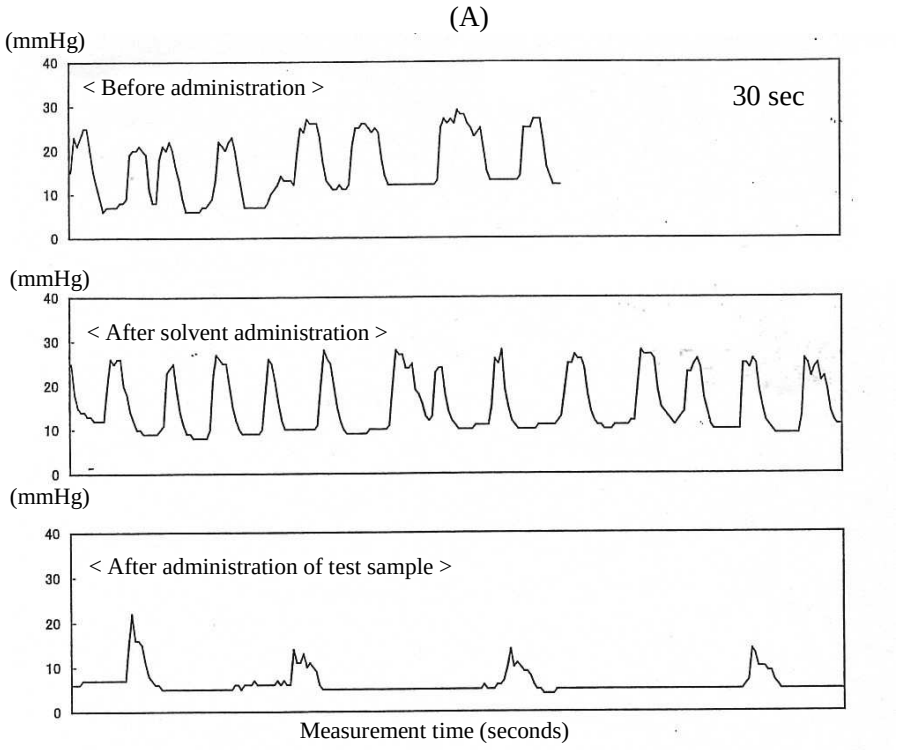
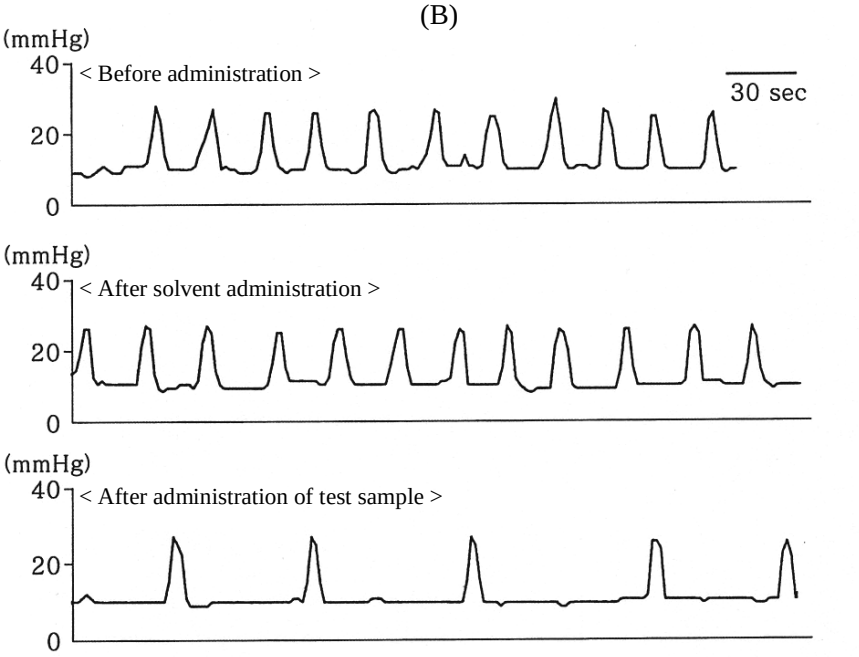


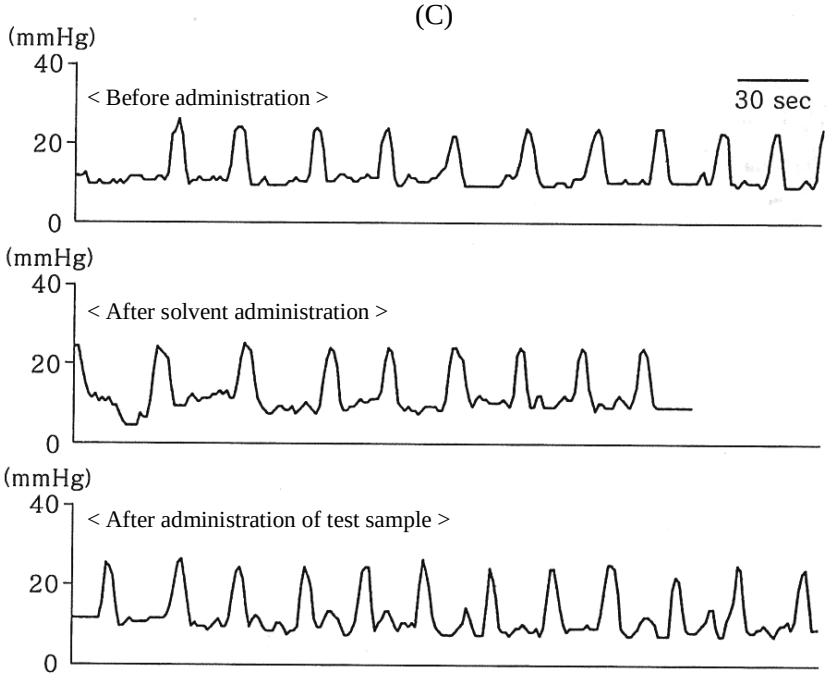
Fig.1 In-bladder pressure in rats
(A) EFLA®940 Pumpkin seed water-soluble extract Lot No. 3038141

(B)



Measurement time (seconds)

(C)



Measurement time (seconds)

Fig.1 In-bladder pressure in rats

(B) EFLA®940 Pumpkin seed water-soluble extract Lot No. 3036525

(C) Soybean germ extract Lot No. 77900303

The vertical line shows in-bladder pressure, and the horizontal line shows measurement time in seconds. The upper, middle and lower rows are urination curves before administration, after solvent administration, and after administration of test sample, respectively.

Table 1 Urination frequency (times/min)

Sample name	Administration concentration	Before administration (n)		After solvent administration (n)		After test sample administration (n)	
Pumpkin seed water-soluble extract (EFLA® 940 Lot No. 3038141)	250 mg/kg	1.62±0.38[a]	(3)[b]	1.51±0.20	(3)	0.58±0.14[c, e]	(3)
Pumpkin seed water-soluble extract (EFLA® 940 Lot No. 3036525)	250 mg/kg	2.61±0.66	(4)	2.41±0.43	(4)	1.13±0.27[d, f]	(4)
Soybean germ extract (ISOMAX-30 Lot No. 77900303)	250 mg/kg	1.61±0.33	(3)	1.51±0.30	(3)	1.51±0.13	(3)

a: Mean±SD

b: The number of animals used for analysis is in the parenthesis.

c: With a statistical significance in comparison to before administration ($p<0.05$)

d: With a statistical significance in comparison to before administration ($p<0.001$)

e: With a statistical significance in comparison to after solvent administration ($p<0.005$)

f: With a statistical significance in comparison to after solvent administration ($p<0.001$)

Table 2 Urination delay index with the urination frequency before administration as one (-fold)

Sample name	Administration concentration	Before administration (n)		After solvent administration (n)		After test sample administration (n)	
Pumpkin seed water-soluble extract (EFLA® 940 Lot No. 3038141)	250 mg/kg	1.00	(3)	1.06±0.15	(3)	2.96±1.19[c]	(3)
Pumpkin seed water-soluble extract (EFLA® 940 Lot No. 3036525)	250 mg/kg	1.00	(4)	1.08±0.19	(4)	2.33±0.35[d, f]	(4)
Soybean germ extract (ISOMAX-30 Lot No. 77900303)	250 mg/kg	1.00	(3)	1.07±0.07	(3)	1.09±0.31	(3)

Notes [a-f]: as shown in Table 1

III. Discussions

Cystometrogram makes possible comprehensive observation of bladder functions including bladder, urinary tract, and neuronal function controlling urination reflex. It therefore is widely used for clinical diagnosis and evaluation of urinary therapeutics using anesthetized animals[5]. To confirm the efficacy of health food PEP® (Tervis Co., Ltd., Tokyo, Japan) for which results of clinical studies have been reported[2)-4)], the present study examined the effects of Pumpkin seed and soybean germ extracts used for PEP® by cystometrogram using rats.

Water-soluble extracts of Pumpkin seeds, both Lot No. 3038141 (EFLA®940) and Lot No. 3036525 (EFLA®940), increased bladder volume. With the administration at 250 mg/kg, urination frequency was reduced to a half of that before administration, while there was no change after administration of solvent alone. On the other hand, urination frequency per one minute was unchanged after administration of soybean germ extract or solvent alone.

The urination delay index (-fold change) was about 2 to 3-fold after administration of Pumpkin seed extract EFLA®940, as compared to before administration, showing a significant increase from before administration. On the other hand, no increase was observed in the urination delay index (-fold change) after soybean germ extract, as compared to before administration and after solvent administration.

These results show that Pumpkin seed extract EFLA®940 decrease in-bladder pressure and increase bladder volume significantly (decrease of urination frequency and increase of urination delay index/-fold change), while soybean germ extract shows no effect on in-bladder pressure. Thus, the active components for reducing in-bladder pressure and increasing bladder volume appear to be present in water-soluble extract of Pumpkin seeds. Therefore, Pumpkin seed water-soluble extract EFLA®940 is a part of components supporting clinical effects of PEP®. According to the analysis by Japan Food Analysis Center and Nikaidoh et al.[6], there are no detectable levels of lignans or isoflavons. Sterols or tocophenols are undetectable. Cerens, which are rich in the prostate tissue and are suggested to contribute to suppression of prostatic hypertrophy, are undetectable. By amino acid analysis, arginine and glutamate are present two to ten-fold the concentrations of other amino acids. Persson et al.[1)7)] and Downie et al.[1)8)] suggested that arginine/NO metabolism is involved, independent of adrenaline and acetycholine, in relaxation of urination muscle at a stage of full bladder. Persson et al.[1)7)] suggested, using female Sprague-Dawley rats, that NG-L-nitroarginine or NG-L-nitroaruginin methylester induces bladder hyperactivity and reduction of bladder volume through tension increase of the bladder by metabolic inhibition of L-arginine/NO pathway. Therefore, arginine contained highly in Pepo Pumpkin seed extract appears to increase the productions of NO (nitrogen monooxide) via the arginine/NO pathway, which contributes to relaxation of the bladder and decrease of in-bladder pressure.

Thus, the present results strongly support the clinical data of PEP®.

Conclusions

To confirm the clinical results of processed food PEP®, a mixture of Pumpkin seed water-soluble extract EFLA®940 and soybean germ extract, the effects of these two components were examined by cystometrogram using rats.
The present study showed the following results;

1) Pumpkin seed water-soluble extract, by the administration at 250 mg/kg, significantly increases bladder volume, decreases urination frequency and increase urination delay index.
2) Soybean germ extract shows no effect on bladder volume.
3) The clinical effects of PEP® are mediated by components present in Pumpkin seed water-soluble extract EFLA®940.

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Cucurbita pepo

Possible influence on hormonal imbalance and incontinence

Pumpkin seeds are used in the modern phytotherapy for benign prostate hyperplasia (BPH) stages I and II. They are used by many men preventively as well as therapeutically. A further application could possibly be the treatment of stress incontinence concerning mainly women during post menopause.

Caesar B. Schmidlin, Matthias H. Kreuter

Introduction

The medical history of cucurbita pepo seeds goes back several centuries into the natives medicine of north and central America, where the medical drug was used as anthelmintic and diuretic.(1) Meanwhile cucurbita pepo is known world-wide, the seeds are mainly recommended for treatment of miction troubles for men with benign prostate hyperplasia in the stages I and II as well as for irritated bladder (2,3). The inability to hold back urine is highly actual, continuous and at the same time a very old suffering.

Urinary incontinence is a frequent, expensive and socially isolating illness. Both sexes and all age groups are concerned; the incidence rises with proceeding age. Concerned persons are often troubled with shame and despair and also with the question how to handle the urinary incontinence or how to find possible treatments. Not or not yet concerned persons are interested in possible measures for prevention and how to avoid or treat urinary disorders, which lead to urinary incontinence. Urinary incontinence has been defined by the International Continence Society as involuntary urine loss and is classified into different forms, among them the stress incontinence and the overflow incontinence (4). Mainly women are suffering from the stress incontinence with proceeding age, as on the other hand, the overflow incontinence is observed mainly in men with BPH (5,6). It is generally assumed that in both cases hormonal imbalance has to be regarded as concurring cause. In case of the BPH concerning elderly men, in the epithelium and in stroma of the prostate, as well as in case of stress incontinence, concerning mainly women during post menopause,
an altered ratio between androgens and estrogens (7,8) is measured. The observable, pathological altered ratio between testosterone and dihydrotesterone in man is accomplished probably through an overproduction of 5-α-reductase (9). Its inhibition leads to a reduced production of dihydrotestosterone, which affects the course of the disease positively. A weakened musculature of the closure apparatus and the pelvic floor is characteristic for stress incontinence. Through the inhibition of the peripheral aromatase and the resulting enhancement of the testosterone level, an anabolic effect in the sense of a muscle stabilisation for the women concerned can be expected. We were able to prove a direct inhibition in vitro and an indirect inhibition in vivo of the 5-α-reductase as well as a direct inhibition of the aromatase in vitro through a new developed lipid free cucurbita pepo extract. Therefore this publication is meant to deal with the influences of cucurbita pepo extracts on the hormonal imbalance in connection with stress incontinence concerning mainly women during post menopause as well as the overflow incontinence in case of BPH.

Methods

Aromatase

Aromatase inhibition was tested with human placenta homogenate containing 10mg/ml cucurbita extract (EFLA®940). The Metabolism products of (1β, 2-β-^{3}H)-Testosterone, catalysed by the Aromatase was measured after incubation with cucurbita pepo extract by measuring the released $^{3}H_20$ by means of liquid-szintillation spectroscopy. Measured according to Hartmann and Batzl (10).

5-α-Reductase in vitro

The inhibition of the 5-α-reductase was tested in human prostate homogenate as well as in prostate homogenate of rats. The prostate homogenate was incubated for 30 minutes at pH 5.5 with (4-^{14}C) testosterone. Prior of incubination following cucurbita pepo extract was

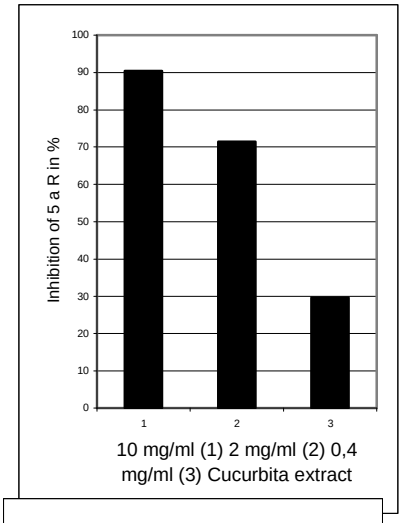
added. Concentrations: 10mg/ml, 2 mg/ml and 0,4 mg/ml.
Dihydrotestosterone (DHT) and testosterone were determined by HPLC. Additionally the inhibition of 5-α-reductase type II, experimented through human-embryonic-kidney-cells (HEK 293) was measured. For that purpose curcurbita extract (EFLA®940) was incubated in concentrations of 50mg/ml, 20mg/ml and 5 mg/ml together with ^{3}H-Androstendion for 150 minutes at 37℃, 5 percent $C0_2$ and 95 percent humidity.

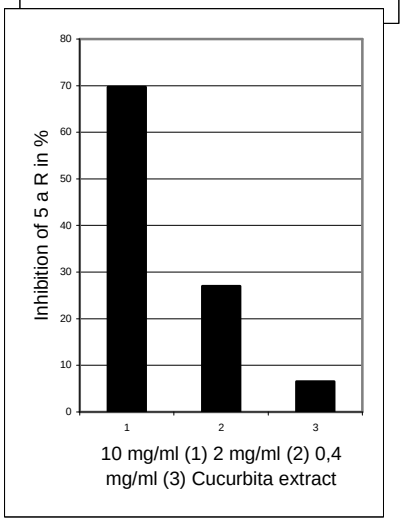
5-α-Reductase in vivo

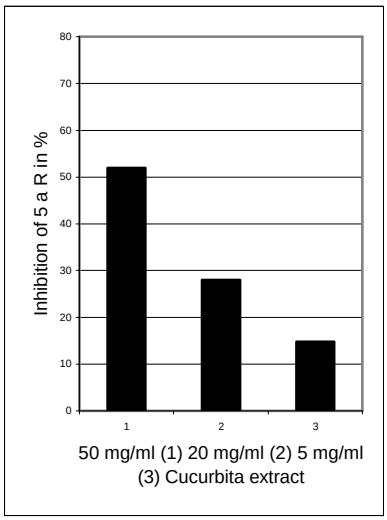
A prostate hypertrophy was induced in castrated, immature, male Sprague Dawley rats by applying subcutaneously 1mg/kg body weight of testosteronpropionate. Cucubita pepo extract was fed for four days at the dosage of 100mg/kg body weight. The influence on the prostate growth was detected considering the prostate weight after the treatment. As negative control group served rats which was treated with the vehicle (0.5 per cent of carboxymethylcellulose) and as positive control group served a group which was treated with finasterid 1mg/kg body weight injected subcutaneously. The animals had free access to water and were fed with standard laboratory food ad libitum.

Results
The measuring of aromatase inhibition was accomplished in three test series, consisting of each two-extract batches. With 10mg/ml Cucurbitae extract it caused a reduction of the aromatase activity of 55.9 (+/-15,7) % and 44,7 (+/-0,2) % for each batch.
The inhibition of 5-α-reductase type II in human prostate-homogenate had following effects: Curcurbitae extract 10mg/ml, 2mg/ml and 4 mg/ml: Activity reductions of 90,4 (+/-2,4) per cent 71,4 (+/-4,1) % and 29,6 (+/-7,9) %. (Image 1).
The inhibition of 5-α-reductase type II in human prostate of rats resulted under similar conditions as for human prostate-homogenate resulted in the activity reductions of the 5-α-reductase of 69,7 (+/-2,8) %, 27,0 (+/-1,5) % and 6,5 (+/-1,1) % (image 2).
The inhibition of the activity of 5-α-reductase type II, synthesised from HEK cells, by cucurbita extract in concentrations of 50 mg/ml, 20mg/ml and 5 mg/ml caused activity reductions of 52,0 (+/-2,4) %, 28,0 (+/-2,4) % and 14,8 (+/-7,8) % (image 3).
Castrated male rats treated with testosterone 1 mg/kg body weight, had a prostate weight of 41,6 (+/-1,9) mg/100g body weight after four days. Inhibition of the 5-α-reduktase with cucurbita extract 100mg/kg body weight or finasterid
1 mg/kg body weight caused a prostate weight of 32,8 (+/-2,0) mg/100g body weight respectively 20,5 (+/-1,5) mg/100g body weight. Vehicle treated rats had a prostate weight of 13,7 (+/-1,9) mg/100g body weight after four days. If the difference of the prostate weights of the testosterone treated group and the vehicle treated group is
100%, then the reduction of prostate weight by finasterid is 76 % ($p<0,01$, $n=7$) and by cucurbitae extract 31 per cent ($p<0,05$,$n=7$) (image 4)

Image 1
Image 2
Image 3



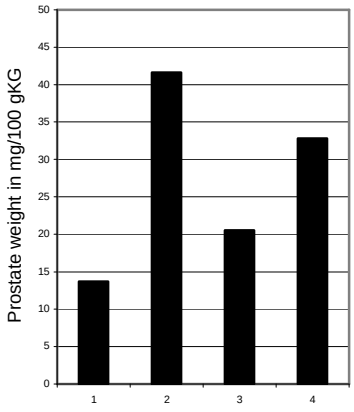




Discussion

The benign prostate hyperplasia stage I and II has been treated for some time palliative with cucurbita

pepo seed extracts. The therapeutical
Image 4
effect is achieved, as mentioned by an author, among other things, by



I: castrated rats, 2: castrated rats + Testosteron 1 mg/kg BW, 3: castrated rats + Finasterid 1 mg/kg BW, 4: castrated rats + Cucurbita extract 100 mg/kg BW

delta-7-phytosteroles which are occurring in cucurbita pepo in trace levels (11). Newer data point out that phytosteroles are absorbed only in very small quantities, which does not particularly support the assumption that phytosteroles are the main active principle of the medicinal pumpkin (Cucurbita) (12,13). The results of pharmacological studies, represented in this publication, which was accomplished with a lipid free cucurbita seed extract (EFLA®940), are indicating rather to polar principles to be effective. The results show clearly a inhibition of the 5-α-reductase in vitro in a standard animal model indirectly as well as in vivo. Finasterid as verum control additionally strongly supports the hypothesis; that also in case of cucurbita seed extract the observed growth inhibition of the prostate tissue in vivo is obtained through inhibition of 5-α-reductase.
A possible effect mechanism of the lipid free cucurbita seed extract in connection with BPH stages I and II can thus be assumed in the inhibition of the prostate connected 5-α-reductase . In addition the data shows that the growth of the prostate in the model of the prostate hyperplastic rat is slowed down. To what extent this retardation is achievable in humans, cannot be assessed at present, but it however is
not considered to be impossible anymore. A polar group of substances, which could have this inhibitory effect as a characteristic, is the recently discovered phenoglycosides in cucurbita, which are made responsible for the inhibition of the aromatase (14). As in the described experiments with EFLA®940 also an inhibition of the aromatase in vitro could be shown, it should be examined in what respect cucurbita seed preparations can be applied for succesful treatment of stress incontinence during post menopause. A recently performed open, clinical study in Japan, testing lipid free cucurbita seed extract, with 39 women suffering from stress incontinence during post menopause indicated in this direction (15). During the 8-week study, the number of daily episodes of incontinence dropped during the treatment continuously from 7,3 at the beginning of the study to 1,5 at the end of the study significantly. The achieved results make us reconsider the effective principles of cucurbita postulated so far in connection with BPH of the stages I and II. They are indicating an effect mechanism and are proposing to examine cucurbita seed preparations in detail for the treatment of stress incontinence in women.

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Maturitas. 2006 May 20;54(2):135-40. Epub 2005 Nov 16. ELSEVIER FULL-TEXT ARTICLE Links

The effect of a soy-rich diet on urogenital atrophy: a randomized, cross-over trial.

Manonai J, **Songchitsomboon S**, **Chanda K**, **Hong JH**, **Komindr S**.

Department of Obstetrics and Gynaecology, Faculty of Medicine, Ramathibodi Hospital, Mahidol University, Bangkok, Thailand. rajmo@mahidol.ac.th

OBJECTIVE: To evaluate the effect of a soy-rich diet on urogenital symptoms, vaginal health index, and vaginal cytology in perimenopausal and postmenopausal women. MATERIALS AND METHODS: Thirty-six perimenopausal and postmenopausal women (mean age 52.5+/-5.1 years) participated in a randomized, cross-over trial with two 12-week diet periods and two 4-week washout periods before and between treatments. The study diet consisted of a control diet (soy-free diet) and an isocaloric soy-rich diet (25 g soy protein in various forms of soy food containing more than 50 mg/day of isoflavones substituted for an equivalent amount of animal protein). Subjects were assessed for urogenital symptoms, vaginal health index, vaginal pH and vaginal cytology. The single physician and the single cytopathologist were blinded with regard to onset, period and randomization number. Statistical analyses were performed using paired t-test or Wilcoxon Signed Ranks Test, significance was set as $P<0.05$. RESULTS: Good compliance to the diet was shown by the significant elevation of serum levels of daidzein and genistein during the soy-rich diet period. The symptoms of urge incontinence and vaginal dryness had significantly increased after 12-week of soy-free diet. All other urogenital symptoms did not change in both periods. The vaginal health index, the vaginal pH, the karyopyknotic index, and the maturation value were not significantly changed in both periods. CONCLUSION: A soy-rich diet did not relieve the urogenital symptoms or restore the vaginal epithelium or improve the vaginal health in perimenopausal and postmenopausal Thai women.

Maturitas. 2007 Nov 20;58(3):249-58. Epub 2007 Oct 29. ELSEVIER FULL-TEXT ARTICLE Links

Efficacy and safety of a soy isoflavone extract in postmenopausal women: a randomized, double-blind, and placebo-controlled study.

Nahas EA, **Nahas-Neto J**, **Orsatti FL**, **Carvalho EP**, **Oliveira ML**, **Dias R**.

Department of Gynecology and Obstetrics, Botucatu Medical School, Sao Paulo State University-UNESP, Rubiao Junior, Botucatu, Sao Paulo 18618-970, Brazil. epetri@fmb.unesp.br

OBJECTIVE: To investigate the efficacy of soy isoflavone on climacteric symptoms in postmenopausal women. DESIGN: In this double-blind, randomized, placebo-controlled study, a total of 80 women (mean age = 55.1 years), who reported 5 or more hot flush episodes per day, were randomized to receive either 250 mg of standardized soy extract (Glycine max AT) a total of 100mg/day of isoflavone (n = 40) or placebo (n = 40). Exclusion criteria included: contra-indication for hormone therapy (HT), chronic gastrointestinal diseases, and users of HT within the preceding 6-months. For 10-months, climacteric symptoms were evaluated using a score card and the menopausal Kupperman index. Compliance and safety were also assessed. At baseline and the end of the study, lipid and hormonal profiles, as well as vaginal, mammographic and ultrasonographic parameters were measured. The t-test, Wilcoxon test and ANOVA were used in the statistical analysis. RESULTS: At baseline, the mean number of hot flushes was 9.6 +/- 3.9 per day in the isoflavone group and 10.1+/-4.9 in the placebo group ($p>0.05$). After 10 months, there was a significant reduction in frequency of hot flushes among isoflavone users when compared to those on placebo (3.1 +/- 2.3 and 5.9 +/- 4.3, respectively) ($p<0.001$). Kupperman index mean values showed a significant reduction in both groups. However, soy isoflavone was significantly superior to placebo, in reducing hot flush severity (69.9% and 33.7%, respectively) ($p<0.001$). Endometrial thickness, mammography, vaginal cytology, lipids and hormonal profile did not change in both groups. No serious adverse event related to isoflavone treatment was reported. CONCLUSIONS: The soy isoflavone extract exerted favorable effects on vasomotor symptoms and good compliance, providing a safe and effective alternative therapeutic for postmenopausal women.

Menopause. 2007 Nov-Dec;14(6):1006-11. Wolters Kluwer | Lippincott Williams & Wilkins Links

Comment in:
Menopause. 2007 Nov-Dec;14(6):976-7.

Endometrial safety assessment of a specific and standardized soy extract according to international guidelines.

Palacios S, **Pornel B**, **Bergeron C**, **Chantre P**, **Nogales F**, **Aubert L**, **Vazquez F**, **Eden J**, **Mares P**.

Instituto Palacios, Madrid, Spain.

OBJECTIVE: To assess the effects of an oral soy isoflavone extract (Phytosoya) on endometrium (evaluated by biopsy and ultrasonography) in postmenopausal women treated for 12 months. DESIGN: A total of 395 postmenopausal women were included in this international prospective, open-label study. The women were treated for 12 months with a specific standardized soy isoflavone extract (total of 70 mg/d). Endometrial biopsy and transvaginal ultrasonography were performed before and after 12 months of treatment according to European guidelines. RESULTS: A total of 301 assessable biopsy specimens were obtained from women treated for 12 months; the results were 99.67% atrophic/inactive endometrium and 0.33% proliferative endometrium. No case of hyperplasia or carcinoma was diagnosed, demonstrating the endometrial safety of this extract (point estimate: 0.0; upper limit of 95% CI: 0.012). Endometrial thickness did not show any increase after 12 months of treatment (2.2 mm at inclusion and 2.12 mm at the end of the study). Only eight women reported some kind of bleeding as an adverse event during the study. CONCLUSIONS: These results of endometrial biopsy and endometrial thickness suggest that daily administration of 70 mg of a specific and standardized isoflavone extract for 12 months does not stimulate the endometrium.

Age Ageing. 2009 Jan;38(1):86-93. Epub 2008 Dec 2.

A preliminary study of the safety, feasibility and cognitive efficacy of soy isoflavone supplements in older men and women.

Gleason CE, **Carlsson CM**, **Barnet JH**, **Meade SA**, **Setchell KD**, **Atwood CS**, **Johnson SC**, **Ries ML**, **Asthana S**.

Section of Geriatrics and Gerontology, Department of Medicine, University of Wisconsin, Madison, WI 53705, USA. ceg@medicine.wisc.edu

BACKGROUND: a small number of reports exist on the cognitive effects of soy isoflavones, the findings from which are mixed. Isoflavone efficacy is dependent upon conversion of glycosides contained in soy foods and supplements to the biologically active aglycons. Of particular interest is the production of the metabolite, equol, which is dependent upon intestinal microflora and an integrous digestive system, both being altered by age and age-associated conditions. Unfortunately, few studies enrolled adults over the age of 70, and none included older men. OBJECTIVE: we examined safety, feasibility and cognitive efficacy of soy isoflavone administration in older nondemented men and women (age 62-89 years). DESIGN AND METHODS: in this randomised, placebo-controlled, double-blind pilot study, subjects ingested either 100 mg/day soy isoflavones (glycoside weight) or matching placebo tablets for 6 months. RESULTS: active and placebo-treated subjects exhibited a comparable side-effect profile. Plasma levels of genistein and daidzein ($P < 0.001$), but not equol, increased with isoflavone administration. While similar at baseline, the two groups differed across 6 months of treatment on 8 of 11 cognitive tests administered. Isoflavone-treated subjects improved on tests of visual-spatial memory ($P < 0.01$) and construction ($P = 0.01$), verbal fluency ($P < 0.01$) and speeded dexterity ($P = 0.04$). Placebo-treated participants were faster than isoflavone-treated subjects on two tests of executive function ($P < 0.05$). CONCLUSIONS: these data suggest that administration of 100 mg/day of isoflavones was well tolerated. Plasma genistein and daidzein levels, but not equol, increased with isoflavone administration. Finally, data support the potential cognitive effects of soy isoflavones in older adults.

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GoLess™ reference list of major studies

Last update: June 2011

Study title	Year	Country of research	Publication	Frutarom Study Sheet
Go-Less™ – High effectiveness in key symptoms of female overactive bladder syndrome and top level of patients' satisfaction	2010	Korea	Unpublished data	06.11
Effects of EFLA®940 / Soybean Germ Extract combination on pollakiuria in elderly men	2004	Japan	Terado T et al., Jpn J Med Pharm Sci, 2004 Vol 52 (4)	07.07
Effects of EFLA®940 / Soybean Germ Extract combination on stress urinary incontinence in an open clinical study	2003	Japan	Yanagisawa Ei et al., Jpn J Med Pharm Sci, 2003 Vol 50 (3)	07.07
Effects of EFLA®940 / Soybean germ Extract combination on nocturia in an open clinical study	2001	Japan	Sogabe H and Terado T, Jpn J Med Pharm Sci, 2001 Vol 46 (5)	07.07
Studies (in-vivo)				
Effects of Pumpkin Seed Extract on urinary bladder function in anesthetized rats	2005	Japan	Hata L et al., Jpn J Med Pharm Sci, 2005 Vol 54 (3)	07.07

STUDY SUMMARY

Go-Less™ – High effectiveness in key symptoms of female overactive bladder syndrome and top level of patients' satisfaction

- **EFLA®940 Pumpkin seed extract – Cucurbitae Sem extr.s.sicc**
- **SoyLife® 40 – Soy germ extract**

To investigate the efficacy and safety of a product consisting of a defined mixture of EFLA®940 and SoyLife® 40 (Soy germ extract) a placebo-controlled, randomized clinical trial was conducted in Korea on women suffering from bladder related voiding dysfunction.

Study Design

Subjects: 120 women aged 35-70 years (60 placebo/60 test group)

Test substance: 500 mg Tablets containing 218.75 mg of Pumpkin seed extract (EFLA®940) and 31.25 mg of Soy germ extract (SoyLife® 40)

Dosage: 4 Tablets per day

Duration: 12 weeks
Subjects were asked to maintain a healthy daily lifestyle including diet and exercise; health functional foods, medicines or non-medicine products that could affect the research results were prohibited.
A bladder diary was evaluated to measure the day and night amounts and frequency of urination and the frequency of urgency and incontinence. At each clinic visit flow rate and remaining urine were measured, a survey was conducted using OAB-q V8 (Overactive Bladder Questionnaire V8 Symptom Irritation scale) to evaluate the improvement of overactive bladder syndrome and quality of life, and SQoL-F (sexual quality of life questionnaire – female) to evaluate the improvement of the quality of sexual life.
For safety evaluation, vital signs, blood and urine tests were performed at each visit. The presence of unexpected reactions and adverse effects were evaluated through interviews with the individual subjects.

Statistical analysis: Changes in blood, overactive bladder syndrome and urine dysfunction of each group by period were analyzed using a paired t-test, and a comparison between groups about the improvement level of each element at week 12 after taking the trial product was conducted through an unpaired t-test. In each statistical analysis, $p<0.05$ was considered to be statistically significant. Only those subjects that were compliant to the treatment and completed all measurements including the 12 weeks measurements were included in the statistical analyses for all time points.

Results

There is a significant decrease of the key symptoms of overactive bladder syndrome and voiding symptoms, demonstrated by a decrease of the daily average frequency of urination, a decrease of the daily average frequency of urgency and a decrease of average frequency of nocturia. These decreases amount for each parameter at an average of 30% are shown graphically in the following way.

- Decrease of the daily average **frequency of urination** with statistical significance in the test group **(- 27%)**

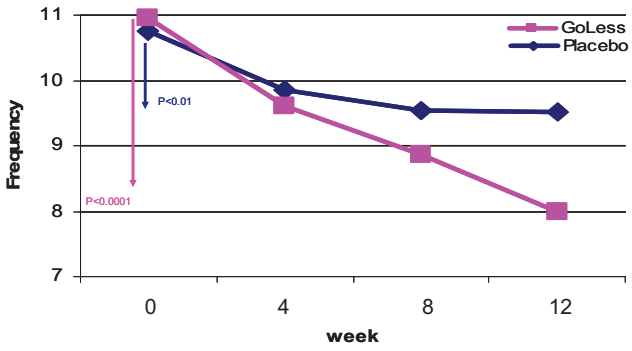


Fig 1. Changes of daily average frequency of urination (times)

- Significant decrease of the daily average **frequency of urgency** in the test group **(- 31 %)**

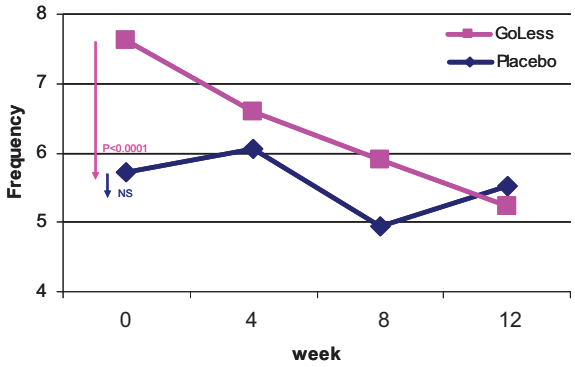


Fig 2. Changes of daily average frequency of urgency (times)

- Decrease of the average **frequency of nocturia** with statistical significance in the test group **(- 31 %)**

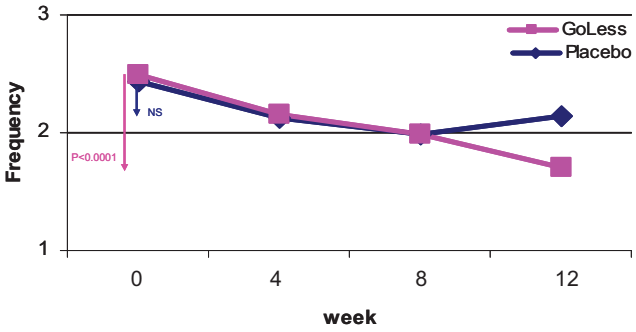


Fig 3. Changes of daily average frequency of nocturia (times)

Not only symptoms of overactive bladder syndrome, but also the quality of life and the satisfaction level were positively assessed by the patients.

- significant improvement of quality of life (22%), measured on the OAB-q V8 Symptom Irritation Index at week 12
- high satisfaction level of the treated patients: positive response by 90.5% of the test group and will to continuation of therapy by 95.2% (compared to 56.1% rsp. 46.3% of the placebo group)

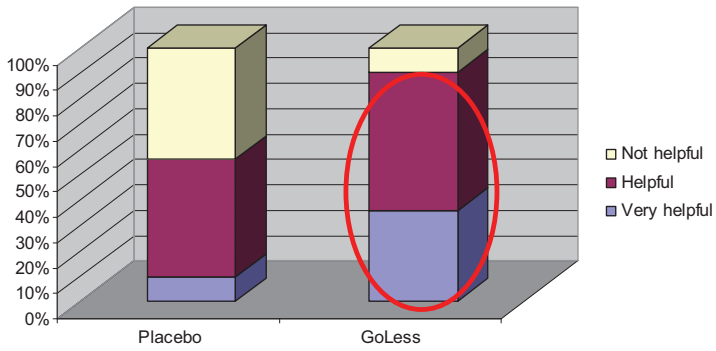


Fig 4. Subjective satisfaction level of participants in the trial (week 12)

Safety

- No particular findings in blood and urine tests
- All adverse reactions were temporary and minor, and none lead to drop outs from the trial

Conclusion

12-week Supplementation with the mixture of Pumpkin seed extract EFLA®940 and Soylife® 40 Soy germ extract lead to statistically significant improvements from placebo in urination frequency, nocturia frequency, frequency of urgency, urgency score and overactive bladder irritation scale.
The most important symptom in the definition of overactive bladder syndrome is urgency. Furthermore, the most important key element of the success of the improvement of overactive bladder syndrome is the improvement of elements related to urgency, since the biggest inconvenience, in practice, for people suffering from overactive bladder syndrome is urgency. In this clinical trial, there was a statistically significant improvement at week 12 in the average frequency of urgency and the average urgency score for the test group, compared to placebo.
Women with overactive bladder syndrome were able to confirm the functionality of EFLA®940 and SoyLife® 40 in voiding health through this human trial, and the role of these products can be positively considered, regarding the costs, adverse effects, reduced compliance and recurrence rate of the pharmacological treatment and behaviour modification treatment that has become popular recently.
In conclusion, the combination of EFLA®940 and SoyLife® 40 provides fundamental assistance to the voiding health of women with overactive bladder syndrome.

06.11

STUDY SUMMARY

Effects of EFLA®940 / Soybean Germ Extract combination on pollakiuria in elderly men

- EFLA®940 Pumpkin Seed Extract - Cucurbitae Sem extr.s.sicc
- Soybean germ extract

To investigate the efficacy and safety of a health food product containing EFLA®940 and soybean germ extract in the treatment of pollakiuria at night, an open clinical study was conducted in Japan on elderly men suffering from the disease.

Study design

Subjects: 45 men (> 65 years) suffering from pollakiuria at night. Study duration: 6 weeks. Daily dosage: 875 mg EFLA®940 and 167 mg soybean germ extract during weeks 1-2, 525 resp. 100 mg during the following weeks. Participants were asked to record diurnal and nocturnal urination frequency and subjective degree of satisfaction daily. Efficacy was rated as one of four grades by the investigator. Statistical significance was deter-mined by comparison of the mean before (initial) and after dosing using paired t-test. Laboratory tests and recorded vital signs and adverse events were analyzed for safety and tolerance assessment.

Results

Change in urination frequency

For all analysis 3 groups were built; Total, group A (with concurrent use of pollakiuria drugs) and group B. Within Group B and Total the frequency of nocturnal urination already markedly improved at week 2 of treatment, with a further decrease after 6 weeks of treatment (both $p<0.01$). Whereas, in group A no differences were seen.

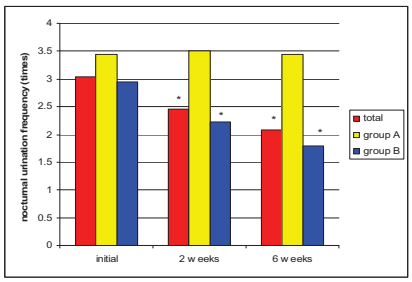


Fig.1: Times of nocturnal urinary frequency throughout the study period (n=45) (group A n=8, group B n=37). Data represent mean values, * $p<0.01$.

Sleeping satisfaction

The sleep satisfaction level was scored the following; satisfied 2 points, slightly satisfied 1, slightly unsatisfied -1, unsatisfied -2. A significant improvement was seen in the Group B and the Total ($p<0.01$).

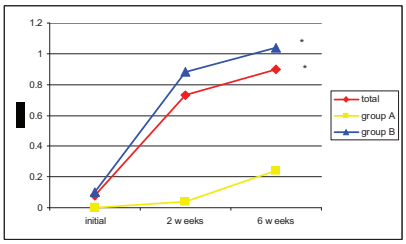


Fig.2: Sleep satisfaction improvement. Data represent mean values, * $p<0.01$.

Total rate of improvement

EFLA®940/soybean extract combination also significantly improved the quality of life, as concluded from the participants' subjective judgment.

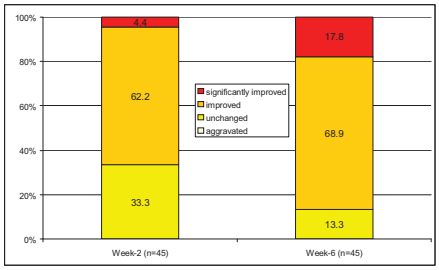


Fig. 3: Changes in subjective symptoms improvement.

The improvement level of patients who suffered from complications from prostatic hypertrophy remained unchanged whereas patients suffering from prostatic hypertrophy in the early stage showed increased levels of improvement.

Safety

The study product showed high compliance and demonstrated high safety and tolerance.

Conclusion

In conclusion, the present study demonstrated the beneficial effects of the food supplement containing a mixture of EFLA®940 Pumpkin Seed Extract and soybean germ extract in elderly men with pollakiuria and early prostatic hypertrophy. The intake of the EFLA®940 containing preparation quickly and significantly improved objective and subjective symptoms resulting in an enhanced quality of life.

Reference:

Terado, T et al (2004). Clinical study of mixed processed food containing pumpkin seed extract and soybean germ extract on pollakiuria in night in elderly men. Jpn J Med Pharm Sci; 52(4): 551-561.

STUDY SUMMARY

Effects of EFLA®940 / Soybean Germ Extract combination on stress urinary incontinence in an open clinical study

- EFLA®940 Pumpkin Seed Extract - Cucurbitae Sem extr.s.sicc
- Soybean germ extract

To investigate the efficacy and safety of a health food product containing EFLA®940 and soybean germ extract in the treatment of urinary incontinence, an open clinical study was conducted in Japan on pre- and postmenopausal women suffering from stress urinary incontinence.

Study design

Subjects: 50 women (aged 35-84) suffering from stress incontinence. The study was performed on two study sites. Study duration: 6 weeks. Daily dosage: 875 mg EFLA®940 and 164 mg soybean germ extract (30 % soy isoflavones) during weeks 1-2, 525 mg respectively 100 mg during the following weeks.
Participants were asked to record incontinence episodes and subjective degree of satisfaction daily. Efficacy was rated as one of four grades by the investigator. Statistical significance was determined by comparison of the mean before (initial) and after dosing using paired t-test. Laboratory tests and recorded vital signs and adverse events were analysed for safety and tolerance assessment.

Results

Incontinence episodes

The frequency of urination incontinence per day already markedly improved at week 2 of treatment, with a further decrease after 6 weeks of treatment.

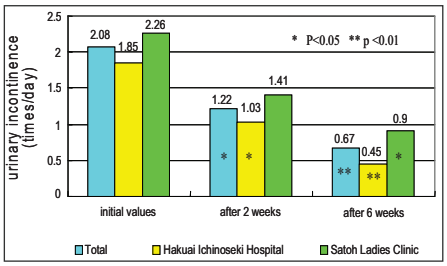


Fig.1: Number of urinary incontinence episodes per day throughout the study period (n=48). Data represent mean values.

Dividing the subjects into groups, based on the frequency of incontinence before treatment, resulted in a significant decrease in incontinence in the group of patients showing the highest initial number of incontinence episodes.

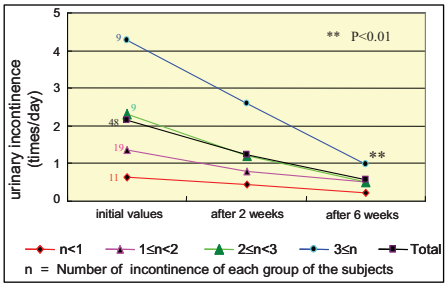


Fig.2: Number of urinary incontinence throughout the study period, dividing subjects into groups. Data represent mean values.

Total rate of improvement

EFLA®940/soybean germ extract combination also improved the quality of life, as concluded from the participants' subjective judgement.

All of the favorable effects described above were reflected by a high general improvement rate, as confirmed by the overall efficacy judged by the investigator based on all data. From a total improvement rate of 87.5% after two weeks of treatment, 10.4% of the patients were graded "Markedly improved". After six weeks of treatment the percentage of this "Markedly improved"-group even increased to 27.1% whereas the total improvement rate stayed at 87.5%.

Safety

The study product showed high compliance and demonstrated high safety and tolerance.

Conclusion

The present study demonstrated the beneficial effects of the food supplement containing a mixture of EFLA®940 Pumpkin Seed Extract and soybean germ extract in pre- and postmenopausal women with stress urinary incontinence. The intake of the EFLA®940 containing preparation quickly and significantly improved objective and subjective symptoms of women with stress urinary incontinence, resulting in an enhanced quality of life.

Reference:
Yanagisawa, Ei et al (2003). Study of Effectiveness of Mixed Processed Food Containing Cucurbita Pepo Seed Extract and Soybean Seed Extract on Stress Urinary Incontinence in Women.
Jpn J Med Pharm Sci; 14(3): 313-322.

STUDY SUMMARY

Effects of EFLA®940 / Soybean Germ Extract combination on nocturia in an open clinical study

- EFLA®940 Pumpkin Seed Extract - Cucurbitae Sem extr.s.sicc
- Soybean germ extract

To investigate the efficacy and safety of a health food product containing EFLA®940 and soybean germ extract in the treatment of nocturia, an open clinical study was conducted in Japan on postmenopausal women suffering from stress incontinence.

Study design

Subjects: 39 postmenopausal women (aged 52-86 years) suffering from increased night urinary frequency. Study duration: 6 weeks. Daily dosage: 875 mg EFLA®940 and 164 mg soybean germ extract (30 % soy isoflavones) during weeks 1-2, 525 mg resp. 100 mg during the following weeks. Participants were asked to record nocturnal and diurnal micturitions, incontinence episodes and subjective degree of satisfaction daily. Efficacy was rated as one of four grades by the investigator. Statistical significance was determined by comparison of the mean values before (pre) and after dosing using paired t-test. Laboratory tests, vital signs and adverse events recording were analysed for safety and tolerance assessment.

Results

Frequency of urination

The frequency of urination during night and daytime already markedly improved at week 1 of treatment. Night urinary frequency continuously improved thereafter until week 6, daytime values remained stable at the level reached at week 2.

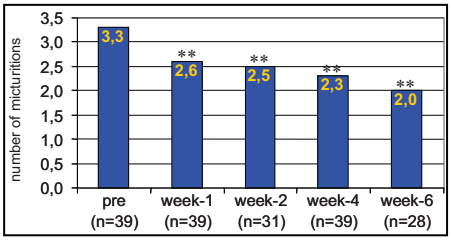


Fig.1: Number of nocturnal micturitions throughout the study period. Data represent mean values: ** $p < 0.01$

Incontinence episodes

Frequency of incontinence markedly diminished continuously during the study period, in the group of 16 subjects who experienced episodes of incontinence during the observation period.

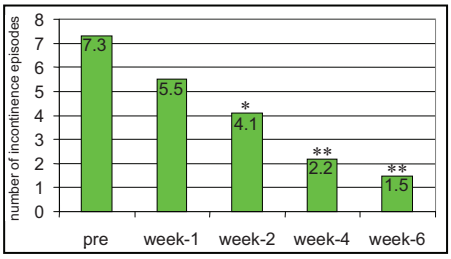


Fig.2: Number of incontinence episodes (n=16). Data represent mean values: ** $p < 0.01$, * $p < 0.05$

Subjective improvement

EFLA®940/soybean germ extract combination improved patients' subjective sleeping satisfaction.

Total rate of improvement

All the favorable effects quoted above were reflected by a high general improvement, as confirmed by the overall efficacy judged by the investigator based on all data. Total improvement was rated for almost 75 % of patients as "Markedly improved" and "Improved" after 6 weeks. In the subgroup of 33 subjects with 2 to 4 episodes of nocturia per night, the improvement rate was even higher (> 80%).

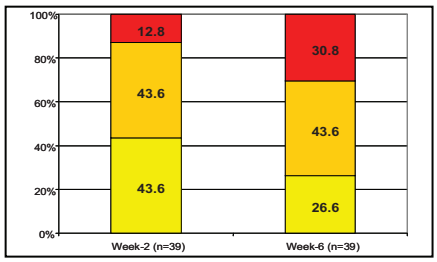


Fig.4: Total ratio of improvement rated as "Markedly improved" (■), "Improved" (■), "No change" (■) and "Worsened" (■).

Safety

The study product demonstrated high safety and tolerance.

Conclusion

The present study demonstrated the beneficial effects of the food supplement containing a mixture of EFLA®940 Pumpkin Seed Extract and soybean germ extract in postmenopausal women. The intake of the preparation quickly, continuously and significantly improved objective and subjective symptoms of women with urinary problems, resulting in an enhanced quality of life.

Reference:

Sogabe H, Terado T, 2001. Open clinical study of effects of pumpkin seed extract/ soybean germ extract mixture-containing processed food on nocturia. Jpn J Med Pharm Sci; 46(5): 727-37

07.07

STUDY SUMMARY

Effects of EFLA®940 on *in situ* urinary bladder function in anesthetized rats

- EFLA®940 Pumpkin Seed Extract - Cucurbitae Sem extr.s.sicc
- Soybean germ extract

Efficacy for EFLA®940 in the treatment of urinary incontinence and overactive bladder has been obtained in clinical trials in a combination with soybean germ extract. To separately study the effect of both components on the muscle function of the bladder, a rat study was conducted.

Study design

Test animals were divided into 3 groups: - 3 rats 250 mg/kg EFLA®940 Batch#A.
- 4 rats 250 mg/kg EFLA®940 Batch#B.
- 3 rats 250 mg/kg soybean germ extract.

All samples were dissolved in 1% DMSO and applied intravenously. Rats were deeply anesthetized and in-bladder pressure as well as urination frequency and urination delay index were measured by cystometry. Statistical significance was determined by comparison of the mean before and after administration of the test samples using ANOVA and post-fox test by Fisher's PLSD method.

Results

Bladder pressure curves

The obtained cystometograms showed no difference in pressure curves before and after the injection of the solvent, 1% DMSO. Therefore, it can be concluded that the results after the administration of EFLA®940 and the soybean germ extract are related to the substances and not to the solvent and therewith confirm the method's reliability.

The urination frequencies significantly decreased after administration of both batches of EFLA®940 compared to before administration. No effects were seen after the administration of soybean germ extract.

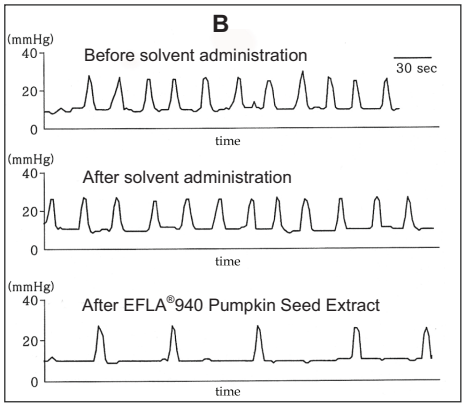


Fig.1a: Cystometogram: Group B, (Group A not shown)

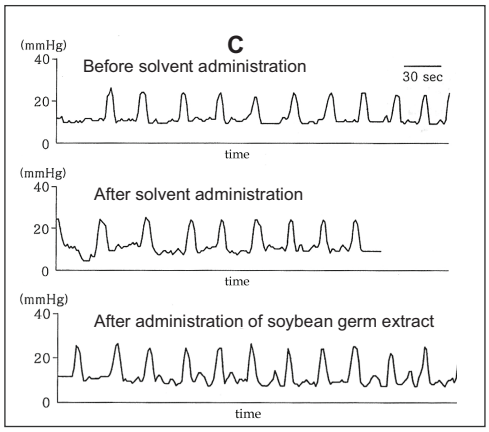


Fig.1b: Cystometogram: Group C; in-bladder pressure (mmHg) vs. measurement time (seconds).

Urination delay index

The urination delay index (-fold change) was about 2 to 3-fold after administration of EFLA®940, as compared to the baseline before solvent administration, showing a significant increase. After the administration of soybean germ extract no increase in urination delay was found.

Sample	Before administration	After solvent administration	After test sample administration
EFLA®940 Batch 3038141	1	1.06 +/- 0.15	2.96 +/- 1.19*
EFLA®940 Batch 3036525	1	1.08 +/- 0.19	2.33 +/- 0.35**
Soybean germ extract	1	1.07 +/- 0.07	1.09 +/- 0.31

Fig.2: Urination delay index with the urination frequency before administration as one (-fold). * With statistical significance compared to before administration. * $p<0.05$; $p<0.001$

Conclusion

Only EFLA®940 was responsible for a reduction of in-bladder pressure and a decrease in urination frequency. Its mode of action could be related to arginine, which is present at relatively high amounts in EFLA®940. This amino acid appears to increase the production of nitrogen monooxide (NO) via the arginine /NO pathway, contributing to bladder wall relaxation and decrease of in-bladder pressure. Next to having this direct bladder calming effect EFLA®940 has also been shown to mediate a muscle-streng-thening (anabolic) effect on pelvic floor / sphincter muscles *in-vitro*. Soybean germ extract showed no effect on in-bladder pressure. Nevertheless, soybean germ extract may help maintain a healthy estrogen level, due to the phytoestrogenic properties of isoflavones contained in the extract, which is important to support bladder health in (postmenopausal) women. This complementary mode of action makes EFLA®940 lipid-free Pumpkin Seed Extract and soybean germ extract sensible combination partners for targeting overactive bladder and urinary incontinence in aging women and men.

Reference:

Hata, K et al (2005). Effects of Pumpkin Seed Extract on urinary bladder function in anesthetized rats. Medical Science and Pharmaceutical Science 54 (3): 339-345

Go-Less™

Natural Bladder Support for Aging Men and Women

Overactive bladder is a widespread condition, affecting about one in six adults over the age of 40. Overactive bladder is defined as having an urgent need to empty the bladder, more frequent urination during the day and night, and incontinence.[1,2] Urinary incontinence, or the accidental leakage of urine affects 13 million Americans and occurs twice as often in women than men.[3] Although incontinence occurs more often in older individuals, it is not considered a normal part of the aging process.

Overactive bladder and incontinence are often embarrassing for those affected by the condition. Having to go to the bathroom frequently or leaking urine can interfere greatly with daily activities and researchers have found that about one-third of individuals with the condition report feeling depressed or stressed.[1] In addition, frequent trips to the bathroom at night can decrease sleep quality for both the individual and their partner or caretakers. Drugs that treat overactive bladder and incontinence are available; however, less than half of people affected would consider seeing a doctor about their problem.[1] Therefore, natural supplements that can improve symptoms of overactive bladder and incontinence have the potential to significantly improve quality of life for individuals affected.

Frutarom has developed *Go-Less*™, a proprietary blend of EFLA® 940 special pumpkin seed extract and SoyLife® soy germ isoflavones to support bladder health in aging men and women. Such a blend of pumpkin seed extract and soy isoflavones has been clinically studied and proven to be effective in addressing the cause of overactive bladder as well as improving its symptoms. This document reviews recent information on urinary incontinence and overactive bladder and summarizes the most up-to-date research on *Go-Less* and how it can provide natural bladder support for aging men and women.

What is overactive bladder?

Overactive bladder is actually a form of urinary incontinence. Symptoms of overactive bladder include an urgent feeling to urinate, increased frequency of urinating during the day and night and incontinence. Urinary incontinence is the accidental leakage of urine. This often occurs when individuals cough, laugh, sneeze, or have sudden urges to go to the bathroom and can't get there in time. Typically, urinary incontinence does not cause major health problems, but it can be embarrassing and affect self-esteem and quality of life. There are different kinds of incontinence[3]:

- **Stress incontinence** is urine loss during physical activity that increases abdominal pressure, such as sneezing, coughing, laughing, etc.
- **Urge incontinence or overactive bladder** is an urgent need to urinate that is so strong that individuals often cannot make it to the toilet in time. Also called overactive bladder, urge incontinence occurs when your bladder contracts when it shouldn't. This can happen even when there is only a small amount of urine in the bladder.
- **Overflow incontinence** is leakage that occurs when the bladder fails to empty properly, due to a blockage or weak bladder muscle contractions. Obstruction is usually related to either enlargement of the prostate or narrowing of the urethra from scar tissue.
- Stress and urge incontinence often occur together in women. This combination is sometimes referred to as **"mixed incontinence."**

In **men**, urinary incontinence is often related to a problem involving the prostate gland, such as enlargement of the prostate (benign prostatic hyperplasia, or BPH). Hormonal imbalances are a well-known cause of BPH in aging men. Hormone imbalances are also associated with a weakening of the pelvic floor in postmenopausal **women**. Stress incontinence in women can also result from childbirth, weight gain, or other conditions that stretch the pelvic floor muscles.

GoLess™ background

Go-Less™ is a proprietary blend of EFLA® 940 special pumpkin seed extract and SoyLife® 40% soy germ isoflavones. The seeds of the medicinal pumpkin (*Cucurbita pepo L*.) have been used for centuries as a natural remedy for urination problems. EFLA® 940 is a water-soluble pumpkin seed special extract that contains the complete spectrum of polar and semi-polar constituents of the seed. Frutarom uses a proprietary EFLA® HyperPure process that ensures highly selective removal of the fat-soluble components from the extract. Being virtually fat free, EFLA® 940 allows for increased stability and solubility and absence of rancidity.

Soy isoflavones are a class of phytoestrogens, or plant estrogens, which are suggested to help balance hormone levels in the body. SoyLife® is a soy germ isoflavone extract containing a standardized amount of isoflavones, as well as other phytonutrients in soy that are associated with improved health. SoyLife is also the only patent protected soy germ isoflavone ingredient on the market for use in dietary supplements.

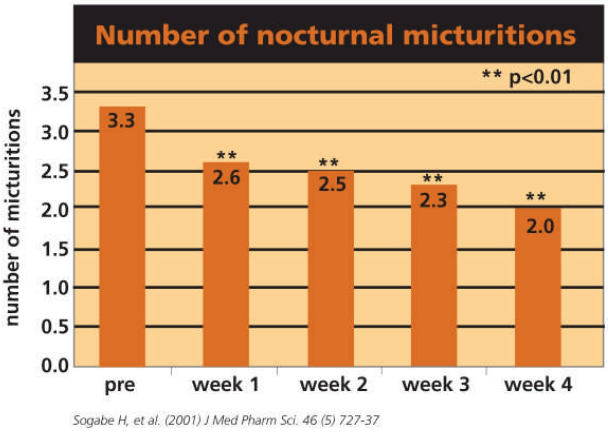
Summary of studies

The combination of EFLA® 940 special pumpkin seed extract and soy germ isoflavones has been shown to be effective in the treatment of urinary disorders associated with hormonal imbalances as demonstrated by pre-clinical in vivo research.

Effects on urination at night

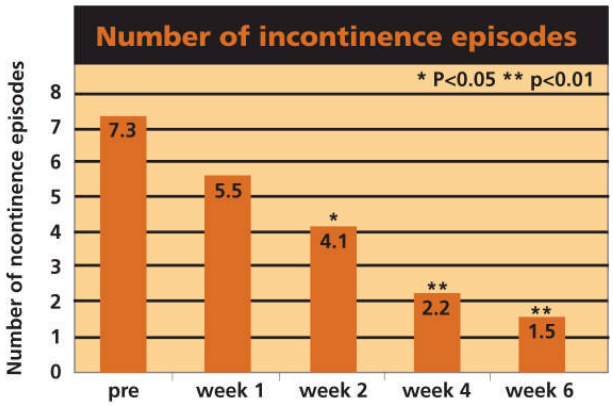
In a study of 39 women aged 52 to 86 years, it was found that a supplement containing EFLA® 940 and soy germ isoflavones decreased the frequency of urination during the day as well as at night (nocturia). Subjects also reported improved sleep satisfaction. The study consisted of a one-week pre-trial observation period followed by six weeks of supplement intake. Subjects recorded the frequency of urination during the day and night and their degree of sleep satisfaction. Researchers found that the frequency of urination was significantly improved at week 1, followed by continued improvement (Figure 1). The degree of sleep satisfaction was also improved at week 1 with additional improvement at week 2. Urinary incontinence was decreased significantly by week 2 and further at week 4 (Figure 2). [4]

Figure 1: *frequency of urination at night*



Sogabe H, et al. (2001) J Med Pharm Sci. 46 (5) 727-37

Figure 2: *frequency of incontinence*

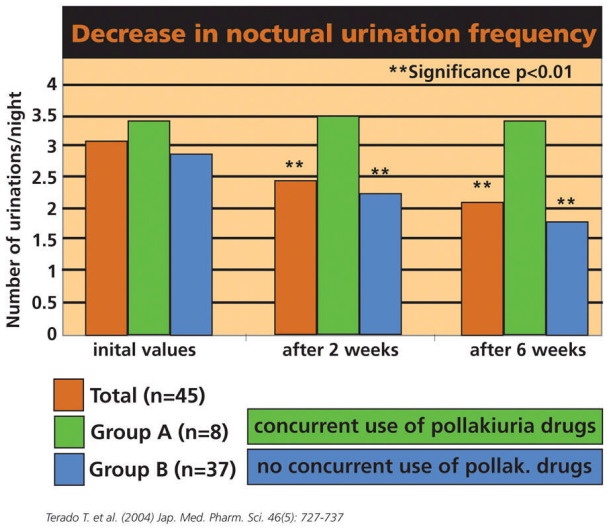


Sogabe H, et al. (2001) J Med Pharm Sci. 46 (5) 727-37

Pollakuria is defined as the condition in which one is awakened to urinate at night. In a study of 45 males over the age of 65 suffering from pollakuria, it was shown that a supplement containing EFLA® 940 and soy germ isoflavones reduced pollakuria and improved sleep satisfaction. The subjects were divided into two groups; one with concurrent use of therapeutic drugs for pollakuria at night (group A) and those without use of drugs of pollakuria (group B). The study consisted of a one-week pre trial observation period, followed by 6 weeks of supplement intake. Subjects recorded the number of times they urinated during the day, night, any adverse symptoms as well as their sleep satisfaction. The frequency of urination at night started to decrease within the first week of taking the supplement and had decreased by approximately 40% after 6 weeks. Researchers concluded that the

frequency of urination at night was significantly reduced compared to before taking the supplement (Figure 3).

Figure 3: *frequency of nocturnal urination*



Terado T. et al. (2004) Jap. Med. Pharm. Sci. 46(5): 727-737

The supplement was also found to increase sleep satisfaction. More than 86% of subjects also reported that their symptoms improved or significantly improved.[5]

Effects on stress incontinence

A supplement containing EFLA® 940 and soy germ isoflavones was found to reduce urinary incontinence in women suffering from overactive bladder and stress incontinence. Researchers studied 50 women aged 35 to 84 with overactive bladder and stress urinary at two different clinics in Japan. The study involved a one-week observation period (pre-trial), followed by six weeks of supplement administration. During the test period, patients recorded the frequency of urination during the day and at night. They also recorded the number of incontinence episodes and rated their satisfaction with the treatment. At the end of the study it was shown that the episodes of incontinence improved significantly (Figure 4). In particular, patients with the largest number of incontinence episodes showed the greatest improvement.[6]

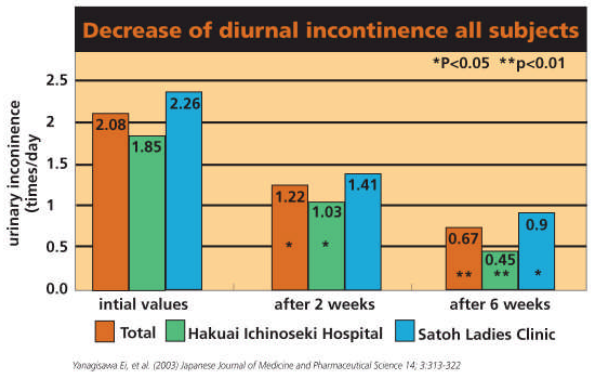
Mechanism of Action

Frutarom has performed several *in vitro* and *in vivo* experiments to determine the mechanism of action for how *Go-Less* contributes to bladder health. Results of the experiments suggest that EFLA® 940 special pumpkin seed extract and soy germ isoflavones exert beneficial activity in two ways: one on the hormonal level, resulting in anabolic, muscle strengthening effects; the other on a direct muscle relaxing effect resulting in a decreased urination frequency of the bladder.

In vitro experiments have shown that the ingredients in *Go-Less* inhibit enzymes involved in hormone metabolism. Human **5-alpha reductase** is an enzyme involved in the conversion of testosterone to di-hydrotestosterone, DHT. An overproduction of this enzyme is thought to play a role in the development of BPH. Inhibition of 5-alpha reductase may therefore lead to balanced levels of testosterone and DHT. EFLA® 940 pumpkin seed extract was found to inhibit 5-alpha reductase in a dose dependent fashion.

During menopause, women experience estrogen deficiency, which leads to symptoms of menopause such as hot flashes and night sweats and increases a woman's risk of developing osteoporosis and heart disease. In addition, estrogen deficiency has been linked to the reduction of vaginal and periurethral collagen content. Therefore, menopause is associated with urogenital complaints, including stress urinary incontinence and urgency.[10] Isoflavones are classified as phytoestrogens. They have a chemical structure similar to human estrogen, which allows isoflavones to attach to estrogen receptors and exert weak estrogenic and anti-estrogenic effects. *In vitro* studies by Frutarom have shown that isoflavones and EFLA®940 pumpkin seed extract inhibit **aromatase**, an enzyme involved in the conversion of testosterone to estradiol. In women, a decrease in this conversion can help maintain healthy testosterone levels, supporting the strengthening of pelvic muscles.

Figure 4: *frequency of incontinence*



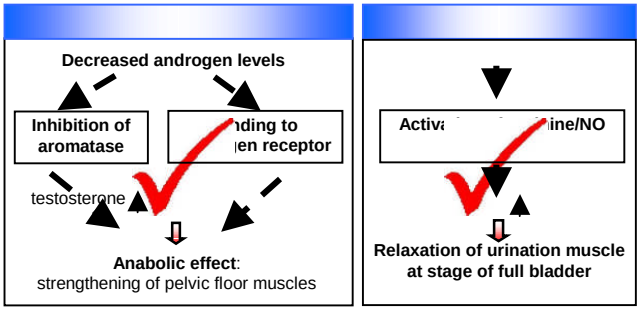
Yanagisawa Ei, et al. (2003) Japanese Journal of Medicine and Pharmaceutical Science 14; 3:313-322

Go~Less®

Another *in vitro* study found that EFLA® 940 pumpkin seed extract binds to androgen receptors. The androgen receptor is a ligand-activated nuclear transcription factor that mediates responses to androgens (testosterone and its metabolite DHT) in a variety of tissues. In addition to being strongly present in male sexual organs, androgen receptors are also present in muscle tissues and female reproductive organs.[7] Androgens produce direct anabolic effects on skeletal muscle. Recently it has been observed that androgens may potentially play an important role in the pelvic-floor and lower urinary track disorders because certain muscles in the urinary tract are sensitive to androgens and contain large number of androgen receptors.[8,9]

Findings of an *in vivo* experiment in rats also suggest that the clinical effects of EFLA 940 might also be mediated by an activation of the arginine/ nitric oxide pathway, resulting in an increased production of the muscle relaxing nitric oxide.[11]

Figure 5: *mechanisms by which Go-Less supports bladder health*



In Summary

In summary, clinical studies in men and women have found a reduction in the frequency of urination at night as well as during the day. Incontinence episodes in women were also shown to decrease and subjective improvement in symptoms of overactive bladder were reported. Greater than 60% of subjects reported improvements within two weeks of taking the supplement and more than 80% had improved after 6 weeks. The studies show high compliance and confirm the safety and tolerance of preparations with EFLA®940 pumpkin seed extract and soy germ isoflavones.

Dosage Recommendations

A daily dose of 600 mg of *Go-Less*, providing 525 mg of EFLA 940 and 75 mg SoyLife 40% is recommended to provide the amount of pumpkin seed extract and soy germ isoflavones used in the human studies. Point of consideration: the studies used a higher dose for the first two weeks, followed by a reduced dose for the last four weeks. To mirror the studies, you may suggest 1000 mg of *Go-Less* for the first two weeks, followed by 600 mg of *Go-Less* in future weeks.[4,5,6]

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1. Irwin DE, et al. (2006). Impact of overactive bladder symptoms on employment, social interactions and emotional well-being in six European countries. *BJU Int*. 97(1):96-100.

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5. Terado T, et al. (2004). Clinical Study of mixed processed foods containing pumpkind seed extract and soybean germ extract on pollikiuria in night in elderly men. *Jap J Med Pharmaceut Sci*. 52(4): 551-561.

6. Yanagisawa, EI et al. (2003). Study of effectiveness of mixed processed food containing cucurbita pepo seed estract and soybean seed extract on stress urinary incontinence in women. Japanese Journal of Medicine and Pharmaceutical Science 14;3:313-322.

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11. Hata K et al. (2005) Effects of pumpkin seed extract on urinary bladder function in anesthetized rats. *Medical Science and Pharmaceutical Science*. 54(3): 339-345.

PRODUCT FLYER

EFLA®940 Pumpkin Seed Extract 0085940

Natural Bladder Support for Women

Every fifth, after menopause even every fourth woman is suffering from a weakening of bladder function and incontinence respectively. Incontinence is associated with the weakening of pelvic floor and sphincter musculature as well as a shift in hormonal imbalances, particularly in women suffering from (postmenopausal) stress incontinence. The seeds of the medicinal pumpkin (*Cucurbita pepo L.*) were already used by the North American Indians as a natural remedy for the prevention and treatment of female urinary bladder problems.

EFLA®940 is a water soluble Pumpkin Seed Extract containing the complete spectrum of polar and semi-polar constituents of the seed. EFLA®HyperPure, Frutarom's proprietary process technology, ensures the highly selective removal of all lipophilic constituents from the extract. Being virtually lipid-free EFLA®940 offers key advantages compared to oily pumpkin seed extracts: pharmacological activity, increased stability and solubility, no rancidity, no formation of potentially harmful degradation products.

According to comprehensive pharmacological and pre-clinical evidence, the beneficial properties of EFLA®940 as a multicomponent system are being mediated at least in two different ways: one at the hormonal level, resulting in an anabolic, strengthening effect on pelvic floor and sphincter musculature, the other one through a direct relaxing effect on the muscles of the bladder wall.

The efficacy, safety and tolerance of lipid-free EFLA®940 Pumpkin Seed Extract has been confirmed in several clinical trials in women suffering from irritated bladder and (postmenopausal) stress incontinence.

EFLA®940 Pumpkin Seed Extract – natural bladder support for women.

Information on the drug

Indication according to official references	**German Commission E** Irritable bladder, micturition problems associated with BPH, stages I and II. **Merck Index** Anthelmintic.
Folk medicine applications	Prevention and treatment of irritable bladder conditions and urinary bladder problems, childhood enuresis nocturna. Anthelmintic and bladder disease agent.
Description of the herbal drug	Medicinal cultivars of *Cucurbita pepo L. var. styriaca;* cultivated in Austria.

Information on "EFLA®940 Pumpkin Seed Extract"

Technology	ISO, PIC-GMP conform, patented EFLA®HyperPure manufacturing technology.
Standardized on	• Phenolic derivatives (as Enterodiol/UV) 2.0 – 4.0 % (m/m) • Adenosin (HPLC) > 0.100 % (m/m)
Drug–extract ratio	15 – 25 = 1 (20 = 1)
Type of extract	Powder
Studies with EFLA®940	*in-vitro* Anabolic effect: significant inhibitory activity on the enzyme aromatase. Significant binding to androgen receptor. *in-vivo (rats)* Direct muscle-relaxing effect on bladder wall: significant decrease in urination frequency, probably mediated by an increased production of nitric oxide (NO). *clinical* (Postmenopausal) women with stress incontinence and irritated bladder: significant decrease of incontinence episodes, nocturnal / diurnal micturitions, subjective improvement.
Dosage recommendation	500 mg extract / day corresponding to 10 g coarsely ground or well chewed seeds, taken with fluids (German Commission E).

The brands EFLA / Flachsmann stand for high-quality herbal extracts that mirror all characteristics of the plant substances thanks to an optimized extraction technology.
Innovation. Science. Quality.

09.09

PRODUCT FLYER

EFLA®940 Pumpkin Seed Extract

0085940

Natural Prostate and Bladder Support for Men

More than half of all men over age 50 are affected from benign prostatic hyperplasia (BPH) and irritable bladder. Hormonal imbalances are a concomitant cause of BPH / irritable bladder and the resultant overflow incontinence. The seeds of the medicinal pumpkin (*Cucurbita pepo L.*) were already used by the North American Indians as a natural remedy for the prevention and treatment of male micturition problems.

EFLA®940 is a water soluble Pumpkin Seed Extract containing the complete spectrum of polar and semi-polar constituents of the seed. EFLA®HyperPure, Frutarom's proprietary process technology, ensures the highly selective removal of all lipophilic constituents from the extract. Being virtually fat free EFLA®940 offers key advantages compared to oily pumpkin seed extracts: pharmacological activity, increased stability and solubility, no rancidity, no formation of potentially harmful degradation products.

According to comprehensive pharmacological and pre-clinical evidence, the beneficial properties of EFLA®940 as a multicomponent system are being mediated at least in two different ways: one at the hormonal level, resulting in a normalisation of testosterone levels, the other one through a direct relaxing effect on the muscles of the bladder wall.

The efficacy, safety and tolerance of lipid-free EFLA®940 Pumpkin Seed Extract has been confirmed in animals as well as in a clinical trial in men suffering from irritable bladder.

EFLA®940 Pumpkin Seed Extract – natural prostate and bladder support for men.

Information on the drug

Indication according to official references	**German Commission E** Irritable bladder, micturition problems BPH, stages I and II. **Merck Index** Anthelmintic.
Folk medicine applications	Prevention and treatment of irritable bladder conditions and micturition problems related to BPH, childhood enuresis nocturna. Anthelmintic and bladder disease agent.
Description of the herbal drug	Medicinal cultivars of *Cucurbita pepo L. var. styriaca;* cultivated in Austria.

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Technology	ISO, PIC-GMP conform, patented EFLA®HyperPure manufacturing technology.
Standardized on	• Phenolic derivatives (as Enterodiol/UV) 2.0 – 4.0 % (m/m) • Adenosin (HPLC) > 0.100 % (m/m)
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Type of extract	Powder
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Dosage recommendation	500 mg extract / day corresponding to 10 g coarsely ground or well chewed seeds, taken with fluids (German Commission E).

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Innovation. Science. Quality.

Sevurin™

Grazie ai suoi
ingredienti naturali
aiuta a favorire
la normale funzionalità
del sistema urinario

HILTON
PHARMA

Pumpkin Seed and Hormonal Imbalance

Matthias-H. Kreuter, PhD

Caesar B. Schmidlin, PhD

Directory

- Short Introductions to
  - Urinary Incontinence and
  - Benign Prostate Hyperplasia (BPH)
- Pumpkin Seed: Selection of Compounds
- Mode of Action and Active Fraction
- Pre-Clinical and Clinical Trials
- Technical Background, Quality and Safety
- Final Summary of Effects of EFLA®940

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

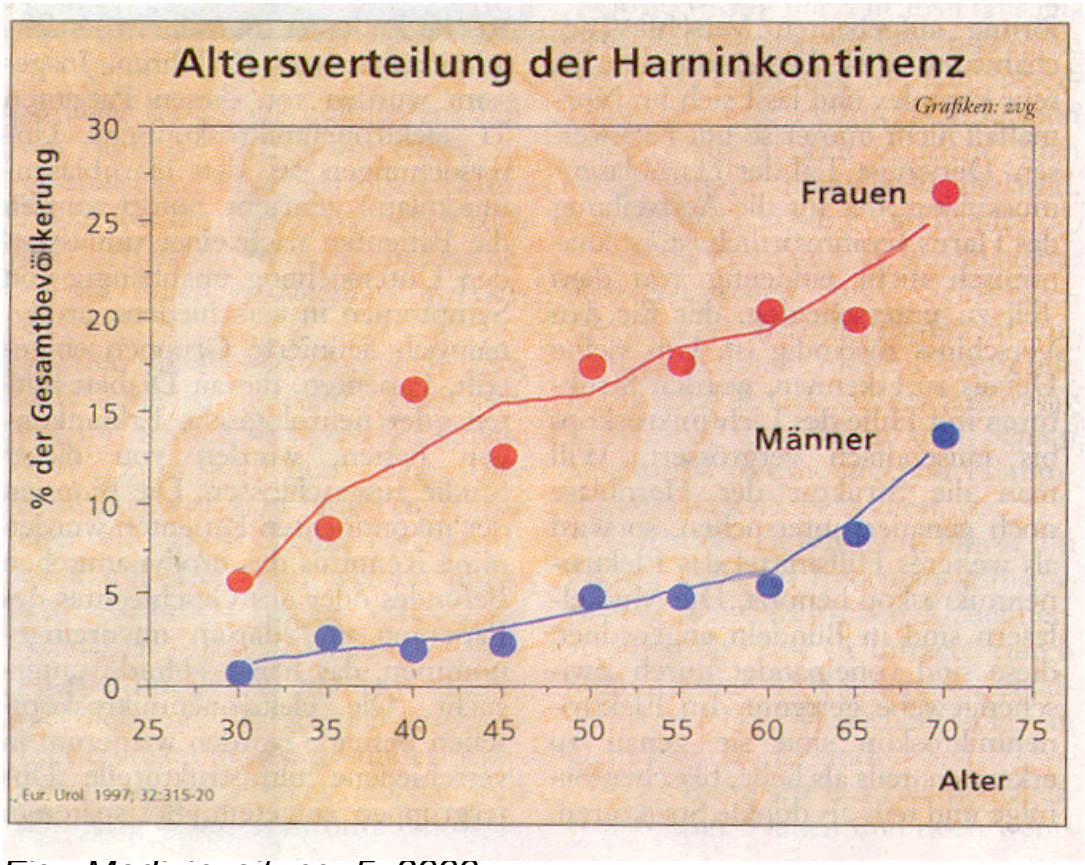


Fig.: Medizinzeitung, 5, 2000

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

  → **aromatase**

overflow incontinence:

benign prostate hyperplasia in men:

- testosterone deficit

  → **5α-reductase**

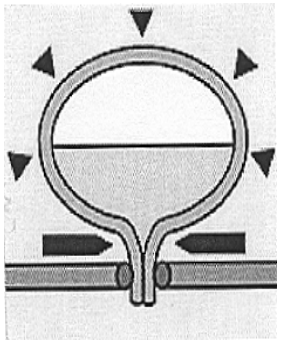
Urinary Incontinence: Pathophysiology

Overflow incontinence

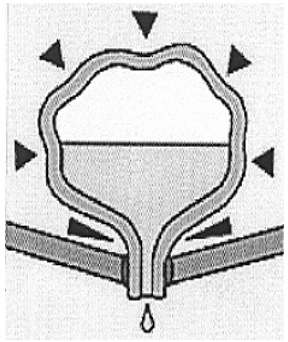
functional	obstructive
weakness of bladder muscle	narrowing of the urethra caused by prostate growth
overstretching of bladder wall	**benign prostate hyperplasia (BPH)**
both sexes affected	***men at advanced age***
metabolic disturbance, side effects of remedies, parkinson etc.	cause not clear yet,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

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

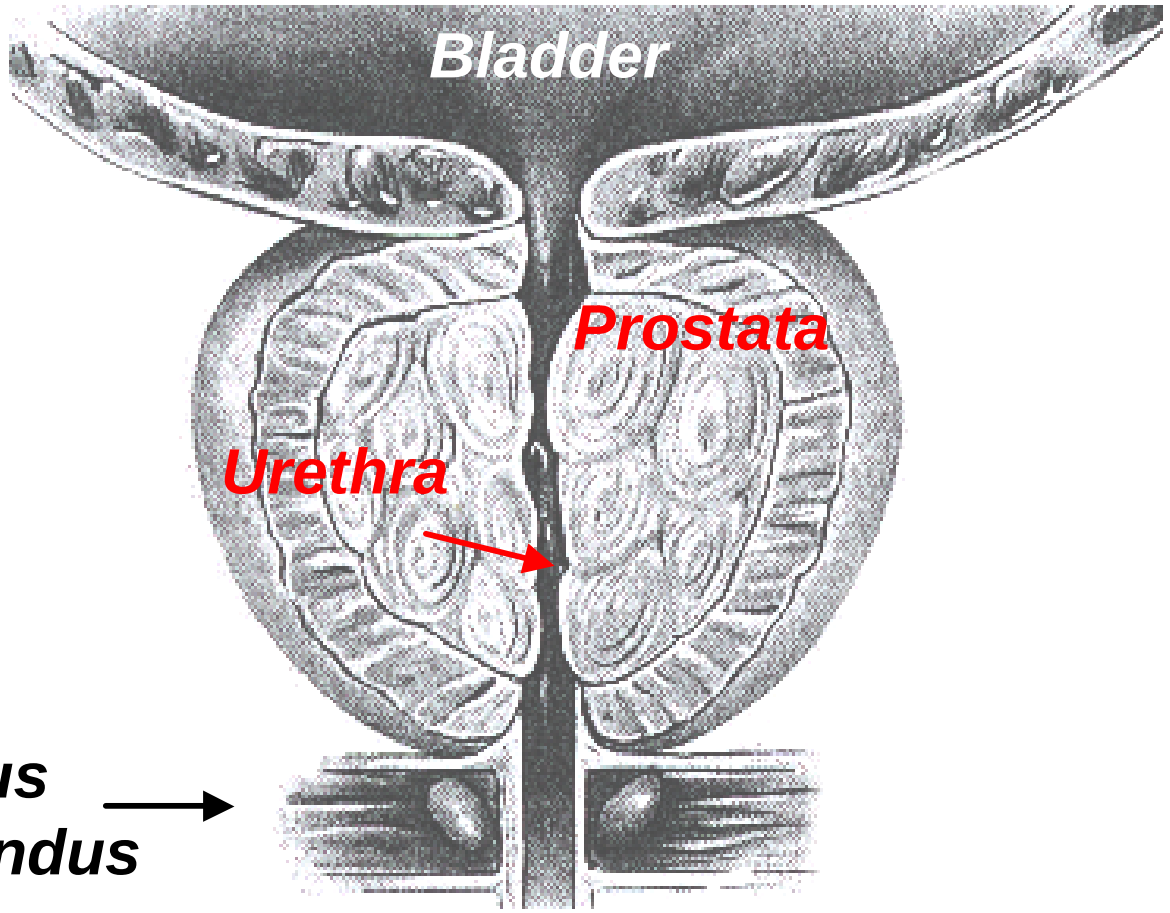
Benign Prostate Hyperplasia: General Data

- 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.

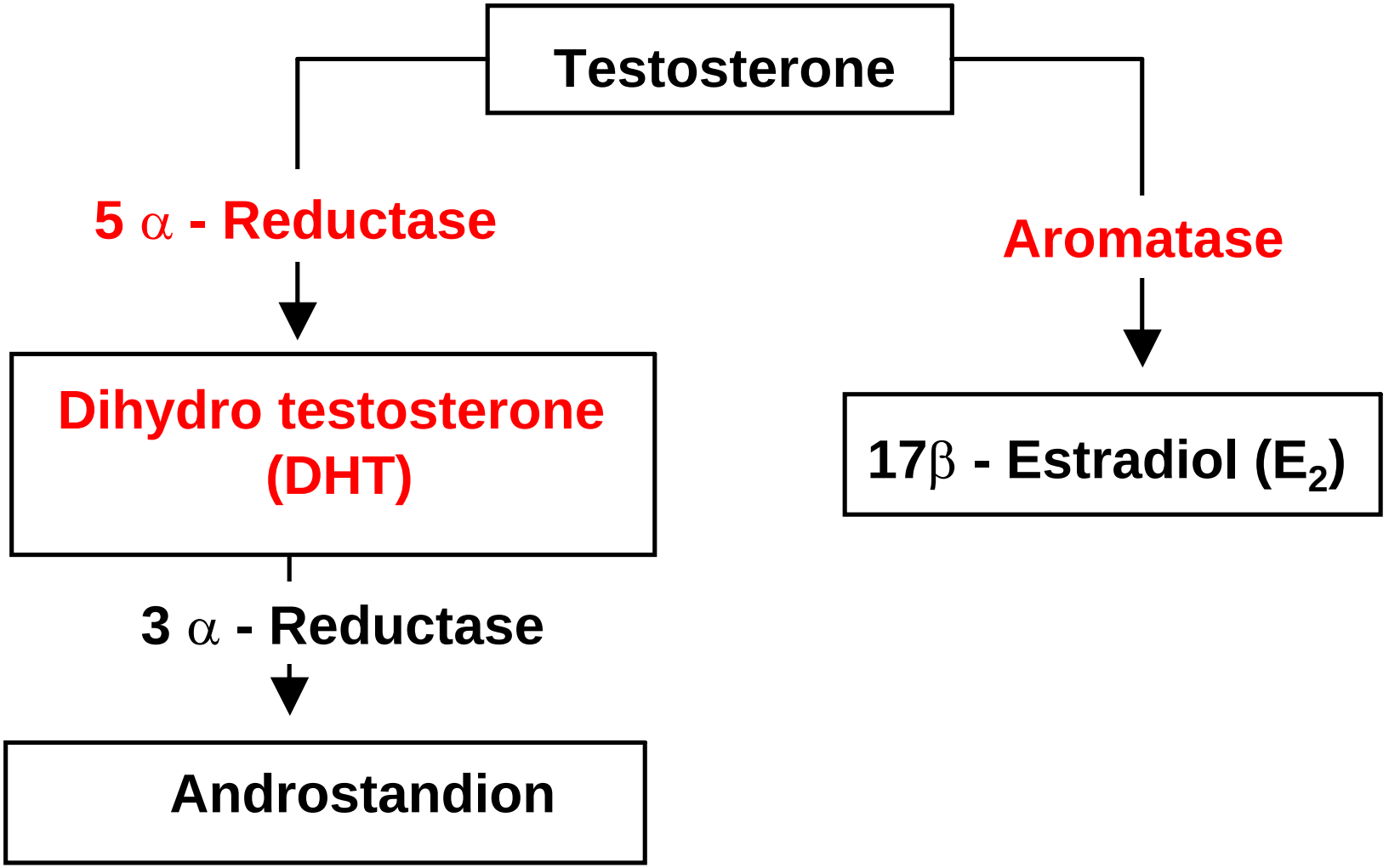
Benign Prostate Hyperplasia: Pathophysiology



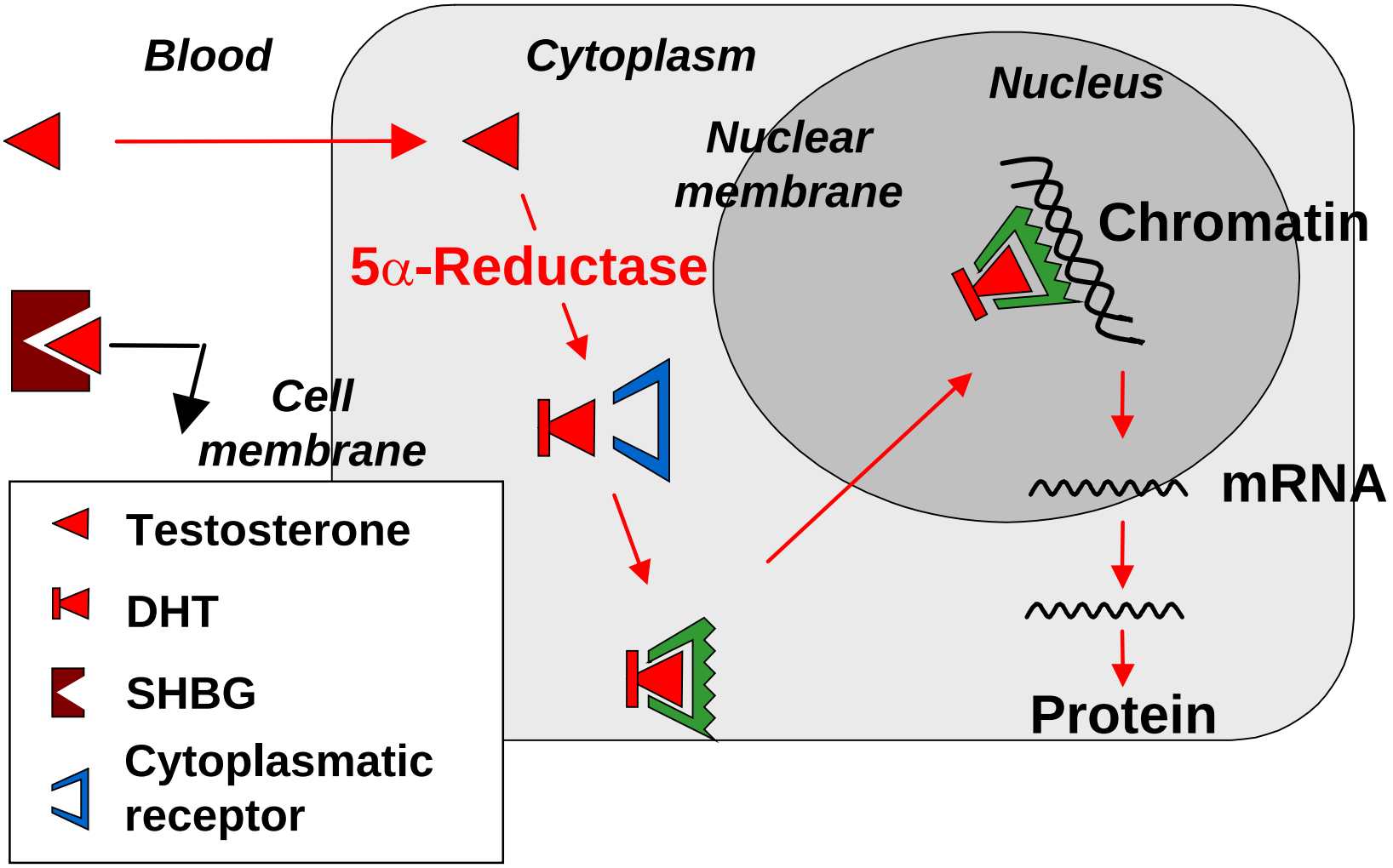
M. transversus perinei profundus →

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

Benign Prostate Hyperplasia: Pathophysiology



Benign Prostate Hyperplasia: Pathophysiology



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

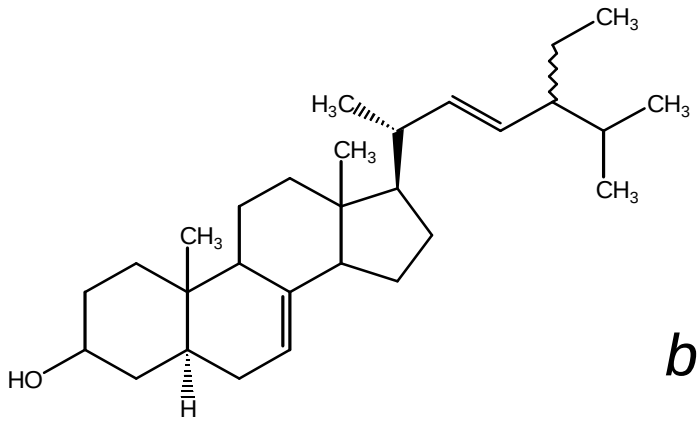
secoisolariciresinol

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

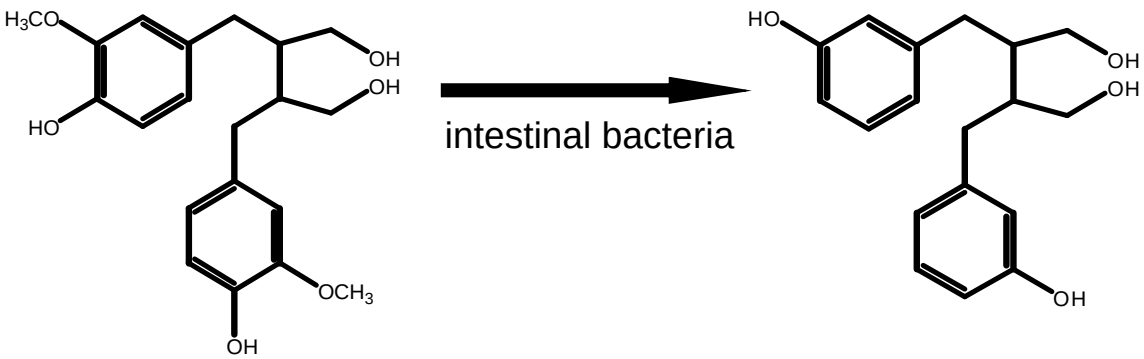
→ no rational therapy with phytosterols

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***

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

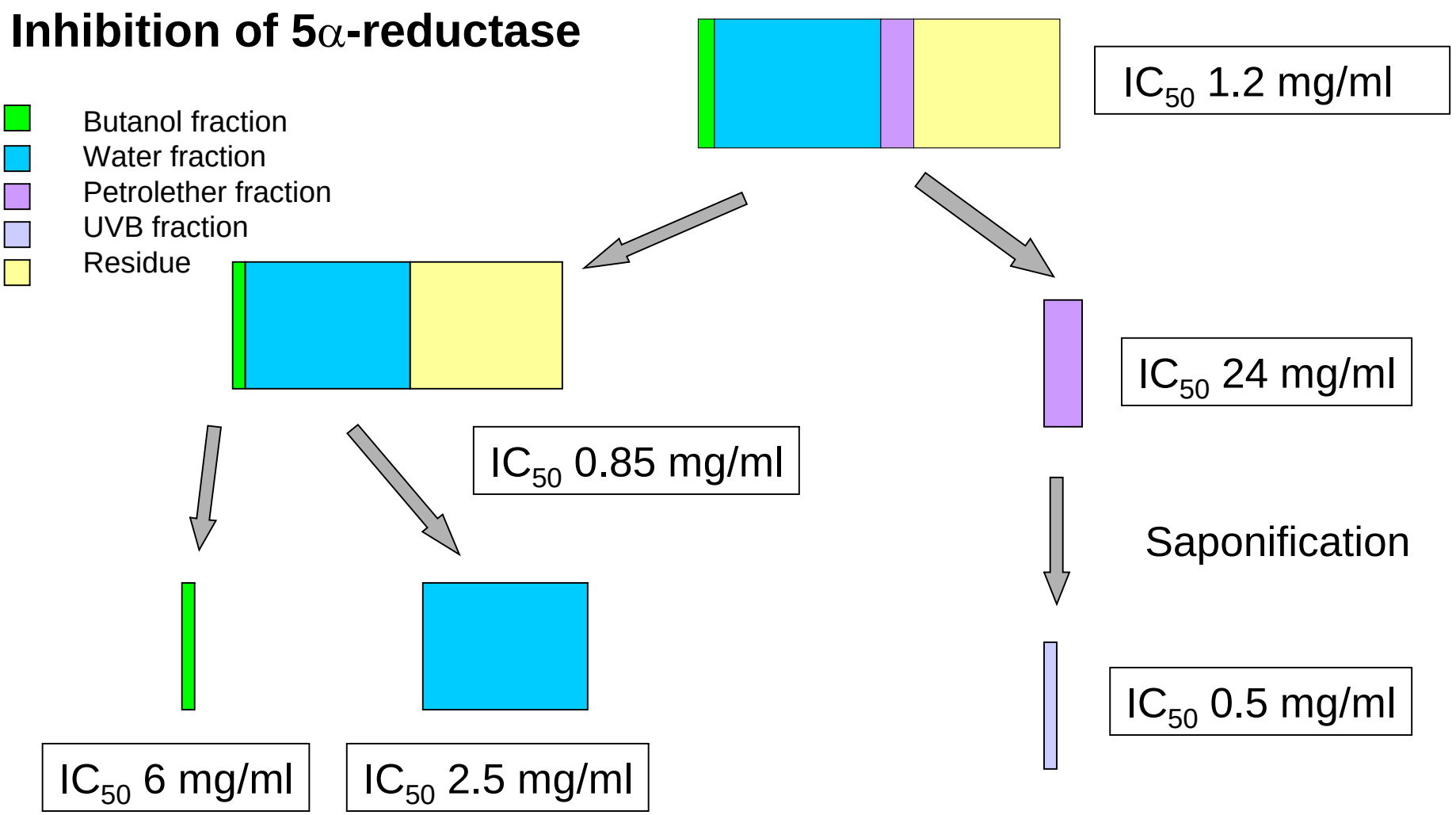
Schottner M, et al. (1998) *J Nat Prod.* 61(1): 119-21

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

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

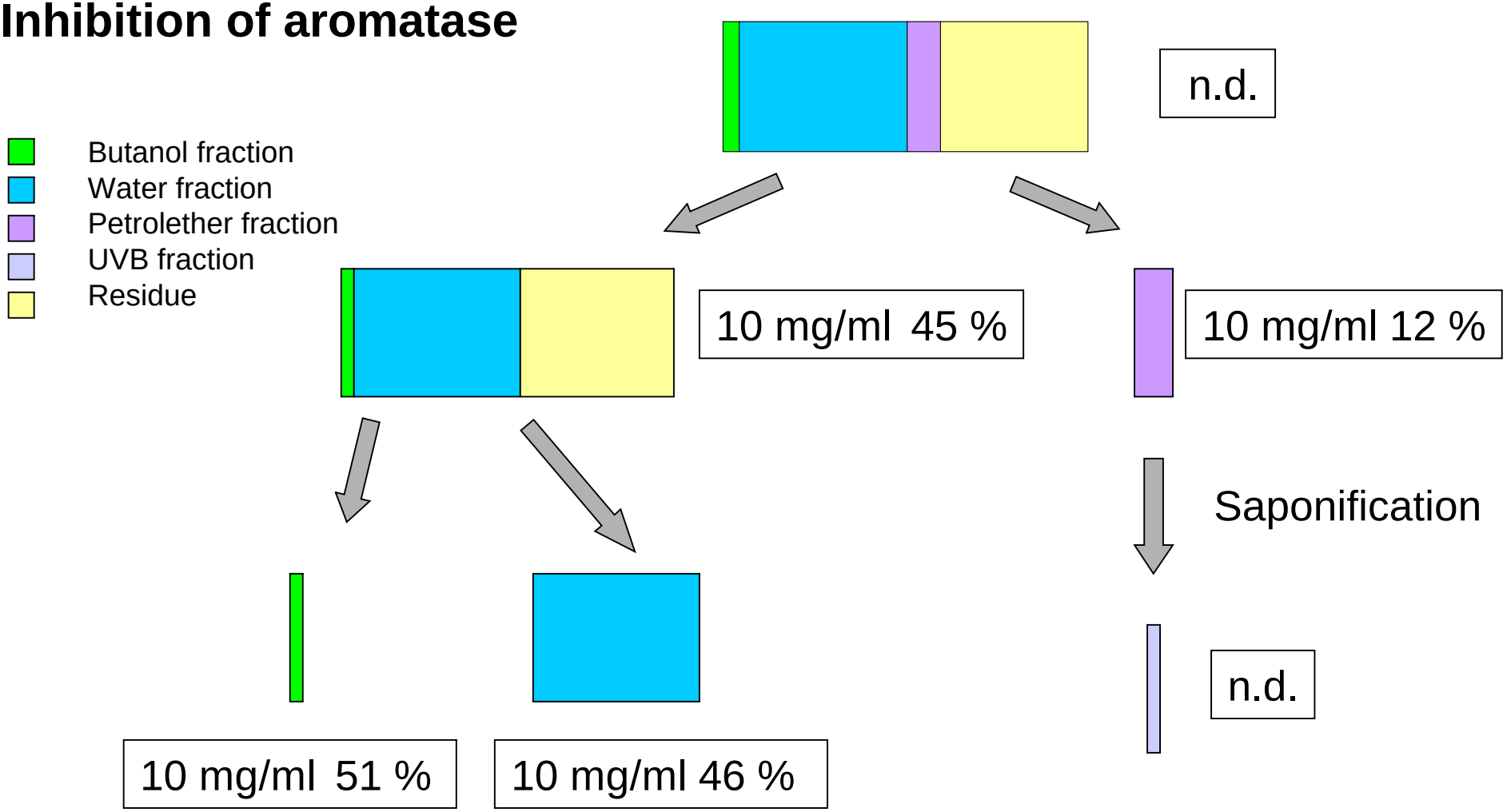
Martin ME, et al. (1996) *Life Sci.* 58(5): 429-36.

Pumpkin Seed: Active Fraction



Pumpkin Seed: Active Fraction

Inhibition of aromatase



Pumpkin Seed: in vitro Inhibition of Aromatase

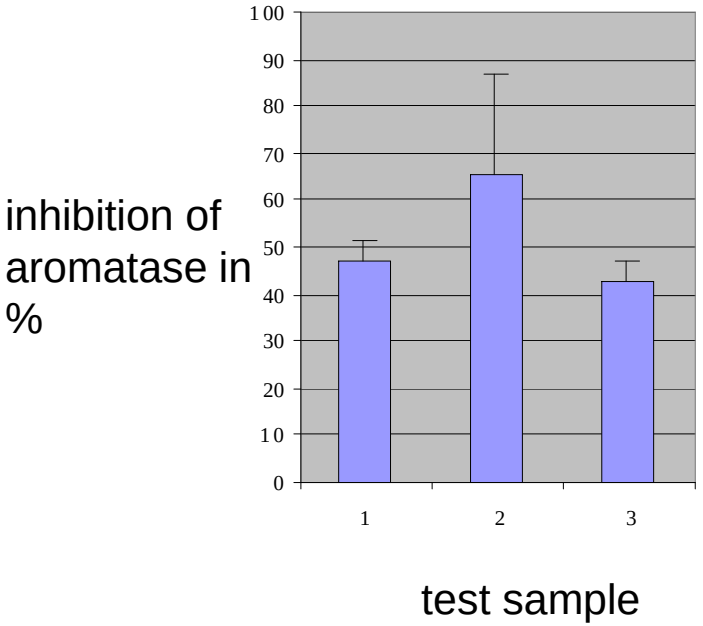
Experimental design:

- 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



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

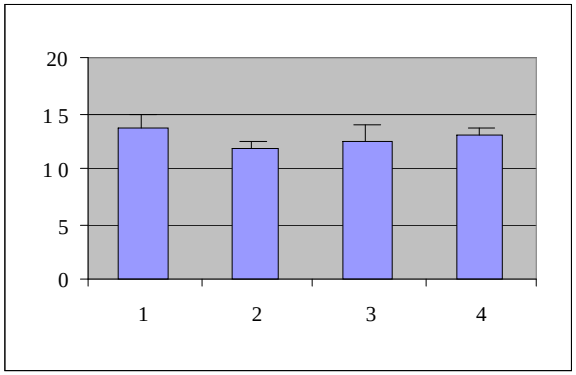
Treatment		Uterine wet weight (mg/100 g b.w.) Mean ± SEM	Uterine dry weight (mg/100 g b.w.) Mean ± SEM
Vehicle treated control (CMC)		108.8 ± 22.0	17.5 ± 3.1
85940 (mg/kg)	5	128.5 ± 32.1	18.5 ± 4.0
	10	129.5 ± 34.9	21.1 ± 4.9
	20	182.6 ± 65.7	23.6 ± 6.4

Pumpkin Seed: in vivo Phytoestrogenic Effect

Result: experiment I

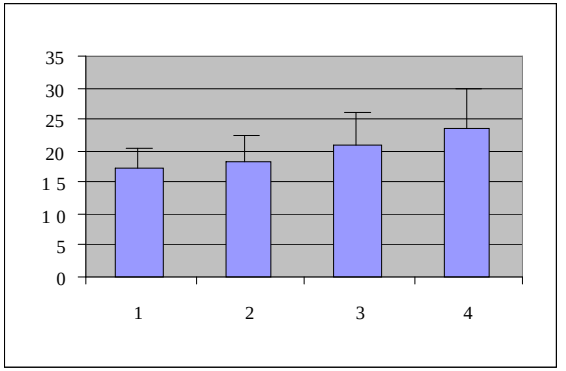
- 4 days of treatment reveals no effect
- 8 days of treatment shows an increase of the dry weight of uterus of 34.9 %

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.

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

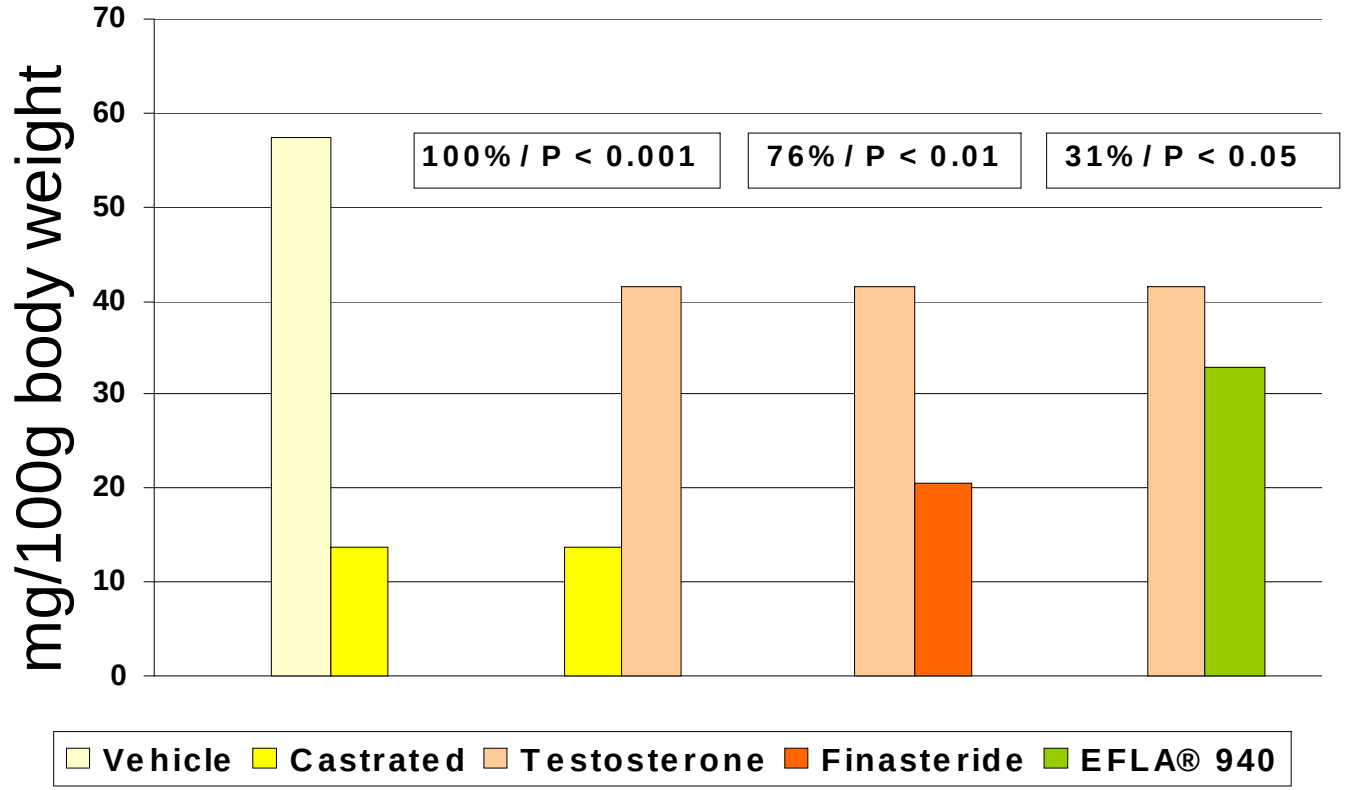
Design:

- 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	+	+	+	-	+

Pumpkin Seed: in vivo Inhibition of 5α-Reductase

Result



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

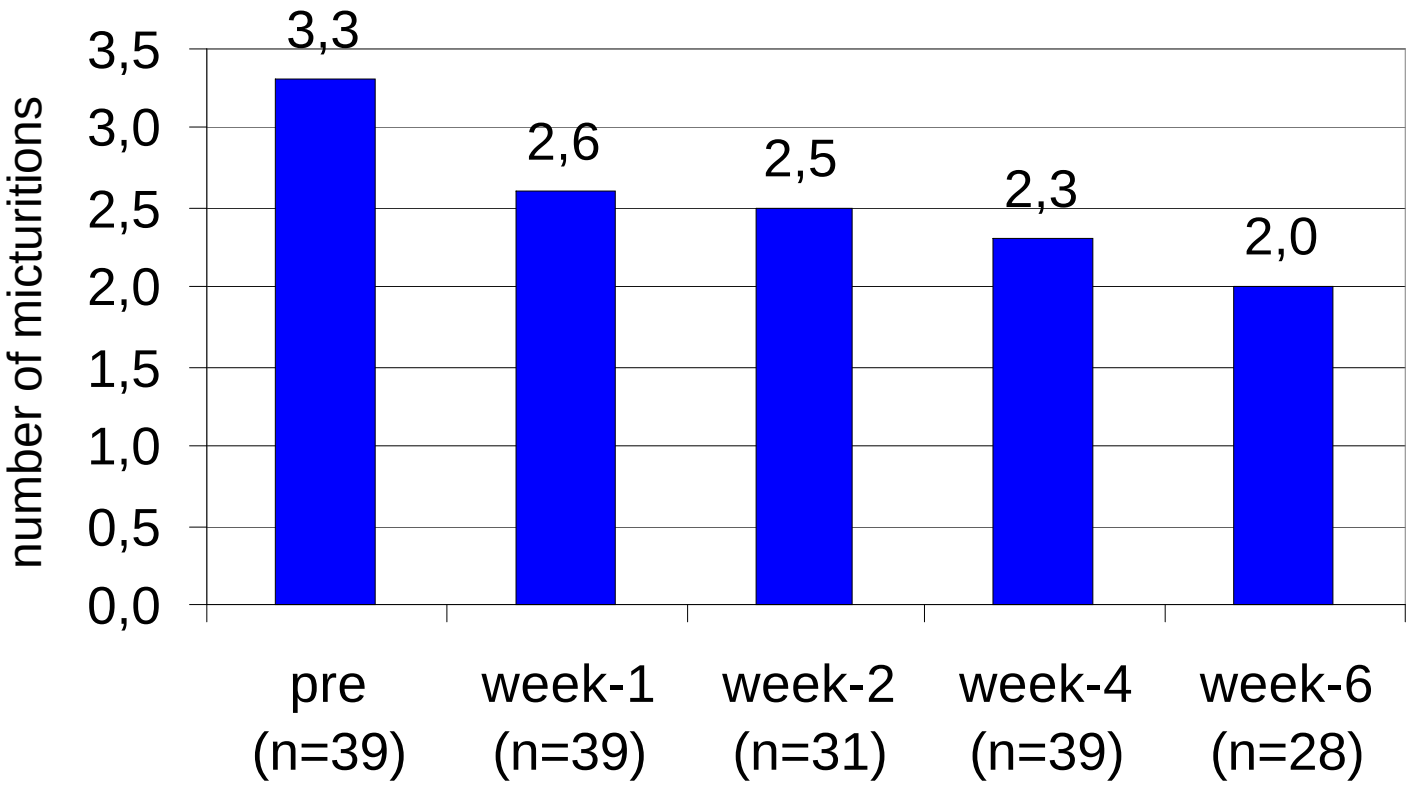
Urinary Incontinence: Open Clinical Trial

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)

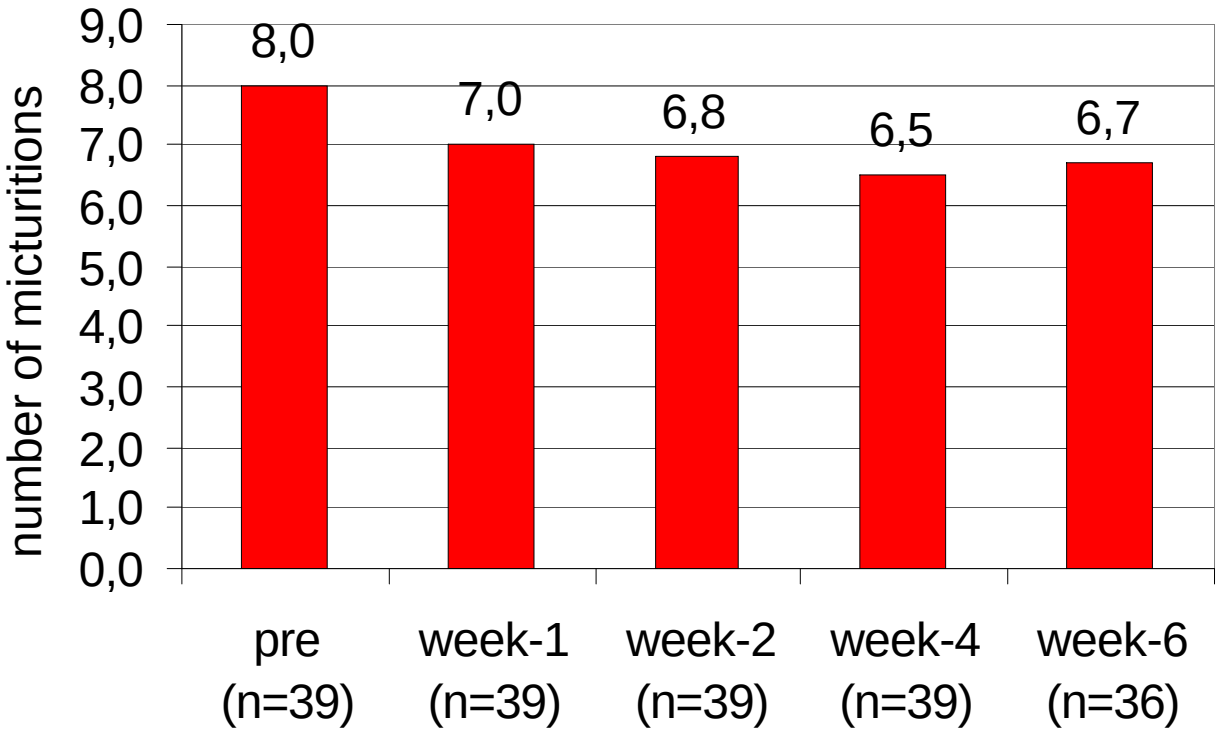
Urinary Incontinence: Open Clinical Trial

Results: number of nocturnal micturitions



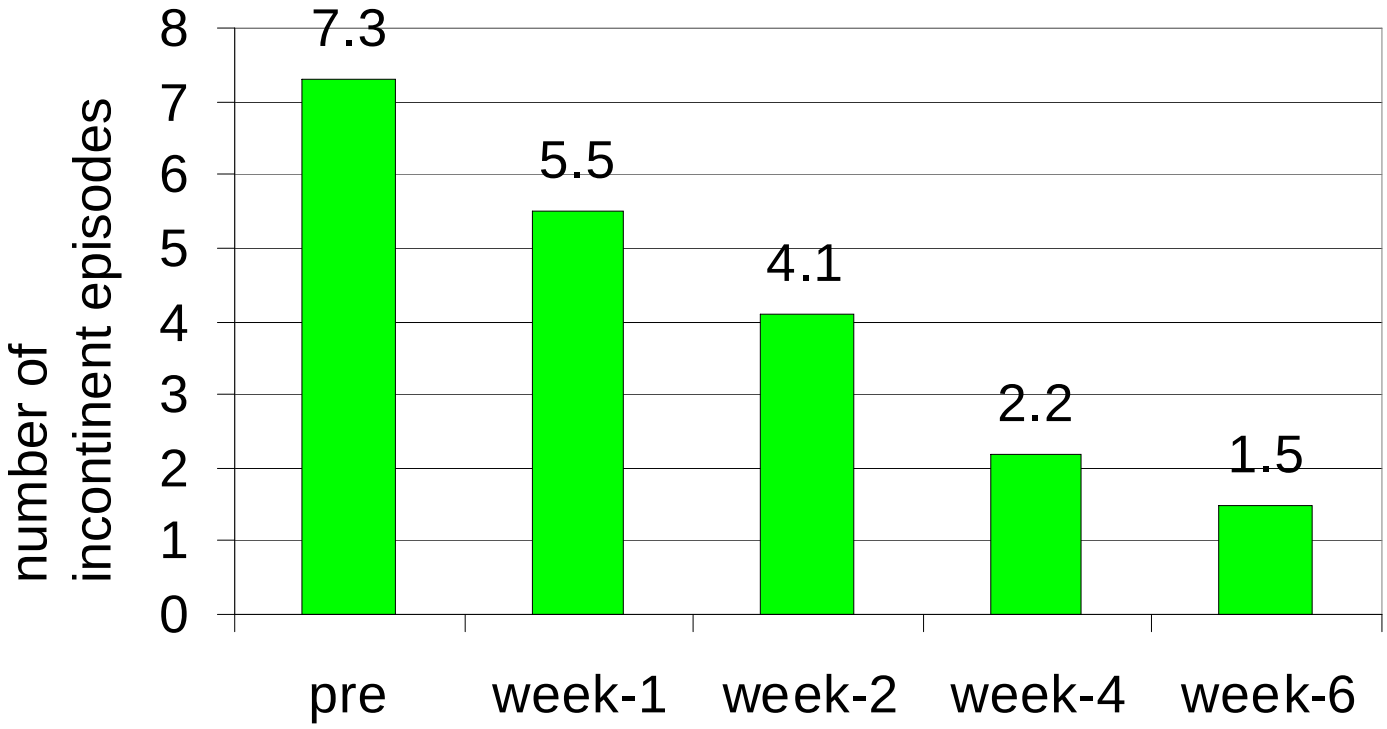
Urinary Incontinence: Open Clinical Trial

Results: number of diurnal micturitions



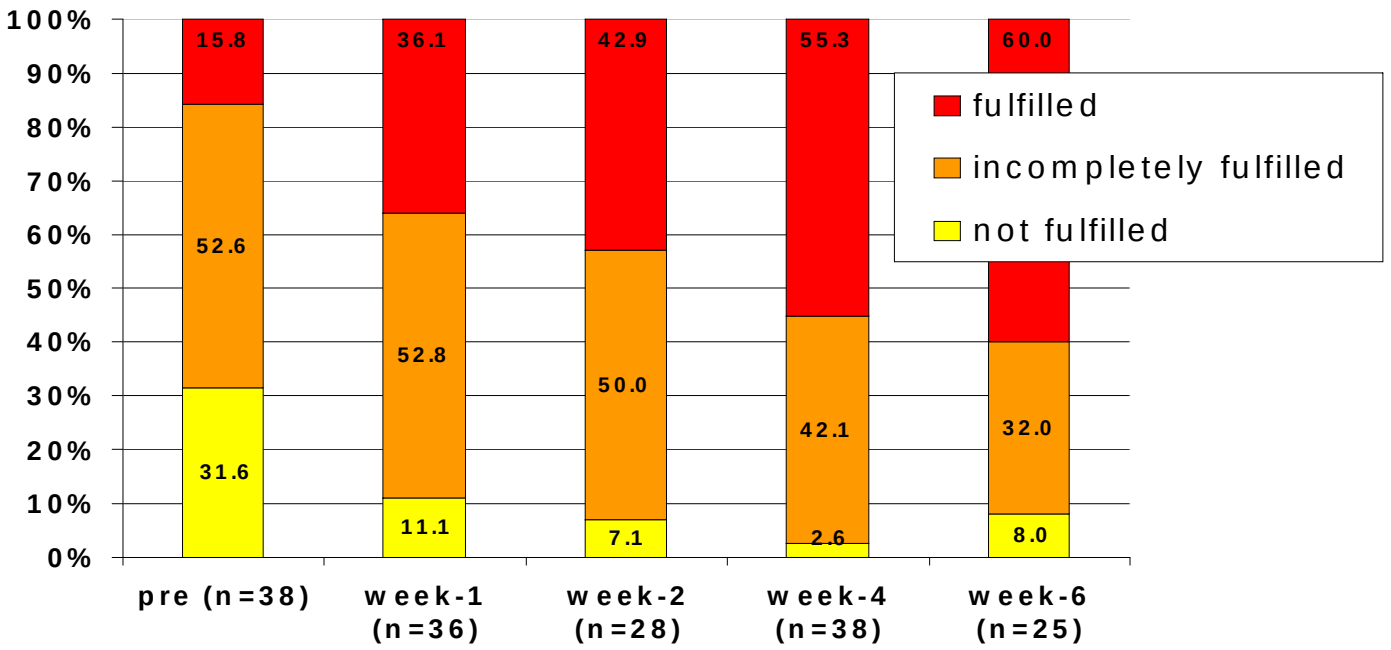
Urinary Incontinence: Open Clinical Trial

Results: **number incontinent episodes**



Urinary Incontinence: Open Clinical Trial

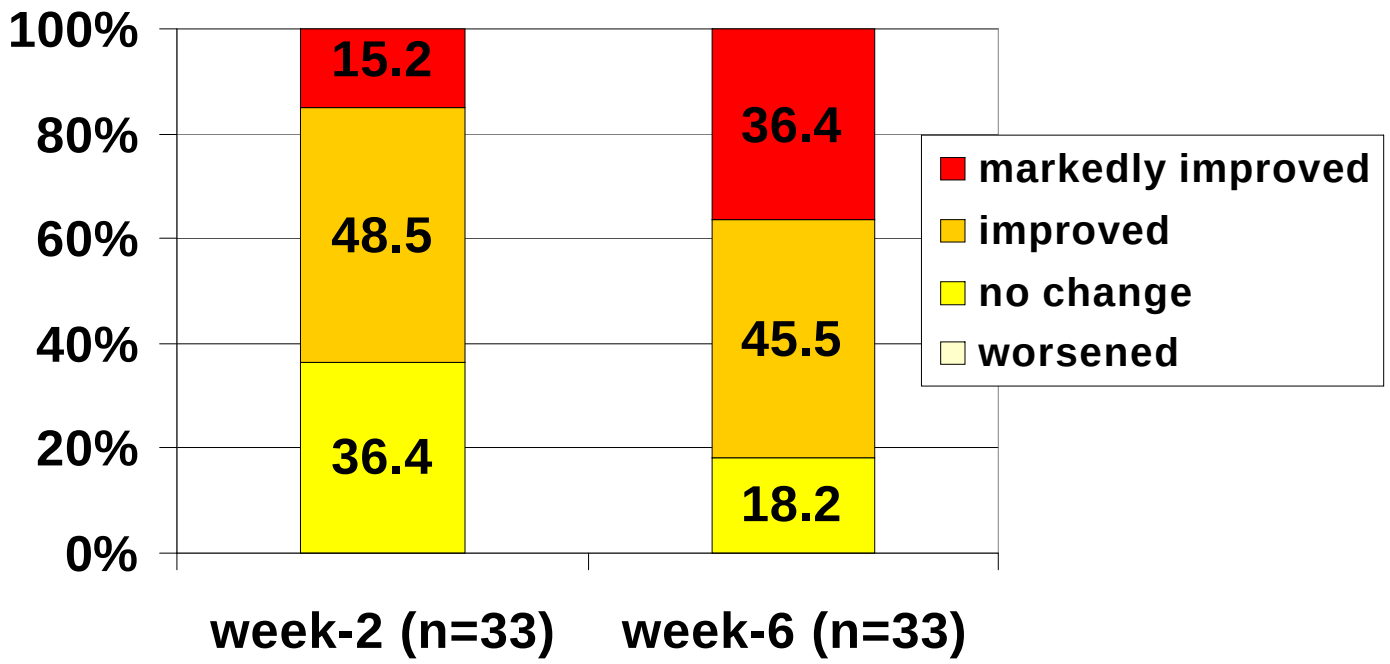
Results: subjective improvement



Urinary Incontinence: Open Clinical Trial

Results: **total ratio of improvement**

subgroup of 33 individuals with 2 to 4 micturitions per night



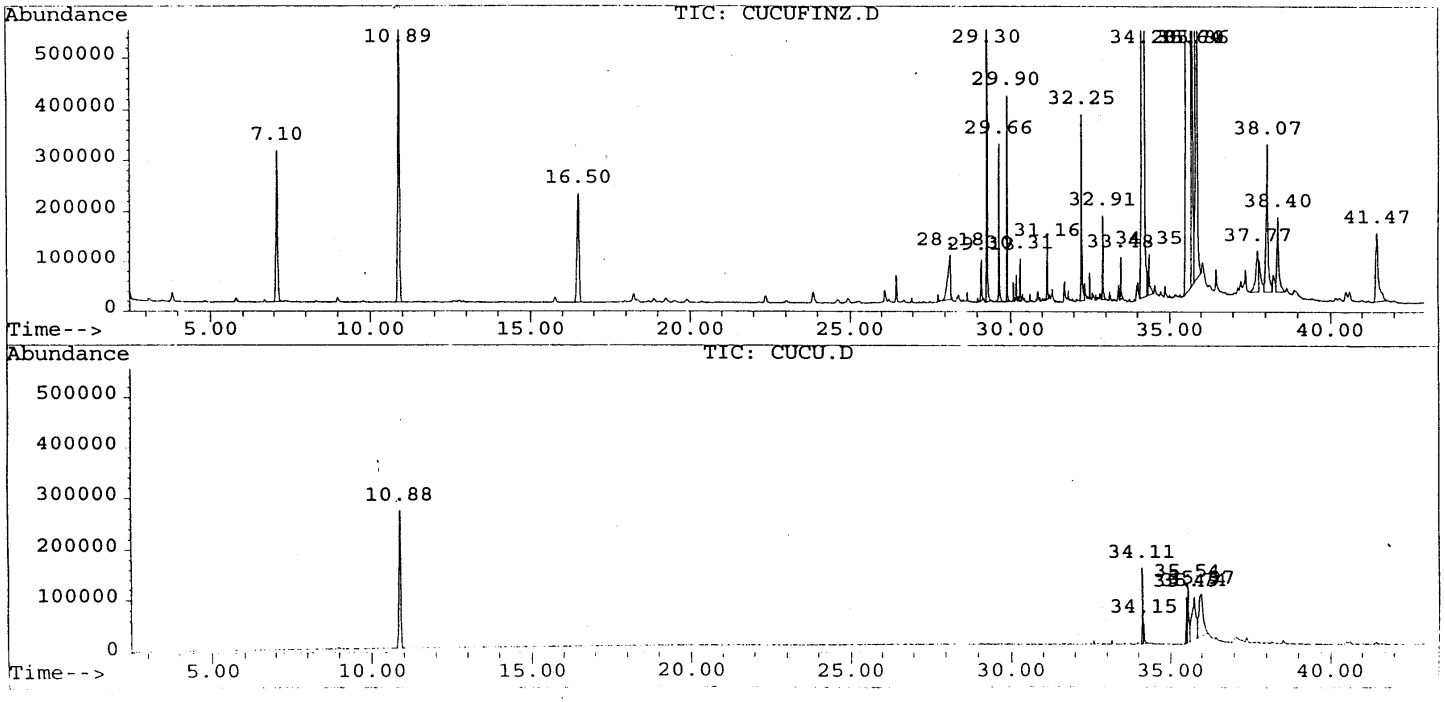
Urinary Incontinence: Open Clinical Trial

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 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