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

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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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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).

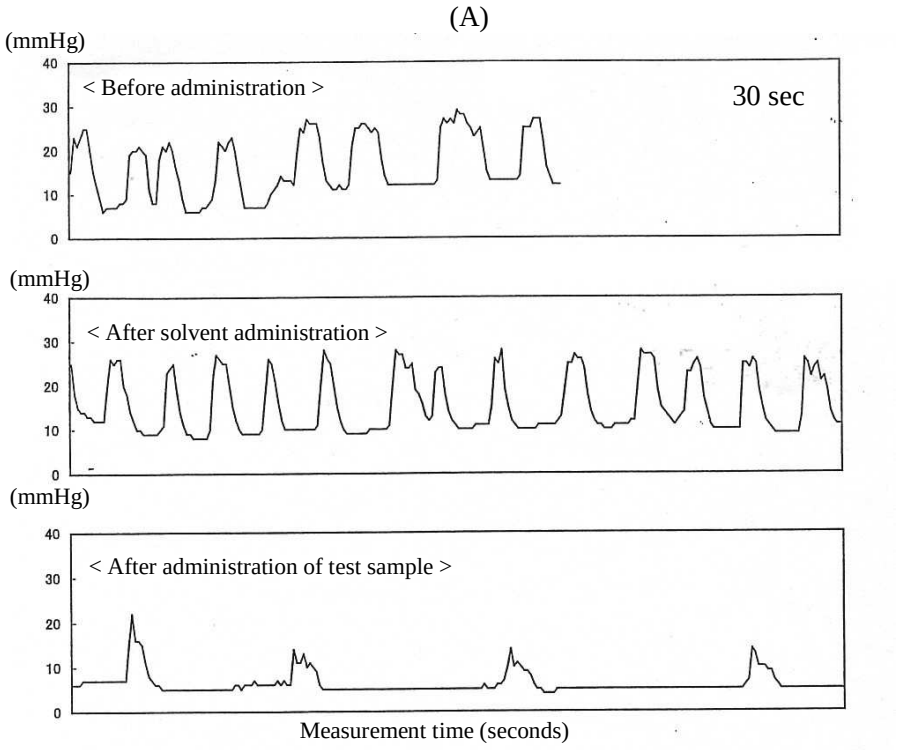
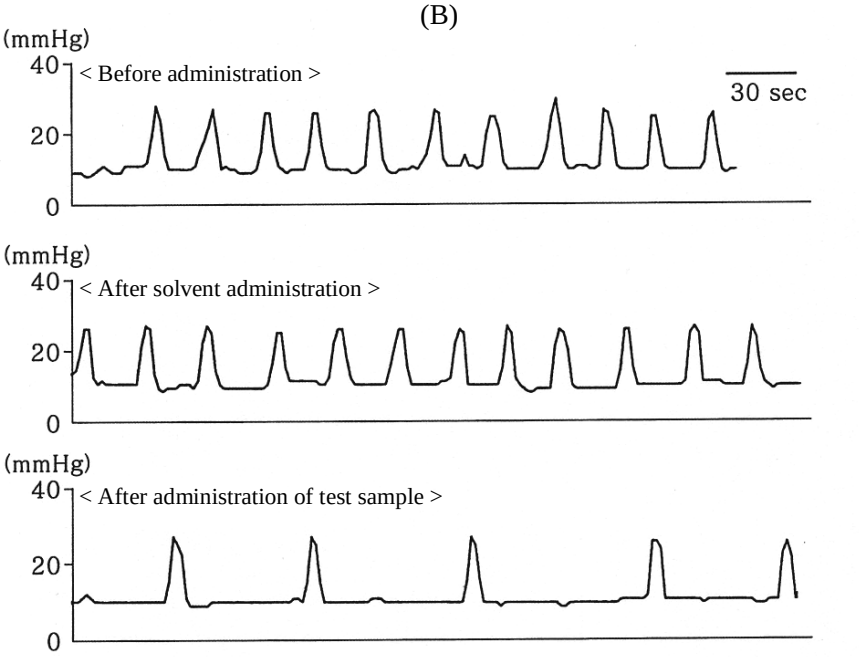


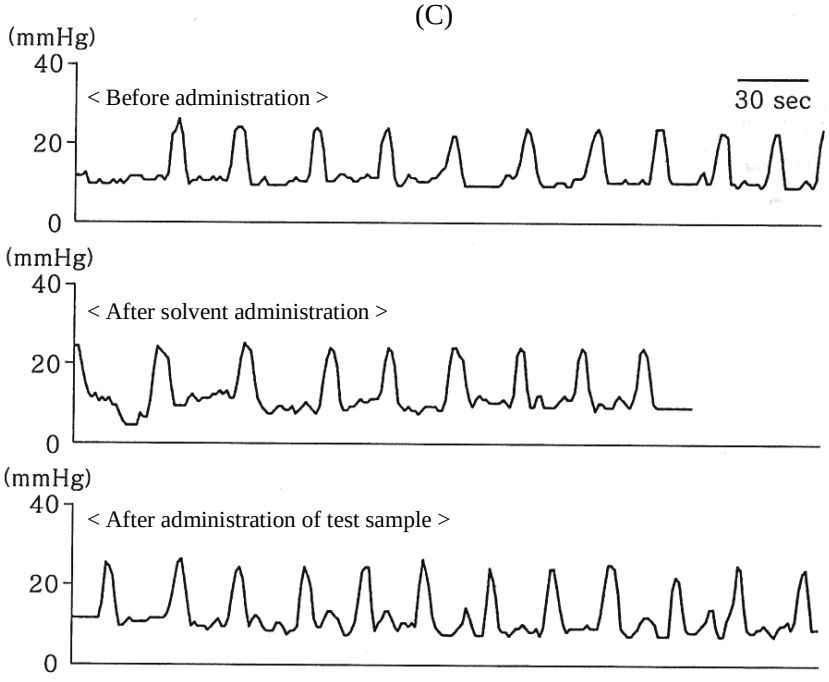
Fig.1 In-bladder pressure in rats
(A) EFLA®940 Pumpkin seed water-soluble extract Lot No. 3038141

(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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