SUPPLEMENTARY MATERIAL

Effect of a mixture of botanicals extracts plus mannitol on Hydration and Bloating Sensation. An open label study in women with high extra cellular water.

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ABSTRACT
The aim of the study is to evaluate the effectiveness of 4 and 8 week supplementation with highly standardized formula with *Fraxinus Ornus* L., plus *Ananas comosus* L., concentrated juice, *Betula Pendula* R., *Equisetum Arvense* L., *Urtica Dioica* L. and *Pilosella Officinarum* L. Vail dry extract, on the state of hydration and bloating sensation in subjects with high and moderate extra cellular water (ECW). 19 women (mean age 35ys and Body Mass Index 22.82 kg\m2) with Extra Cellular Water over 45% completed the study and their data were analysed at baseline, at 30 and 60 days. Bio-impedance, SF36 and anthropometric parameters were assessed. The ECW decreased of -1.97% (at 30 days) and -2.30% (60 days) (p<0.01). Also fat mass decreased of -1.58% (at 30 days) and -2.21% (60 days) (p=0.057). An improvement of free fat mass was assessed (p<0.05) but not on bloating sensation questionnaire at 60 days (p=0.422).

Keywords
Mannitol; bloating sensation; extra cellular water; water retention; hydration.
3. Experimental

3.1 Subjects

Consecutive patients with self reported water retention and bloating sensation to afferent at our endocrinology medicine division (Santa Margherita Institute), during the period of January 2018 to June 2018, were invited to participate in the study after a diagnosis made by a senior Endocrinologist.

All the patients did not have received previous treatments with drugs or botanical supplements in the last six months.

The patients had to fulfill the following five inclusion criteria:

(I) Patients with Severe or moderate water retention, assessed by BIVA: (extracellular water > 45%)

(II) Young adults: Age between 18 and 50 (fertile age)

(III) Availability to take oral nutritional supplements

(IV) Availability to participate by providing written informed consent

(V) Body Mass Index (BMI) between 18.5 kg/m² and 35 kg/m²

Exclusion criteria were as follows:

Current signs or symptoms of severe, progressive, or uncontrolled hepatic, hematological, pulmonary, cardiac, neurological, or cerebral disease; ongoing or past serious infection; pregnancy or breast feeding; current malignancy or history of malignancy within the past five years; congestive heart failure; allergy to components of supplementation.

This study was approved by the Ethics Committee of University of Pavia and informed consent was obtained from all patients.

3.2 Study design

The primary endpoint was to determine the proportion of improvement (in decrease) from baseline of the Extra Cellular Water and improvement of Bloating sensation.

The secondary endpoints of the study were to determine the improvement from baseline to day 60 in the following parameter: hydration parameters by bioimpedence evaluation, health-related quality of life assessed using the Short Form- (SF-) 36 questionnaire, body composition by anthropometric measures.

3.3 Dietary supplement

All subjects were instructed to take immediately before starting breakfast a formulation based on the intake of 15 ml diluted in half a liter of water of XANADREN MD® (Promopharma SPA) which has the following formulation: Mannitol 375 mg by Fraxinus ornus L. (mannitol 70%);
water; *Ananas comosus* L. concentrated juice (13.87%/100 ml) *Betula Pendula* R. dry extract 75 mg (Hyperoside 1%); *Equisetum Arvense* L. dry extract 75 mg (minerals silicon 10%); *Urtica Dioica* L. dry extract 75 mg (Beta-Sitosterol 4%) *Pilosella officinarum Vaill* L. dry extract 75 mg (vitisin 1%); Ascorbic acid (1%) Citric acid (0.3%) Potassium sorbate (0.2%)

### 3.4 Safety

The participants were asked to report any adverse event occurring during the procedures or during the interval between them.

### 3.5 Procedures

Each participant underwent three evaluations, at baseline, at 30-day interval and after 60 days (table 1).

### 3.6 Measurements

*Bio-impedance analysis*

Impedance measurements were conducted in all participants with the same Akern – BIA 101 body composition analyser. Out of body mass components the level of the following components were assessed: fat mass (FM), fat free mass (FFM), total body water (TBW), and extra-cellular water (ECW). Water with its dissolved ions, which is a good electricity conductor, is an indicator of the level of fat and fat free content in the body. Mutual relations between resistance and reactance are established on the basis of phase dependence equations.

In the current study the percentage content of components in total body mass was used.

*Body composition*

Body weight was measured to the nearest 0.1 kg on a precision scale with the participants wearing light clothing, without shoes, with the use of a standardized technique. Waist measurements were taken at the midpoint between the lowest rib and the top of the hip bone (iliac crest), with the use of a standardized technique. Also other anthropometric measurements (Arm Circumference, Calf circumference, Hips circumference, WHR, knee circumference) was done by a standardized technique and waist to hip ratio (WHR) were calculated. (Mazess RB 1990)

*Assessment of quality of life*

The participants were tested with the Short-Form 36-Item Health Survey (SF-36) to assess their quality of life. The SF-36 scales were summarized in 2 dimensions. The first 5 make up the
“physical health” dimension, and the last 5 the “mental health” dimension (MCS, mental component summary). The vitality and general health scales are parts of both dimensions. Thus, each dimension includes 3 specific and 2 overlapping scales. (Ware 1992)

*Characteristics of the sensation of bloating*

The score of the sensation of bloating was assessed by the bowel symptom questionnaire (BSQ) included inquiries about bowel habits, associated abdominal and nonabdominal symptoms, severity of illness, food sensitivity, and health care use. (Munakata 1997)

Patients were asked to indicate all viscera-sensory symptoms that they had experienced over the previous 8 wk.

The symptom list was a compilation of the most commonly reported viscero-sensory symptoms in our clinic: pain behind the chest bone, pressure behind the chest bone, sensation of fullness, sensation of gas, sensation of bloating, bloating with distension of the belly, sensation of fullness in the rectum after a bowel movement, nausea, and belly pain.

*Statistical Analysis*

Data were analyzed by descriptive statistics. Intra-group comparisons was conducted using the Student’s t-test, with a p value <0.05 considered statistically significant. All analyses was performed using SPSS version 21. In addition we evaluated effectiveness time for treatment with general linear models analysis at repeated measures.
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