Efficiency evaluation of innovative herbal compositions with inulin

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Abstract. This work examines the issues of obtaining compositions with adaptogenic and prebiotic effects, quality control, as well as assessment of their biological effects. Inulin was analyzed by reverse-phase HPLC with refractometric detection. In vivo studies were performed using 50 male Wistar rats, 6 weeks old, with an initial body weight of 158 ± 3 g. To study the intestinal microbiota, DNA was extracted using the DNA-Sorb-S kit. As a result, compositions have been developed that include functional food ingredients based on spinach leaves and quinoa grains, containing at least 96.0% inulin. The established beneficial effect of adaptogenic compositions with inulin on the intestinal microbiota opens up the potential for their use to maintain the optimal composition of the intestinal microbiota.

1 Introduction

Many factors in modern realities influence the state of biological status, effective performance and psycho-physiological state. These include, first of all, the diet, the level of physical and nervous stress, and the environmental conditions. To maintain body tone and remain active for a long time, it is necessary to be more demanding when it comes to nutrition. This problem is solved by including complex products with adaptogenic and prebiotic effects into the diet [1].

Among the plant sources of adaptogens, the well-studied one is maral root (*Rhaponticum carthamoides* (Willd.) Iljin), which component is 20-hydroxyecdysone (20E), belonging to the class of phytoecdysteroids. In the above-ground part of the *Serratula coronata* L., the 20E content reaches up to 1.0% in terms of dry weight, which is an order of magnitude higher than in the rhizomes of *R. carthamoides*. [2]. Currently, more than 100 different ecdysteroid-containing dietary supplements and specialized food products based on extracts of *R. carthamoides*, *Ajuga turkestanica* (Regel) Briq.), Brazilian ginseng (*Pfaffia glomerata* (Spreng.) Pedersen), Chinese plant *Cyanotis vaga*, etc. have appeared on the world market [3-5]. Since the use of extracts obtained from the leaves and stems of medicinal plants, or plants that do not have a tradition of food use, in food products is limited (including by law), the search for food plants – new sources of phytoecdysteroids is of interest. Screening targeted studies have made it possible to identify

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phytoecdysteroids in food plants: spinach (*Spinacia oleracea* L.) and quinoa (*Chenopodium quinoa* Willd.), although in significantly smaller quantities than in wild medicinal plants [6-7].

Almost unstudied issues of possible synergism in the manifestation of a wide range of pharmacological effects determined by the combination of phytoecdysteroids and polyphenols that make up these food plants are of particular interest.

Intestinal microbiota is one of the most important key factors in maintaining the health of the entire body. Inulin belongs to the group of soluble dietary fibers that can suppress the activity of pathogenic intestinal microflora at the same time stimulates the development of beneficial microbiota such as *Lactobacillus* and *Bifidobacterium* [8-10].

This work examines some technological issues of creating new compositions based on phytoecdysteroids and polyphenols extracted from quinoa grains and spinach leaves, their complexation with the plant prebiotic inulin, monitoring their quality, as well as in vivo assessment of their effect on the state of the intestinal microbiota of laboratory animals.

2 Materials and methods

2.1 Preparation of compositions with inulin and extracts from spinach and quinoa

To obtain the compositions, a mechanical method was chosen, consisting of joint grinding of dry components (functional food ingredient (FFI) and inulin). The following substances were used: inulin “fibrulin instant”, native highly purified inulin from chicory, not subjected to any fractionation (Novaproduct, Belgium), and functional food ingredients obtained in laboratory conditions based on spinach leaves (FFI 1) and quinoa grains (FFI 2). The components were mixed and then thoroughly ground in a porcelain mortar for 20 minutes at 20°C.

2.2 Control of inulin content in compositions

Inulin was analyzed by reverse-phase HPLC with refractometric detection. Agilent 1260 chromatograph, software – ChemStation (ver. A.09.03) were used. Refractometric detector 1260 RID (G1362A). Autosampler 1260 ALS (G1329B). Column thermostat 1266 TCC (G1316A). Chromatographic column for HPLC Sugar-Pak (WATERS, USA), 300 mm long and 6.5 mm internal diameter, filled with microcrystalline cation exchange gel in calcium form. For free sugars, the elution mode is isocratic (mobile phase – purified water with the addition of Ca-EDTA 0.05 mg/ml). Flow rate – 0.5 ml/min, column t = 80 °C. The volume of the injected sample is 10 µl. 5 g of the composition was dissolved in 100 ml of water, 1 ml was taken into a centrifuge tube and centrifuged for 10 minutes at 3600 rpm. The supernatant is transferred into a vial for chromatography. The inulin content is determined in accordance with MI No. 0152/ROSS RU.0001.310430/2020 “Determination of inulin content in food products and dietary supplements for food.”

2.3 Evaluation of the modulating effect of compositions on the intestinal microbiota

The study was carried out using 50 male Wistar rats aged 6 weeks with an initial body weight of 158±3g. The animals were obtained from the laboratory animal nursery of the Stolbovaya branch of the Federal State Budgetary Institution of Science "Scientific Center for Biomedical Technologies of the Federal Medical and Biological Agency." Animal

studies were approved by the Ethics Committee of the Federal State Budgetary Institution "Federal Research Center for Nutrition and Biotechnology", extract from the protocol of the Ethics Committee Meeting No. 4 dated June 14, 2022. Animals were kept under controlled environmental conditions (temperature 20-26°C, relative humidity 30-60%, 12 hour light cycle). The animals were randomly divided into 5 groups according to body weight: K1, G2, G3, G4 and G5 (n=10). During the 14th day of the experiment, animals of all groups received a standard semi-synthetic diet [11] and water *ad libitum*; in a day food intake was recorded. The body weight of the animals was measured weekly. The diet of animals in groups G2 and G4 was additionally supplemented with FFI1 (spinach leaves extract enriched with 20E and flavonoids) and FFI2 (quinoa grain extract enriched with 20E and flavonoids), respectively, based on the dose of 20-hydroxyecdysone - 2.5 mg/kg of body weight of animal. Compositions 1 and 2: complexes of spinach or quinoa extracts with inulin, were additionally added to the diets of animals in groups G3 and G5,respectively, in an amount of 5.0 g/100g of diet.

On the 14th day of the experiment, rats of all groups (deprived of hunger for 12 hours) were removed from the experiment by decapitation under light ether anesthesia. A pathological autopsy of the animals was performed; the cecum was collected to evaluate the effect of FFI and compositions on the intestinal microbiota.

To study the intestinal microbiota, DNA was extracted using the DNA-Sorb-S kit (FBUN Central Research Institute of Epidemiology). The microbiota was studied by real-time PCR using the Colonoflor 16 Premium test system (Alfa Labs) in accordance with the manufacturer's instructions. DNA extracts were diluted 10-fold with DNA dilution buffer (Evrogen). Amplification and detection were carried out using a CFX96 Real Time System amplifier (Bio-Rad), threshold cycle values (Cq) were calculated automatically by the CFX Manager software, the results were interpreted using the supplied Colonoflor software, the results were expressed as decimal logarithms from the number of genome equivalents CFU/g. Additionally, the ratio of lg levels of *B. fragilis* group/*F. prausnitzii* was calculated.

Statistical processing of experimental data was carried out using Microsoft Office Excel 2007, IBM SPSS Statistics 20.0, the mean value (M), standard error of the mean (m), and median were calculated. To analyze the statistical significance of differences between independent samples on a quantitative basis, the nonparametric Mann-Whitney test was used when comparing 2 groups. Differences were considered statistically significant if the probability of the null hypothesis of no differences was $p \leq 0.05$.

3 Results and Discussion

During quality control of compositions with inulin and PPI, it was found that the inulin content in the samples of the compositions was at least 96.0%.

The general condition of all animals in appearance, coat quality, food and water consumption and behavior during daily examination throughout the experiment was satisfactory.

Figure 1 shows the results of determining the content of microorganisms in the contents of the cecum (genome-eq/g).

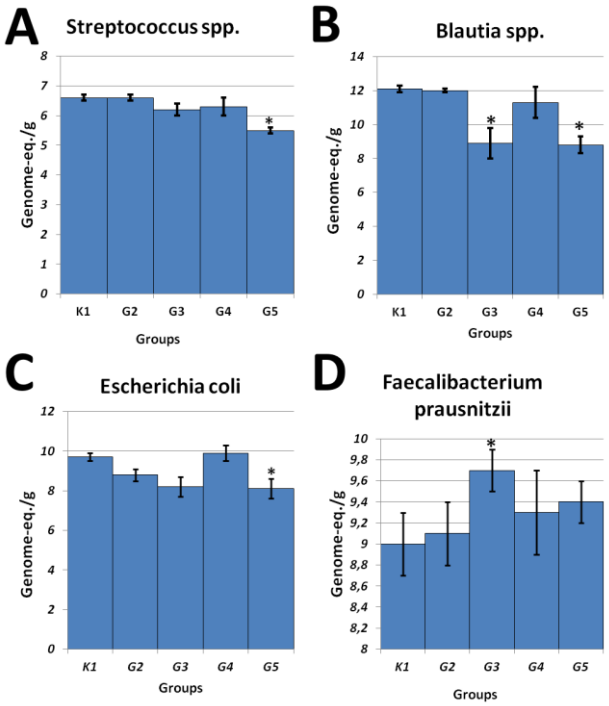

Fig. 1. Levels of microorganisms in the contents of the cecum (genome-eq./g).

When composition 1 and composition 2 were introduced into the diet, a decrease in the levels and frequency of *Blautia* spp was observed, which is a positive change in the microbiota, since there is evidence that Blautia levels correlate positively with metabolic disorders in cardiovascular diseases. The introduction of composition 2 led to a decrease in the content of *Escherichia coli*.

The introduction of composition 1 led to an increase in the content of protective *F. prausnitzii*, a decrease in the ratio of lg levels of *B. fragilis* group/*F. prausnitzii*, high values of which are a potential biomarker of pro-inflammatory dysbiosis (Figure 2).

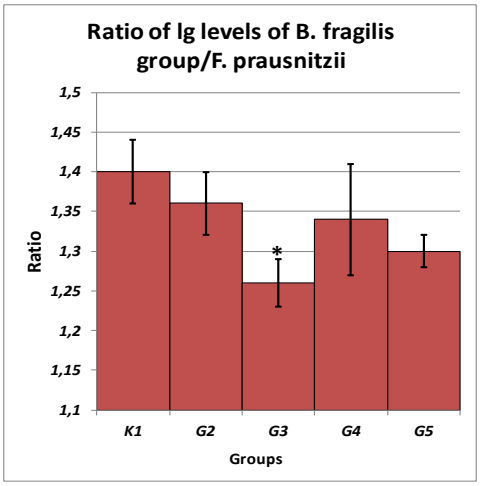

Fig. 2. The ratio of lg levels of *B. fragilis* group/*F. prausnitzii.*

4 Conclusion

Adaptogenic compositions have been developed, including FFI based on spinach leaves and quinoa grains, containing at least 96.0% of inulin. The demonstrated beneficial effect of the developed compositions on the microbiota opens up the potential for their use to maintain the optimal composition of the intestinal microbiota. The principle of creating compositions of FFI and inulin, based on theoretical assumptions, was confirmed in a biological experiment.

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