Evaluation of the effectiveness of new inulin complexes with extracts from quinoa grains and spinach leaves

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Abstract. Consumption of plant-derived prebiotics can help reduce the body's susceptibility to chronic fatigue syndrome, psycho-emotional stress, and the negative effects of increased physical and cognitive overloads. Inulin, a polysaccharide obtained from chicory roots, has beneficial effects on the intestinal microflora. It seems promising to combine inulin with functional ingredients from spinach leaves and quinoa grains. Spinach leaves contain biologically active substances such as carotenoids, phenolic compounds and flavonoids. Quinoa grains contain protein (12.9%), with a high content of lysine and methionine, are rich in fiber, minerals, more than 20 phenolic compounds have been identified in their composition, tannins, saponins, sterols, phytic acid and ecdysteroids are present in small quantities. The aim of this study was to evaluate the effectiveness and safety of the prophylactic administration of the developed complexes to the diet of experimental male Wistar rats. The experiment was carried out during 14 days. Consumption of spinach leaves extract and a complex of spinach extract with inulin by animals decreased the concentration of corticosterone in the blood while simultaneously increasing the content of prostaglandin E2. Consumption of quinoa grain extract and a complex of quinoa extract with inulin significantly reduced only blood corticosterone levels. The beneficial effects of the developed complexes with inulin on the intestinal microbiota have been shown.

1 Introduction

Adequate consumption of plant-based prebiotics, which have a beneficial effect on the state of the intestinal microflora, also helps to reduce the body’s susceptibility to chronic fatigue syndrome, psycho-emotional stress, and the negative effects of increased physical and cognitive overloads [1].

Inulin is found in a fairly large number of plants: its sources can be garlic, artichoke, Jerusalem artichoke tubers, elecampane or burdock root. However, the largest amount of inulin is contained in chicory roots (at least 30%) and that is why this plant serves as the

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main industrial raw material for its production. Once in the stomach, inulin completely dissolves, forming a gel. In the large intestine, inulin is completely fermented exclusively by bifidobacteria, which make up 80-90% of the intestinal microflora of a healthy person. Inulin selectively stimulates the growth of bifidobacteria and lactobacilli in the human intestine, without requiring the introduction of microorganisms from the outside [2]. It is known that bifidobacteria have an enzyme system of beta-fructosidases, which allows them to break down inulin. Bifidobacteria and certain types of lactobacilli multiply very intensively in the presence of inulin: it accelerates, stabilizes and enhances the proliferation of these bacteria in the gastrointestinal tract [3].

Spinach (Spinacia oleracea L.), a dark green leafy vegetable, is a member of the Amaranthaceae family, and there are over 80 unique spinach varieties including Amsterdam Giant, Victoria, and others. Spinach is widely cultivated throughout the world; since 1970, global spinach production has increased by more than 400%. Spinach contains 2.9% protein, 3.6% carbohydrates and 0.4% fat. The water content of spinach is more than 91%. Biologically active substances in spinach are represented by carotenoids, phenolic compounds: (as secondary metabolites in response to various stressors), flavonoids (in particular, quercetin, kaempferol), flavonoid derivatives of patuletin, spinacetin, spinatoside, jaseidin and flavone. The predominant phenolic acids in spinach are ferulic acid and p-coumaric acid. In spinach leaves, the content of 20-hydroxyecdysone is about 0.01% (in terms of fresh weight), which is significantly lower than in medicinal plants [4].

Quinoa (Chenopodium quinoa Willd.) belongs to the subfamily Chenopodiaceae of the Amaranthaceae family. The plant is highly resistant to weather, climate and soil conditions. Quinoa leaves and grains are used for food. Quinoa grains contain carbohydrates (77.6%), protein (12.9%) high in lysine and methionine, fat (6.5%), are rich in fiber, minerals (3.0%), and the content of potassium, calcium, magnesium, phosphorus and iron is much higher than that of traditional cereals. More than 20 phenolic compounds in free or conjugated forms have been identified in quinoa grains, most of which are phenolic acids, as well as flavonoids and their glycosides. Quinoa grains contain small amounts of tannins (0.5%), saponins (mainly oleanolic acid), sterols, phytic acid and ecdysteroids [5].

Accordingly, it seems justified and promising to combine minor biologically active substances extracted from spinach leaves and quinoa grains with a food matrix of a polysaccharide nature - inulin.

The aim of this study was to evaluate the effectiveness and safety of the prophylactic administration of the developed complexes to the diet of experimental male Wistar rats.

2 Materials and methods

2.1 Preparation of extracts from spinach leaves and quinoa grains and their complexes with inulin

Extracts from spinach leaves and quinoa grains were obtained using modern extraction methods, including membrane processing, concentration using reverse osmosis, preparative chromatographic purification, followed by lyophilization. The content of 20E in the extracts of spinach leaves and quinoa grains was 10.5 and 29.2 mg/g, respectively. Complexes of extracts with inulin were obtained by dry mixing of the preparations. The concentration of 20E in the complexes was 0.6 mg/g complex. The resulting dietary supplements were fine powders ranging from light brown to brown in color.
2.2 Experimental animals

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 carried out in accordance with the requirements set out in the National Standards of the Russian Federation GOST 33647-2015 “Principles of Good Laboratory Practice” and GOST 33216-2014 “Guide to the care and maintenance of laboratory animals. Animals were kept under controlled environmental conditions (temperature 20-26°C, relative humidity 30-60%, 12 hour light cycle).

2.3 Experimental design

The animals were randomly divided into 5 groups according to body weight: K1 (n=10), G2 (n=10), G3 (n=10), G4 (n=10) and G5 (n=10) (Table 1).

Table 1. Dividing animals into groups.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Animal group</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>K1 (n=10)</td>
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<tr>
<td>Body weight, g</td>
<td>158.5±5.9</td>
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Note: FFI – functional food ingredient, FS – food supplement.

During the 14 days of the experiment, animals of all groups received a standard semi-synthetic diet [6] 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 FFI 1 (functional food ingredient, spinach leaves extract enriched with 20E and flavonoids) and FFI 2 (quinoa grain extract enriched with 20E and flavonoids), respectively, based on the dose of 20-hydroxyecdysone - 2.5 mg/kg animal body weight. FS 1 and FS 2 (food supplements) - complexes of spinach or quinoa extracts with inulin - were additionally added to the diet 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 food 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 FS on the intestinal microbiota. Cecal samples were stored at –70°C. The blood collected after decapitation of the animal was incubated at a temperature of 2-8°C for 3 hours, centrifuged for 30 minutes at 3000 rpm at a temperature of 4°C, and the resulting serum was stored at -20°C. Using competitive ELISA, the content of corticosterone and prostaglandin E2 was determined in blood serum according to the manufacturer’s method (Elabscience, USA).

The microbiota was studied by real-time PCR using the Colonoflor 16 Premium test system (Alfa Labs), the objects of the study were samples of the contents of the cecum of experimental rats.

Statistical processing of the obtained results was carried out using the SPSS Statistics 20 software package (IBM, USA), using the nonparametric Mann–Whitney U-test and the Student t-test. The mean (M), standard deviation (SD), standard error of the mean (m), and median were calculated. Data are presented as M±m. The critical significance level of the null statistical hypothesis (p) was taken equal to 0.05.
3 Results

The general condition of all animals in appearance, fur quality, food and water intake and behavior during daily examination throughout the experiment was satisfactory. There were no significant differences in food intake between animals of all groups. There were no significant differences in body weight gain between animals of all groups.

Table 2 shows the results of determining the levels of corticosterone and prostaglandine E2 in the blood.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Animal group</th>
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<tbody>
<tr>
<td></td>
<td>K1</td>
</tr>
<tr>
<td>Corticosterone, ng/mL</td>
<td>28.8±2.4</td>
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<tr>
<td>Prostaglandine E2, pg/mL</td>
<td>143.3±9.5</td>
</tr>
</tbody>
</table>

Note: 1 – differences are significant against control group K1 (p<0.05); 2 – differences are significant against group G2 (p<0.05); 3 – differences are significant against group G3 (p<0.05).

Significantly lower levels of corticosterone and higher levels of prostaglandin E2 were detected in the blood of animals of group G2, fed with a diet with FFI 1 included, and of group G3, fed with a diet with FS 1 included, compared to animals of the control group K1. In animals of group G4, fed with the diet with FFI 2 included, there was a significant decrease in the blood level of corticosterone compared to the corresponding indicators of animals in the control group K1. In the blood of animals of group G5, which received FS 2 with a diet, a significant decrease in the level of blood corticosterone was also detected compared to animals of group K1. No changes in the level of prostaglandin E2 were shown for animals of groups G4 and G5 compared to intact control animals. When FS 1 and FS 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 positively correlate with metabolic disorders in cardiovascular diseases. The introduction of FS 2 led to a decrease in the content of *Escherichia coli.*

The introduction of FS 1 led to an increase in the content of protective *F. prausnitzii*, a decrease in the ratio of Ig levels of *B. fragilis* group/*F. prausnitzii*, high values of which are a potential biomarker of proinflammatory dysbiosis.

4 Conclusion

Corticosterone is responsible for the body's adaptation to stressful situations. Produced in the adrenal cortex under the control of the hypothalamus and adrenocorticotropic hormone (ACTH), synthesized by the anterior pituitary gland. Prostaglandin E2 is a component of the peripheral part of the stress-limiting system; accordingly, it belongs to the stress system inhibitors, which limit the action of catecholamines and glucocorticoids, activators of the stress system, preventing stress damage. We have shown that consumption of FFI 1 and food supplement FS 1 based on spinach leaves by animals decreased the concentration of the stress system activator corticosterone in the blood while simultaneously increasing the blood content of the stress system inhibitor prostaglandin E2. Consumption of FFI 2 and food supplement FS 2 based on quinoa grains significantly reduced only the level of blood corticosterone compared to intact animals in the control group, without affecting the peripheral part of the stress system. It is known that under the influence of adaptogens, the synthesis of inhibitors and activators of the stress system and the content of these hormones in the blood change, which activates adaptive reactions on the part of homeostatic systems and increases the overall resistance of the body, i.e. the adaptogen itself acts as a stressor.
The developed food supplements have been shown to have a beneficial effect on the intestinal microbiota, which opens up the potential for their use to maintain the optimal composition of the intestinal microbiota.

Acknowledgement

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References


