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Research Article

Experimental research of garden spinach extract as a potential anabolic medicinal product

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Abstract

Disorders of protein metabolism – one of the pathologies, the treatment of which is an urgent problem of modern medicine and pharmacy. The importance of the problem is stipulated due to the prevalence of diseases that are accompanied by a violation of protein metabolism in the body. Restoration of protein resources during such conditions requires adequate intake of structural components from the outside – proteins, amino acids and other biologically active substances-correctors of protein metabolism. In recent decades, much attention has been paid to the discoveries of anabolic agents of natural origin, especially from plants. We conducted research on the study of the anabolic and antioxidant properties of dry extract of spinach garden leaves on the model of food deprivation in rats. Established, that the dry extract at the dose of 100 mg/kg body weight of animals prevents the development of organic disorders of protein metabolism, oxidative stress, prevents a sharp decrease in body weight and slows down the generalized catabolism caused by starvation in rats. Under the influence of the extract on the model of food deprivation, the diuretic function of the kidneys is preserved at the physiological level.

Keywords

spinach garden leaves, dry extract, food deprivation, protein metabolism, spontaneous diuresis, oxidative stress, rats

Introduction

There is evidence in the scientific literature that almost every deviation in the functional state of the organism causes various changes in protein metabolism. Disruption of protein biosynthesis and different levels of regulation of active metabolism can be a trigger in the genesis of many pathological processes and diseases, as well as accompanying symptoms and syndromes (Morrison and Laeger 2015).

It is an indisputable fact that stressful situations for the body, including acute (hepatitis, poisoning, myocardial infarction) and chronic (autoimmune, renal failure, cirrhosis) diseases of internal organs and systems of various etiologies lead to hypoproteinemia, and as a consequence – functional and organic violations. Because it is known that skeletal muscle proteins and partially proteins of visceral organs under stress are subjects to destruction and subsequent use in other organs. Scientists have calculated that the normal protein resources of the human body without replenishment may be enough for 3–4 days, and with severe stress – only a few hours (Eremenko and Ryadnykh 2013).

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PENSOFT

To restore protein resources during such conditions requires adequate intake of structural components from the outside – proteins, amino acids and other biologically active substances-correctors of protein metabolism.

Therefore, the search of new pharmacological agents for the correction of protein disorders is an urgent problem of modern pharmacology and medicine.

The reaction of the animal's body to food deprivation largely depends on the degree of metabolic processes in tissues and organs, which are aimed at ensuring homeostasis and adaptation to the action of extreme factors.

Adequate provision of the body with a balanced diet based on basic nutrients is a necessary condition for maintaining physiological functions and biochemical processes in cells. Prolonged starvation leads to a decrease in glucose, as well as glycolytic intermediates – phosphoenolpyruvate, pyruvate and lactate in the body (Palm and Thompson 2017).

In order to stimulate the processes of regeneration and protein synthesis are used anabolic drugs, the action of which is aimed at accelerating the renewal and formation of structural parts of cells, primarily muscle structures (Liu et al. 2018).

In many pathological conditions (burns, leukemia, severe infections) there is a violation of protein-synthetic processes, which leads to a negative nitrogen balance. It can be corrected in different ways: the use of protein hydrolysates, amino acid mixtures, blood transfusions or the use of steroid anabolic drugs (retabolil, nerobol, phenobolin, etc.). However, anabolic hormones cause many side effects, so in recent decades much attention has been paid to the search for anabolic agents of natural origin, especially from plants (Tiwari et al. 2020).

One of these plants is garden spinach, which contains a large group of biologically active substances and has a wide range of pharmacological activity, including affecting metabolic processes, one aspect of which is protein metabolism.

Garden spinach (*Spinacia oleracea* L.) is the herbaceous plant belonging to the Amaranth family. Spinach is cultivated in Ukraine and is widespread throughout the world. It has antioxidant, anti-inflammatory, hepatoprotective, antitumor effects (Gutierrez et al. 2019; Roberts and Moreau 2016). In addition, it contains a large number of vitamins such as vitamin A, vitamin C, vitamin E, folic acid and minerals such as magnesium, manganese, iron and calcium. Spinach is also a source of chlorophyll and carotenoids, which are also necessary for the human body (Grinenko and Zhuravel 2017). Among the flavonoids found the presence of rutin, quercetin, apigenin, luteolin and hyperoside.

Scientists of the National Pharmaceutical University of Ukraine determined the content of free and bound amino acids, polysaccharides, flavonoids and polyphenolic compounds in the dry extract. In addition, among the amino acids in the dry extract of spinach leaves were found: alanine, leucine, phenylalanine, valine, asparagine, glycine and methionine (Petrovska et al. 2018).

Considering the content of biologically-active compounds, which are present in the dry extract of spinach garden leaves, it was expedient to research its effect on protein metabolism in animals.

Objective

to study the anabolic activity of dry extract of spinach garden leaves on the model of food deprivation in rats.

Material and methods

Studies of pharmacological correction of protein metabolism disorders on the model of food deprivation were performed on white rats, which for 7 days were in conditions of absolute food starvation with sufficient access to water. To assess the anabolic activity of dry extract of garden spinach leaves (DESL) experimental animals were divided into 3 groups: the first – control group – animals under complete starvation, the second – animals that received oral DESL at the dose of 100 mg/kg (Nykyforuk et al. 2020), the third – animals that were orally administered the reference drug – potassium orotate (KO) at the dose of 100 mg/kg (Morrison and Laeger 2015).

Dry extract of spinach garden leaves was obtained by the method: in a ratio of 1:5 (crushed raw materials and extractant). 10.0 g of raw material was poured into 0.1 M NaOH and stirred vigorously for 30 min. The resulting extract was evaporated on a rotary apparatus to dryness. To prevent denaturation of compounds of protein nature, evaporation was performed at a temperature not exceeding 30 °C.

In experimental animals of all groups on the first day of the study, the following parameters were determined: body weight, daily spontaneous diuresis, urea content in serum and urine. During 7 days of the experiment, the dynamics of body weight of rats was evaluated. Euthanasia of animals was performed at 24 hours, 2nd, 3rd, 5th and 7th days. Before euthanasia, diuresis and urea content in urine were again determined (Vlizlo et al. 2012).

Animals were removed from the experiment under thiopental anesthesia in compliance with all rules of work with vertebrates (Gross and Tolba 2015). Then we studied the biochemical parameters: the content of total protein in the serum, muscles and liver (Vlizlo et al. 2012), as well as the content of urea (Vlizlo et al. 2012) in the serum.

The activity of pro- and antioxidant processes in blood serum, liver and muscles was evaluated by the content of lipoperoxidation products (TBA-AP) (Zeb and Ullah 2016), catalase activity (CA) (Hadwan 2018) and the content of ceruloplasmin (CP) (Vlizlo et al. 2012) in blood serum.

Parametric (Student) and nonparametric (Wilcoxon) research methods were used for statistical data processing. Probable changes were considered at $p \leq 0.05$ (Eroglu and Yuksel 2019).

Table 1. Dynamics of body weight of rats (g) with food deprivation and with the use of DESL and KO (M ± m; n = 6).

Experimental conditions	Before starvation	Terms of research				
		24 hours	2nd day	3rd day	5th day	7th day
Control pathology, CP	197.83 ± 3.45	195.33 ± 3.79	187.66 ± 3.32	176.50 ± 3.42*	166.33 ± 3.47*	160.00 ± 4.06*
Mass change, g		-2.5	-10.2	-21.3	-31.5	-37.8
Pathology + DESL, 100 mg/kg	189.33 ± 3.34	187.16 ± 3.05	182.33 ± 3.31	175.17 ± 3.78*	172.50 ± 3.43*	171.00 ± 3.01*
Mass change, g		-2.1	-7.0	-14.2	-16.8	-18.3
Pathology + KO,100 mg/kg	195.16 ± 2.23	193.33 ± 2.18	188.00 ± 2.38	182.33 ± 2.51*	179.00 ± 2.35*	178.50 ± 1.99*
Mass change, g		-1.8	-7.1	-12.8	-16.1	-16.6

Note: * – probable changes ($p \leq 0.05$) in the body weight of experimental animals relative to the initial weight before starvation; CP – control group of animals (control pathology)

Table 2. The effect of DESL and KO on daily diuresis and urea content in serum and urine of animals with 7-day food deprivation (M ± m; n = 6).

Experimental conditions	Daily diuresis, ml		Urea in blood serum, mmol/l		Urea in urine, mmol/l	
	Before starvation	7 days	Before starvation	7 days	Before starvation	7 days
Control pathology	3.95 ± 0.08	2.38 ± 0.06*	5.88 ± 0.17	11.25 ± 0.19*	351.0 ± 7.75	870.4 ± 8.30*
Pathology + DESL, 100 mg/kg	3.95 ± 0.08	3.43 ± 0.04*,#	5.87 ± 0.17	8.32 ± 0.17*,#	351.0 ± 7.75	547.5 ± 19.75*,#
Pathology + KO, 100 mg/kg	3.95 ± 0.08	3.77 ± 0.07#	5.88 ± 0.17	7.77 ± 0.12*,#	351.0 ± 7.75	440.03 ± 9.85*,#

Note: * – probable changes ($p \leq 0.05$) between the indicators of animals of all groups before starvation and after 7 days of starvation; # – probable changes ($p \leq 0.05$) between the indicators of animals of control pathology and animals that received the experimental extract and the reference drug.

Results and discussion

One of the pathological models used in preclinical studies to assess anabolic activity is food deprivation. At complete starvation violations of metabolic processes are observed in rats: inhibition of anabolism and generalized increase in protein catabolism. This is clinically manifested by a symptomatic complex, characterized by abrupt weight loss, agitation, and then depression, decreased diuresis, negative nitrogen balance (Dietze et al. 2016).

Analysis of the results of the effect of DESL on the body weight of animals is shown in Table 1.

Starvation of rats for 7 days led to the decrease in body weight of animals by 37.8 g. Therefore, we can assume that starvation leads to generalized catabolism. The reduction of total body weight is carried out primarily due to adipose tissue and the breakdown of skeletal muscle proteins, which for some time helps to preserve the mass of vital organs (heart, brain) (Dietze et al. 2016).

In the group of animals that received DESL on the background of starvation, weight loss was significantly lower and was 18.3 g at the end of the study. In animals that received DESL for 7 days at a dose of 100 mg/kg on the background of complete starvation, the weight deficit was 2.1 times lower than in rats of the control group. After administration of potassium orotate to starved animals for 7 days, the body weight decreased even less and it was 16.6 g. The body weight deficit in these animals was 2.3 times lower than in control rats.

It was experimentally established that under conditions of starvation in groups of animals treated with DESL and reference drug – KO, there was an increase in total body weight relative to the control group. Thus, a study of the dynamics of body weight of rats showed that DESL, as well as KO, show protective activity, significantly affecting the weight of animals with food deprivation.

Based on the analysis of the results given in Table 2, it was found that in animals of the control pathology group throughout the experiment there was a decrease in daily spontaneous diuresis and by the end of the study it was 40% less than before starvation.

In rats, that were starving and receiving DESL, in 7 days of the experiment the daily diuresis decreased by 13%, in rats that received potassium orotate, the decrease in daily diuresis was 5% from output data.

During starvation was noted a positive effect of the extract on spontaneous daily diuresis, after 7 days of starvation this indicator probably ($p \leq 0.05$) increased compared to the control group (by 27%). After administration of potassium orotate to starving animals, daily diuresis was almost restored and exceeded that of control animals by 35%.

The functional status of the kidneys was assessed by the content of urea in the serum. Urea is the main end product of nitrogen metabolism in the body. With a positive nitrogen balance, urea excretion decreases. In the presence of an anabolic stimulus and a positive response, the urea content is also reduced in the serum. These changes are the manifestation of improved plastic processes in the body (Shatalova 2015).

In this model pathology in experimental animals was researched the content of urea in the serum and urine (Table 2).

It was found that after 7 days of complete starvation in the serum of rats the urea content increased 1.9 times, in the urine – 2.5 times. This may indicate an increase in protein dissimilation during food deprivation and the development of renal failure in the body.

In the group of starving rats, which were receiving the experimental extract, the urea content after 7 days of starvation increased in serum 1.4 times, in urine – 1.6 times compared with this group of animals before starvation. The use of DESL in the model of food deprivation in rats led to a decrease in serum urea by 1.35 times relative to the control pathology ($p \leq 0.05$) and in urine by 1.6 times.

Table 3. The effect of DESL and KO on protein content in serum, liver and muscles of animals with 7-day food deprivation (M ± m; n = 6).

Experimental conditions	Serum, g/l		Liver, g/100 g		Muscles, g/100 g	
	Before starvation	7 days	Before starvation	7 days	Before starvation	7 days
Control pathology	64.42 ± 1.28	58.10 ± 0.81*	24.07 ± 0.80	21.05 ± 0.37*	25.22 ± 0.41	22.08 ± 0.59*
Pathology + DESL, 100 mg/kg	64.42 ± 1.28	64.83 ± 0.29#	24.07 ± 0.80	24.82 ± 0.39#	25.22 ± 0.41	25.78 ± 0.35#
Pathology + KO, 100 mg/kg	64.42 ± 1.28	66.02 ± 0.43#	24.07 ± 0.80	25.15 ± 0.51#	25.22 ± 0.41	26.68 ± 0.24*,#

Note: * – probable changes ($p \leq 0.05$) between the indicators of animals of all groups before starvation and after 7 days of starvation; # – probable changes ($p \leq 0.05$) between the indicators of animals of control pathology and animals that received the experimental extract and the reference drug.

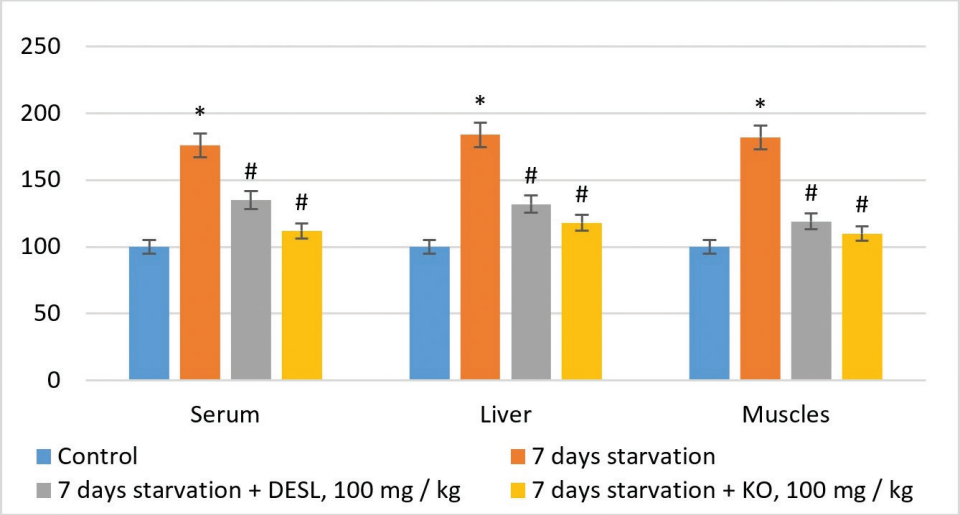

Figure 1. The content of TBA-active products in serum, liver and muscles of rats during starvation and after the use of DESL and KO, %. Note: * – probable changes ($p \leq 0.05$) between the indicators of animals of all groups before starvation and after 7 days of starvation; # – probable changes ($p \leq 0.05$) between the indicators of animals of control pathology and animals that received the experimental extract and the reference drug.

A similar decrease in urea was observed in the serum and urine of rats after administration of potassium orotate on the background of food deprivation, but this decrease was more expressed.

Thus, DESL in the model of food deprivation increases anabolic processes and inhibits catabolic ones.

The presence of a large amount of protein, amino acids, including essential vitamins, micro- and macronutrients (Petrovska et al. 2018) in DESL will help correct protein metabolism in the body, and thus restore its plastic, structural, energy and other functions.

The results, obtained in the determination of total protein in serum, muscles and liver (Table 3), also show that the experimental extract stimulates anabolic processes.

In animals of control pathology, the content of total protein during 7 days of starvation decreased in the serum by 10%. Under the influence of DESL and potassium orotate, there is a probable increase in the total protein content ($p \leq 0.05$) relative to the control group. After administration of the reference drug, this figure exceeded the level of protein that rats had before starvation.

Similar results were obtained in the research of protein content in the liver and muscles.

Starvation for 7 days reduced the protein content of the liver and muscles by 13% compared to output data. Both of our drugs showed an expressed anabolic effect, after their use the experimental indicator increased and slightly exceeded the indicator observed before starvation.

Flavonoids and organic acids contained in spinach leaves and which are characterized by antioxidant, anti-inflammatory, membrane-stabilizing and organ-protective effects, will help to restore the functional activity of vital organs, including the liver, which performs protein-synthetic function, and thus also contributes to the body (Gutierrez et al. 2019).

There are reports in the literature (Gembarovsky et al. 2013) that modeling of food deprivation in rats alters the prooxidant-antioxidant balance towards the development of oxidative stress.

We studied the content of lipoperoxidation products (TBA-AP) in the serum, liver and muscles of rats after 7 days of starvation, as well as the effect on this indicator of DESL and KO (Fig. 1).

Starvation of rats increased serum TBA-AP by 76%, liver by 84%, and muscles by 82%.

This is a consequence of increased activity of oxidative processes, in particular lipoperoxidation, in the body of rats during food deprivation. Our data are consistent with the literature, which shows the development of oxidative stress during starvation of animals for 7 days (Gembarovsky et al. 2013).

Data from an experimental model of food deprivation indicate that lipoperoxidation activation depends on the duration of alimentary starvation. Activation of lipoperoxidation processes may be due to the development of a stress response, which is a reflection of all adaptive reactions of the

Table 4. The effect of DESL and KO on catalase activity in serum, liver and muscles of animals with 7-day food deprivation (M ± m; n = 6).

Experimental conditions	Serum, mckat/l		Liver, mckat/kg		Muscles, mckat/kg	
	Before starvation	7 days	Before starvation	7 days	Before starvation	7 days
Control pathology	0.26 ± 0.02	0.19 ± 0.01*	0.36 ± 0.01	0.24 ± 0.01*	0.30 ± 0.01	0.19 ± 0.01*
Pathology + DESL, 100 mg / kg	0.26 ± 0.02	0.25 ± 0.01#	0.36 ± 0.01	0.32 ± 0.01#	0.30 ± 0.01	0.26 ± 0.01#
Pathology + KO, 100 mg / kg	0.26 ± 0.02	0.26 ± 0.01#	0.36 ± 0.01	0.36 ± 0.01#	0.30 ± 0.01	0.32 ± 0.01#

Note: * – probable changes ($p \leq 0.05$) between the indicators of animals of all groups before starvation and after 7 days of starvation; # – probable changes ($p \leq 0.05$) between the indicators of animals of control pathology and animals that received the experimental extract and the reference drug.

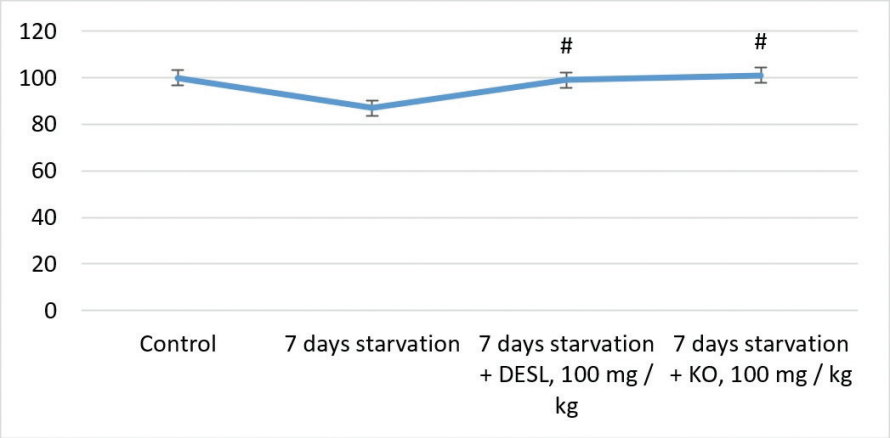

Figure 2. The content of ceruloplasmin in the serum of rats during starvation and after the use of DESL and KO, %. Note: * – probable changes ($p \leq 0.05$) between the indicators of animals of all groups before starvation and after 7 days of starvation; # – probable changes ($p \leq 0.05$) between the indicators of animals of control pathology and animals that received the experimental extract and the reference drug.

body that occur in response to the stimulus (in our case, starvation) and aimed at the implementation of adaptive mechanisms. The stress response is manifested by the growth of catecholamines that activate lipoperoxidation (Dietze et al. 2016). On the other hand, the reduction of oxygen delivery to the organs of the gastrointestinal tract causes the development of hypoxia, which also activates lipoperoxidation.

The experimental extract of spinach leaves led to the decrease in the content of TBA-AP in all studied tissues and organs, which was probable ($p \leq 0.05$). Potassium orotate slightly exceeded DESL in efficiency.

The influence of extreme factors, including starvation, leads to a shift in the balance between pro- and antioxidant systems in the prooxidant direction and the development of so-called "oxidative stress". Namely, under such conditions, oxidative stress develops, which is the result of an imbalance between the excessive formation of the active forms of oxygen and the inability of antioxidant systems to ensure their neutralization.

Increasing the intensity of free radical reactions causes a decrease in the protective and compensatory forces in the affected organism and the inability to eliminate toxic products from it.

In our research it was found that starvation is accompanied by a gradual decrease in catalase activity during the experiment in all organs of rats, which inhibits the process of neutralization of hydrogen peroxide formed as a result of superoxide dismutase reaction and leads to intoxication of the body.

At the end of the experiment (7 days) in animals of the control group was recorded a probable ($p \leq 0.05$) decrease in catalase activity in all studied organs and tissues (Table 4).

In the serum of starving rats for 7 days, catalase activity decreased 1.4 times, in the liver 1.5 times, in the muscles – 1.6 times, which indicates, on the one hand, the activation of oxidative processes in the body, on the other hand, the increase of the processes of protein catabolism during starvation.

After administration of the extract and the reference drug, serum catalase activity approached to output data (before starvation). This confirms the antioxidant and anabolic properties of the extract we studied.

One of the main antioxidants of blood plasma – ceruloplasmin – copper-containing protein of alpha-2-globulin fraction of blood. A feature of this protein is its high stability to the toxic effects of the active forms of oxygen, which allows it to maintain biological activity under conditions of their intensive generation.

The results of the research of the content of CP in the serum of rats after the lesion are shown in Fig. 2.

The content of CP after starvation decreased in the serum of rats by 13%. In experimental rats treated with DESL and KO, ceruloplasmin levels did not differ significantly from the output data.

Thus, DESL on the model of food deprivation increases anabolic processes and inhibits catabolic processes, as well as exhibits antioxidant properties, apparently due to the content of a significant amount of protein, as well as flavonoids and organic acids.

It has been experimentally proven that dry extract of garden spinach leaves under conditions of food deprivation prevents the development of organic disorders of

protein metabolism, oxidative stress, prevents a sharp decrease in body weight and slows down the generalized catabolism caused by starvation in rats. Under the influence of the extract on the model of food deprivation, the diuretic function of the kidneys is preserved at the physiological level.

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