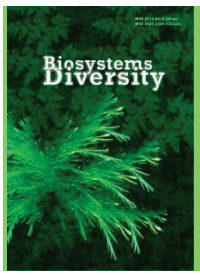

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The effect on the organism of rats of adding *Helichrysum arenarium* inflorescences to a hypercaloric diet, high in sugar and fat

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Obesity increases the risk of developing various pathological conditions such as metabolic syndrome, type 2 diabetes, non-alcoholic fatty liver disease, and cardiovascular disease. For mild correction and even treatment of obesity, drugs based on medicinal plants are increasingly being used. This work aims to determine the overall effect of *Helichrysum arenarium* inflorescences on physiological activity and metabolic processes in model animals fed a diet rich in fat and carbohydrates. In a 27-day experiment on 18 laboratory rats fed a diet with high-fat content and 20% fructose solution, we determined the effect of 0.4% and 4.0% dry inflorescences of *H. arenarium* from the feed mass on the animals' physiological activity and metabolic processes. The body weight of rats in the control group reached 121.7% of the initial body weight; when consuming 0.4% and 4.0% *H. arenarium* in the diet, this indicator was lower – 109.6% and 111.2%, respectively. Dry inflorescences of *H. arenarium* in the rats' diet at a dose of 0.4% caused a decrease in the relative mass of the heart and thymus and an increase in the relative mass of the caecum and colon. A diet with the addition of *H. arenarium* caused a change in blood biochemical parameters: an increase in the urea concentration and urea nitrogen, and a decrease in the glucose concentration and protein coefficient. *Helichrysum arenarium* at both concentrations significantly increased the gamma-glutamyltransferase and alkaline phosphatase activity with a decrease in alpha-amylase activity in the blood. A 0.4% dose of inflorescences of the food mass caused a decrease in AST activity, and 200 g sharply increased blood ALT activity. The consumption of dried *H. arenarium* inflorescences decreased hematocrit, haemoglobin concentration, erythrocyte and platelet counts, and increased the percentage of eosinophils and monocytes. A decrease in physical activity and an intensification of emotional state were found in the animals after eating dry inflorescences of *H. arenarium*, regardless of the dose compared with the control group of animals. Significant changes in the orientation activity of the animals were not observed. The obtained results show that adding dry *H. arenarium* inflorescences as a food supplement to a high-calorie diet is safe, does not cause pathological changes and side effects, and significantly affects metabolic processes. This provides theoretical support for the use of dried *H. arenarium* inflorescences for the production of nutraceutical and pharmacological products for the correction of metabolic disorders in humans and animals. The doses and duration of their use require further research.

Keywords: relative mass of organs; increase in body weight; high-fat diet; phytotherapy; obesity correction.

Introduction

The spread of obesity has become a serious public health problem worldwide. Obesity occurs when energy intake is high and energy expenditure is low. Obesity is a cause of chronic diseases such as type 2 diabetes, insulin resistance, cardiovascular disease, non-alcoholic fatty liver disease and dyslipidemia (Poirier et al., 2006; Fabbrini et al., 2009; Jung et al., 2014). Significant efforts have been made by the scientific community to discover effective drugs for the treatment of obesity and the correction of dyslipidemia. Despite their effectiveness, many methods have bad side effects (Lee et al., 2018; Yang et al., 2020; Lieshchova & Brygadyrenko, 2023a, 2023b). Substantial changes have taken place in modern medicine, the pharmaceutical and chemical industries, but the therapeutic use of medicinal plants is still relevant.

Dwarf everlast or immortelle (*Helichrysum arenarium* (L.) Moench, 1794) is a perennial herbaceous plant of the Asteraceae family. It grows on dry sandy, as well as sandy clay and stony soils. The natural range is the territory of Central, Eastern and South-Eastern Europe, the Northern Balkans, Western Siberia, Central Asia, Mongolia and China (Pljevljakušić et al., 2018). As a medicinal raw material, immortelle flower heads (*Flores Helichrysi arenarii*) are used, which must be collected while they are in full bloom. The collected raw materials are dried in the shade, in a well-ventilated room or in dryers at a temperature not exceeding 40 °C. The chemical composition of inflorescences is complex, the sum of extractive substances is 26.8% of the initial mass of inflorescences. The main active substances of immortelle raw materials include flavonoid glycosides (salipurpuroside, kaempferol and isosalipurpuroside), flavonoids (naringenin and apigenin), sugars (1.2%), vitamins C and K. In addition, phthalides, high molecular weight alcohols, resins (3.66%), steroid compounds, dyes, essential oil (up to 0.05%), inositol, tannins, fatty acids, mineral salts and trace elements (Wierzchowska-Renke & Kosinski, 1994). Chlorogenic acids, naringenin-4′-O-glucoside, tomoroside A, naringenin-5-O-glucoside, isosalipurposide, and naringenin have the strongest physiological activity in *H. arenarium* inflorescences (Ivanović et al., 2022). Most authors confirm that the most important group of compounds responsible for biological activity are flavonoids. It is to flavonoids that Durnova & Kurchatova (2015) attribute the antimutagenic activity of the *H. arenarium* extract. In addition, significant activity of naringenin, one of the main flavonoids of *H. arenarium*, has been reported (Pljevljakušić et al., 2018). Babotă et al. (2018) found that the ethanol extract of *H. arenarium* is dominated by quercetin. Gradinaru et al. (2014) determined the content of phenolic compounds in methanol extract from *H. arenarium* inflorescences with a phenols total content of 160.2 mg/g. *Helichrysum arenarium* contains many trace elements, including silver and gold (Vural & Safari, 2022), but the content of trace elements in raw materials depends on the geological environment of the plant (Vural, 2018).

An essential oil is obtained from immortelle inflorescences, which includes cresol, free acids, including caproic acid (Reidel et al., 2017). Judzentiene et al. (2022) determined that the main components of essential oils were palmitic (≤23.8%), myristic (≤14.9%) and lauric (6.1%) acids,

n-nonanal (10.4%) and trans-β-caryophyllene (≤6.5%). Quantitative analysis of immortelle essential oils by gas chromatography showed that the main components of *H. arenarium* oil are camphor (14.59%) and carboxylic acids (37.02%), as well as 1,8-cineol (5.97%). *Helichrysum nogaicum* Zvelev oil contains camphor (38.33%), borneol (14.21%), 1,8-cineol (11.37%) and terpene-4-ol (5.18%), sesquiterpene compounds (6.28%), and the content of carboxylic acids is negligible – 6.58% (Baimukhambetova et al., 2019). Reidel et al. (2017) determined that sesquiterpenes are the main class of substances that make up the essential oil of most studied species of the *Helichrysum* genus. β-caryophyllene was the key compound of *H. arenarium* and *H. nudifolium* (L.) Less. leaves, even though (E)-2-hexenal showed a high percentage in *H. arenarium* leaves. Zheljazkov et al. (2022) determined that the predominant components of *H. arenarium* essential oil were α-pinene (34.64–44.35%) and sabinene (10.63–11.10%).

Infusion, dry extract, Flamin and Ziflan preparations are also obtained from the inflorescences of this plant, used as choleretic agents for acute and chronic liver diseases, gallbladder and biliary tract, as well as for gastrointestinal tract and kidney diseases (Chinou et al., 1996). Extracts obtained from immortelle improve bile secretion, stimulate the synthesis of bile acids from cholesterol, increase the content of cholates and bilirubin in bile. Immortelle preparations increase the cholate-cholesterol ratio, thus reducing the bile lithogenicity, and gently increase the gallbladder tone (Baimukhambetova et al., 2019). The extract of immortelle also has an antispasmodic effect on the intestinal smooth muscles, biliary tract, gallbladder and blood vessels. These properties are due to the presence of flavonoid compounds in the plant (Kramberger et al., 2021). Grinev et al. (2016) found by molecular absorption spectroscopy that the extract of *H. arenarium* contained 73.48 mg of flavonoids in reference to rutin or 17.94 mg in reference to quercetin per 1 g of dry extract weight.

Immortelle has antibacterial activity, which is associated with the presence of resin acids, it inhibits the growth of staphylococci and streptococci (Bozyel et al., 2021). *Helichrysum arenarium* showed higher antibacterial activity against *Enterococcus faecalis* (Akgun et al., 2022). Skvortsova et al. (2015) showed bacteriostatic and bactericidal activity against *Mycobacterium tuberculosis* strains of aqueous solution of an alcoholic extract from *H. arenarium*, made according to the author's method, which helps to increase the yield of flavonoids. Determination of the antibacterial activity of the methanol extract from *H. arenarium* inflorescences against pathogens of the lower respiratory tract showed that *Staphylococcus aureus* ATCC 25923 is more sensitive to immortelle extract than *Streptococcus pneumoniae* ATCC 49619. The extract itself showed similar antibacterial effects against methicillin-resistant *S. aureus* and penicillin-resistant clinical isolates of *S. pneumoniae*, showing higher activity against the ampicillin-resistant isolate *Moraxella catarrhalis* (Gradinaru et al., 2014). of *H. arenarium* has pronounced antibacterial properties against cariogenic bacteria, such as *Streptococcus mutans* (Demirez Bircan et al., 2022). Kutluk et al. (2018) showed antibacterial, antifungal and antiviral activity of extracts obtained from seven species of the *Helichrysum* genus. It is *H. arenarium* and *H. armenium* that are the most representative species in terms of their biological activity. At the same time, studies by Babotă et al. (2018) showed modest antibacterial and antifungal potential of *H. arenarium* extract. *Helichrysum arenarium* essential oil has also been studied for antimicrobial activity. In an experiment, the minimum inhibitory concentration and the minimum bactericidal and fungicidal concentration of essential oil were determined, and the bacterial sensitivity of seven different bacteria types to it was studied. The results showed that *H. arenarium* essential oil has significant antibacterial activity (Moghadam et al., 2014).

The presence of antitumour activity of *H. arenarium* against transplanted sarcoma 45 while using immortelle extract was also indicated (Grinev et al., 2016). The extract of *H. arenarium* and its active components apigenin and galangin showed a significant protective effect on skin cells exposed to blue light (Park et al., 2022).

Pospelov et al. (2019) studied the biological activity of the native immortelle extract and its fraction with and without lectins in inhibiting the germination of teliospores *Ustilago nuda*. The *in vitro* ovocidal activity of *H. arenarium* essential oil was determined in the nematodes' egg hatch test (EHT), which showed it to be the least effective with an inhibitory effect of 59.8–69.3% on egg hatchability compared to other essential oils (Štrbac et al., 2021). No repellent effects on *Shelfordella lateralis* (Walker, 1868) were exerted by *H. arenarium* inflorescences (Parhomenko et al., 2022). The methanolic extract of *H. arenarium* proved to be the most toxic against *Ixodes ricinus*, causing their death within an hour at the lowest concentration (Smolarz et al., 2013).

Helichrysum arenarium is included in some medications with hepatoprotective properties. The original medication Lavaflam tablets – a combination of immortelle dry concentrate and lavender essential oil in the experiment showed a positive effect on oxidative processes by increasing the compensatory mechanisms of the antioxidant systems, which contributed to the restoration of metabolic and structural damage of the liver caused by chronic hepatitis (Aslanian et al., 2020). *Helichrysum arenarium* as part of a polyherbal composition in the form of an extract (tea) consumed for 45 days showed normalization of liver biochemical parameters and a decrease in hematological and immunological signs of systemic inflammation in patients with hepatobiliary disorders, which made it possible to recommend it as a component of additional therapy for patients with chronic cholecystitis (Gahramanova et al., 2020). One of the most important properties of *H. arenarium* is its antioxidant activity. When studying an aqueous solution of *H. arenarium* ethanol extract, its ability to reduce average-mass (AM) molecules and malonic dialdehyde (MDA) in rat blood plasma was established (Durnova et al., 2015). The anti-atherosclerotic activities of individual flavonoids derived from *H. arenarium* have also been described (Mao et al., 2017).

Yang et al. (2021) identified compounds in the *H. arenarium* extract that exhibited anti-obesity activity and showed that all of the substances found were derivatives of caffeic acid, with isochlorogenic acid showing the most potent activity. *Helichrysum* is rich in polyphenols and their infusions have a beneficial effect on patients with metabolic syndrome. Infusions of this plant can act as prebiotics and thus improve the intestinal environment (Petelin et al., 2022). *Helichrysum arenarium* extracts can scavenge free radicals (Czinner et al., 2000) and reduce lipid peroxidation (Czinner et al., 2000). In clinical trials, consumption of naringenin, the main flavonoid of *H. arenarium*, reduced total and LDL cholesterol and triglycerides and increased HDL cholesterol in overweight/obese adults (Namkhah et al., 2021).

Therefore, the purpose of this study was to determine the effect of dried crushed inflorescences *H. arenarium* on the organism of rats during the consumption of a high-fat diet with the addition of 20% fructose.

Materials and methods

This study has been ethically approved by the Local Animal Experimental Ethics Committee of Dnipro State Agrarian and Economic University (decision number 3/22-23 of 09.16.2022). Adult outbred male rats were used in the experiment. They were divided into three groups: the control group consumed a diet high in fat and fructose; the first experimental group, in addition to this diet, consumed crushed *H. arenarium* inflorescences at a dose of 16 g per 4 kg of feed (0.4%), and the second experimental group at a dose of 160 g per 4 kg of feed (4.0%, Table 1). There were 6 animals in each group and there was no statistically significant difference between groups in body weight. The rats were kept in polycarbonate cages with steel mesh lids and a feeding recess. The room temperature was 20–22 °C, the relative humidity was 50–65%, the light regime was 12 hours of light / 12 hours of darkness. The animals had free access to food and water. The duration of the experiment was 27 days.

The nutrition of the animals was based on a basic diet consisting of 75% grain mixture (corn, sunflower grain, wheat, barley, soybeans), 8% root crops (carrots), 2% of meat and bone meal, 2% of a mineral and vitamin supplement with the addition of 15% sunflower oil. In addition to the diet, all animals received a 20% fructose solution instead of water. Medicinal raw materials in officinal form (dry inflorescences of *H. arenarium* purchased in a commercial pharmacy) were preliminarily crushed and added to the mixture of dry diet ingredients with further preparation of granules in a total amount of 4 kg per animal group. Fresh root crops were given individually every day in the appropriate quantity. The amount of food and water consumed by the animals of each group daily and for the entire period of the experiment was taken into account.

Table 1
Diet characteristics of laboratory animals

Group of animals	Diet			
	basic diet	fat	sugar	medicinal raw material
Control group	+	+15% sunflower oil	+20% fructose solution	–
H. arenarium (0.4% by weight of feed)	+	+15% sunflower oil	+20% fructose solution	+16 g dried *H. arenarium* inflorescences
H. arenarium (4.0% by weight of feed)	+	+15% sunflower oil	+20% fructose solution	+160 g dried *H. arenarium* inflorescences

During the experiment, changes in body weight and consumed food and water were recorded. The total weight gain of the animals and the daily gain in live weight were calculated. Euthanasia was performed by taking blood from the heart under anaesthesia (80 mg/kg ketamine and 12 mg/kg xylazine, intraperitoneally). After the autopsy, the state of the internal organs was visually assessed for the presence of pathological changes, the organs were selected and weighed (heart, liver, lungs, thymus, spleen, stomach, small and large intestines, kidneys) with accuracy of 10 mg. Organs removed postmortem were weighed wet, as soon as possible after autopsy, to avoid drying out, paired organs were weighed together. Orientation-physical activity and the emotional state of experimental animals were studied in the open field test (Seibenhener & Wooten, 2015). We used a setup consisting of a square area of 1 m^2, divided into 16 squares and bordered by an opaque wall 20 cm high. The experiment was carried out in complete silence with intense illumination of the field itself. An animal taken from a cage in a previously darkened room was placed in the center of the field. The exposure time was 2 minutes. Animals were tested for three consecutive days (days 1–3) at the beginning of the experiment and three days at the end (days 25–27). The number of crossed squares was counted: peripheral and central – to evaluate physical activity; peripheral (with support on the wall) and central (without support on the wall) racks – to evaluate orientation activity; the number of grooming acts, defecation and urination – emotional state evaluation (Lieshchova et al., 2021).

Blood samples taken during euthanasia were used for general and biochemical analysis. Blood serum was obtained by maintaining blood for some time and its centrifugation on a CM-3M.01 MICROmed centrifuge (200×g, 5 min; MICROmed, Shenzhen, China). Biochemical parameters were determined on a Miura 200 automatic analyser (Italy) using High Technology reagent kits (USA), PZ Cormay S.A. (Poland) and Spinreact S.A. (Spain). The total protein was determined by the biuret method; globulins and protein coefficient – by calculation; albumin concentration – by reaction with bromocresol green; C-reactive protein by immunoturbidimetry; activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) – by a kinetic method based on the Warburg optical test; alkaline phosphatase – enzymatically by reaction with p-nitrophenyl phosphate; glucose – by the glucose oxidase method (Chawla, 2014). The concentration of total cholesterol was determined – enzymatically using cholesterol oxidase; triglycerides – after cleavage by lipoprotein lipase with detection by the Trinder reaction; HDL and LDL – using selective detergents followed by staining of the enzymatic reaction products, the atherogenic index was also calculated.

The number of erythrocytes and leukocytes, hematocrit and hemoglobin content were determined in the rats' blood after the addition of K3EDTA using a PCV-80 Vet automatic hematology analyser. Blood smears for the leukogram were prepared according to Pappenheim.

The data were analyzed using Statistica 8.0 program (StatSoft Inc., USA). The tables demonstrate the results as x ± SE (mean ± standard error). Differences between the values of the control and experimental groups were determined using the Tukey test (with consideration of Bonferroni's correction), where the differences were considered significant at $P < 0.05$.

Results

During the first five days of experiment, the animals' body weight of the three groups practically did not change, and starting from the sixth day it increased, especially sharply in the control group. Since this trend was observed in all groups, we attribute this to the adaptation of the animals to the modified diet and the replacement of water with a 20% fructose solution. Subsequently, rats fed a high-fat, fructose-supplemented diet increased body weight up to 121.7% of baseline. In groups of rats fed on dry *H. arenarium* inflorescences, the increase in body weight was significantly less, so at a dose of 4.0% of the plant from the weight of the feed it was 111.2%, and at a dose of 0.4% it was 109.6% (Fig. 1).

When *H. arenarium* inflorescences were added to the animals' diet, their feed intake practically did not change in comparison with the control group (Table 2). The addition of 0.4% *H. arenarium* inflorescences to the feed reduced the amount of fructose solution consumed, while the addition of 4.0% did not affect this parameter. Consumption of dried *H. arenarium* inflorescences significantly affected the weight gain of rats. Thus, when 0.4% of *H. arenarium* inflorescences were added to the diet, the daily increase in body weight decreased almost twofold (to 1.23 g/day), and 4.0% decreased by 2.4 times (to 0.99 g/day).

The addition of *H. arenarium* to the animals' diet at a dose of 0.4% caused a significant decrease in the heart relative mass (up to 88.4%) and thymus (up to 76.4% of the control group level, Table 3). Consuming *H. arenarium* inflorescences increased the relative mass of the colon. Against the background of adding 4.0% of inflorescences, the relative weight of the caecum (129.4%) and colon (130.8%) increased, and at a dose of 0.4% – just the colon (up to 138.8% of the control group level, Table 3).

Under the influence of *H. arenarium*, the biochemical parameters of the rats' blood changed (Table 4). In the blood plasma of rats that consumed 0.4% of immortelle inflorescences, there were significant increases in urea concentration (124.2% of the control group), blood urea nitrogen (133.2%), total bilirubin (140.4%), and non-organic phosphorus (126.2%). Enzymatic activity at this dose of the medicinal plant was affected with a decrease in the activity of AST (58.6% of the control group), alpha amylase (77.1%) and a sharp increase in the activity of gamma-glutamyltransferase (137.9%) and alkaline phosphatase (125.1%). At the same time, parameters such as glucose concentration (64.9%), protein coefficient (89.8%) and the ratio of Ca/P (51.5%) significantly decreased compared to the control group (Table 4).

The consumption of *H. arenarium* inflorescences by rats at a dose of 4.0% also caused a significant increase in the concentration of urea (120.5% of the control group), blood urea nitrogen (124.3%), total bilirubin (122.0%), but reduced glucose levels (up to 72.4%) and protein coefficient (up to 85.7% of control). At the same time, the activity of ALT increased (178.6% of the control group), as did that of alkaline phosphatase (157.4% of the control group) and gamma-glutamyl transferase (124.1% of the control group) while alpha-amylase decreased (to a level of 80.1% of the control group).

Under the influence of different *H. arenarium* doses in the rats' diet, there were changes in the general blood test (Table 5). Consuming *H. arenarium* inflorescences caused a decrease in the blood hemoglobin concentration, thus at a dose of 4.0% of the feed weight it fell significantly, to 90.6% of the control group. The hematocrit index also decreased in both groups, but significantly in the group that consumed 0.4% of immortelle inflorescences by feed weight (89.7% of the control group). When eating immortelle inflorescences, the erythrocytes and platelets number significantly decreased in both groups, while the number of leukocytes did not change compared to the control group. On the leukocyte formula, the consumption of immortelle inflorescences was reflected by a strong increase in the number of eosinophils and monocytes per unit volume of blood.

Under the influence of *H. arenarium*, changes in physical activity and emotional state were observed in the rats both at the beginning of the experiment and at the end. At the same time, the orientation activity did not change (Table 6). In rats that consumed 0.4% of *H. arenarium* inflorescences by feed weight, the physical activity in the first three days was significantly lower (almost two times compared to animals in the control group) and this trend persisted by the end of the experiment. In the group of rats that received 4.0% of *H. arenarium* inflorescences by feed weight, physical activity was reduced at the beginning of the experiment by 3.4 times, and at the end by 4.0 times compared with the control.

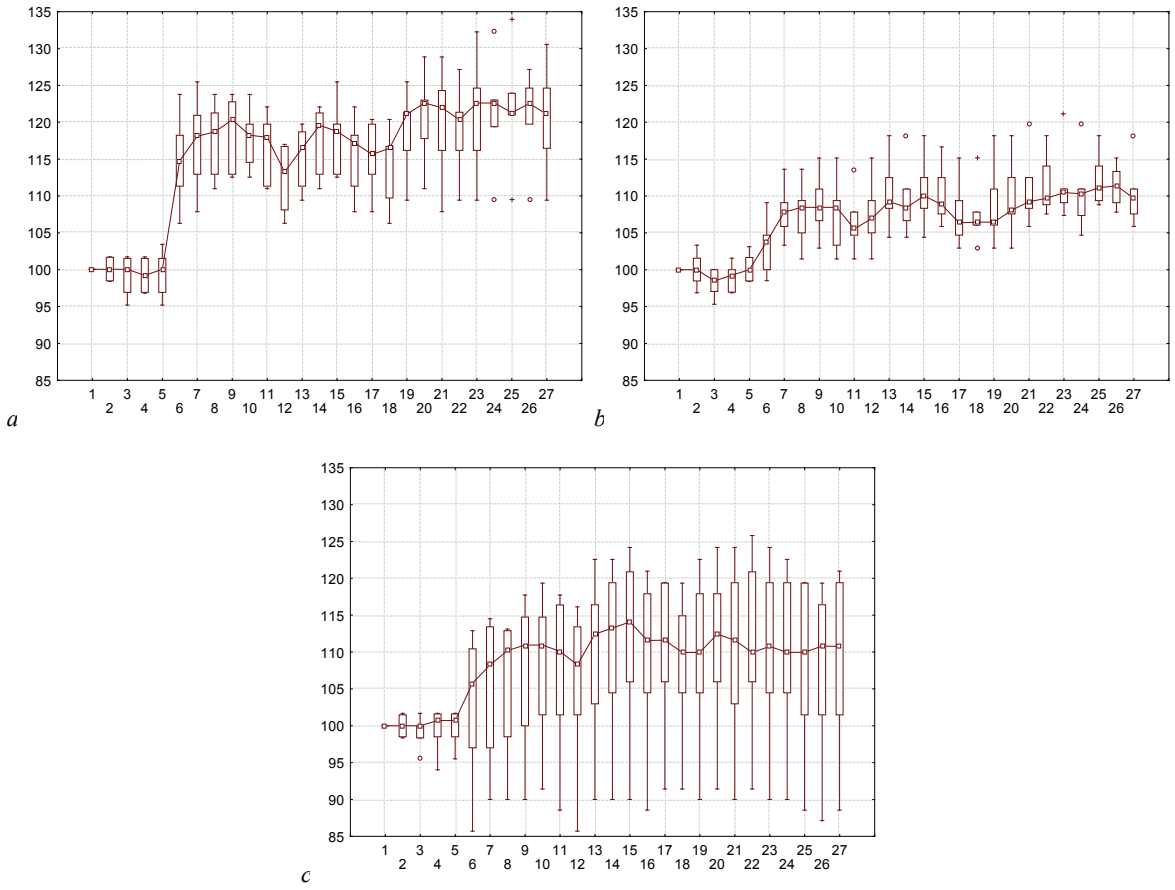

Fig. 1. Change in rats' body weight in the control group (*a*) when *Helichrysum arenarium* (L.) Moench inflorescences were added to their diet at a dose of 0.4% (*b*) and 4.0% of the feed weight (*c*): abscissa shows the day of the experiment; the y-axis is the body weight of the animals (% relative to the initial body weight before the start of the experiment, taken as 100% for each of the experimental animals); small square – median, upper and lower rectangle borders – 25% and 75% quartiles, vertical line – minimum and maximum values, circles – outliers; n = 6

Table 2

Change in the body weight and fodder consumption of young male rats under the influence of addition of *Helichrysum arenarium* (L.) Moench to their diet (x ± SE, n = 6, duration of experiment – 27 days)

Parameter	Control	*H. arenarium* (0.4% of feed weight)	*H. arenarium* (0.4%) compared to the control, %	*H. arenarium* (4.0% of feed weight)	*H. arenarium* (4.0%) compared to the control, %
Consumption of food by animals, g/day	29.3	29.5	100.6	29.6	101.1
Consumption of fructose solution by animals, g/day	36.5	27.6	75.6	36.2	99.3
Change in body weight, g/day	2.38 ± 0.32^{a}	1.23 ± 0.21^{b}	51.7	0.99 ± 0.61^{b}	41.6
Change in body weight, %/day	0.759 ± 0.110^{a}	0.382 ± 0.064^{b}	50.4	0.321 ± 0.187^{b}	42.3

Note: different letters indicate values which reliably differed one from another within one line of the table according to the results of comparison using the Tukey test with Bonferroni correction.

Table 3

Change in relative mass of the organs (%) of young male rats under the influence of addition of *Helichrysum arenarium* (L.) Moench to their diet (x ± SE, n = 6, duration of experiment – 27 days)

Organ	Control	*H. arenarium* (0.4% of feed weight)	*H. arenarium* (0.4%) compared to the control, %	*H. arenarium* (4.0% of feed weight)	*H. arenarium* (4.0%) compared to the control, %
Heart	0.316 ± 0.017^{a}	0.279 ± 0.006^{b}	88.4	0.301 ± 0.020^{ab}	95.5
Liver	3.40 ± 0.21^{a}	3.49 ± 0.16^{a}	102.6	3.32 ± 0.05^{a}	97.8
Lungs	0.646 ± 0.057^{a}	0.618 ± 0.036^{a}	95.7	0.632 ± 0.035^{a}	97.9
Thymus	0.262 ± 0.028^{a}	0.200 ± 0.052^{b}	76.4	0.261 ± 0.025^{a}	99.4
Spleen	0.184 ± 0.020^{a}	0.180 ± 0.024^{a}	98.3	0.218 ± 0.016^{a}	118.7
Stomach	0.455 ± 0.017^{a}	0.435 ± 0.029^{a}	95.7	0.468 ± 0.019^{a}	102.9
Small intestine	1.81 ± 0.11^{a}	1.89 ± 0.17^{a}	104.1	2.01 ± 0.05^{a}	111.1
Cecum	0.209 ± 0.030^{a}	0.207 ± 0.016^{a}	99.0	0.270 ± 0.011^{b}	129.4
Colon	0.224 ± 0.013^{a}	0.311 ± 0.028^{b}	138.8	0.293 ± 0.023^{b}	130.8
Rectum	0.188 ± 0.030^{a}	0.185 ± 0.019^{a}	98.4	0.178 ± 0.021^{a}	94.5
Right kidney	0.288 ± 0.012^{a}	0.285 ± 0.010^{a}	99.1	0.305 ± 0.019^{a}	105.9
Left kidney	0.281 ± 0.009^{a}	0.290 ± 0.015^{a}	103.0	0.305 ± 0.019^{a}	108.7
Brain	0.476 ± 0.016^{a}	0.526 ± 0.008^{a}	110.7	0.539 ± 0.034^{a}	113.4
Testicle	0.471 ± 0.032^{a}	0.474 ± 0.034^{a}	100.7	0.498 ± 0.038^{a}	105.7

Note: see Table 1.

Table 4
Change in blood biochemical parameters of male rats under effect of *Helichrysum arenarium* (L.) Moench supplementation (x ± SE, n = 6, duration of experiment – 27 days)

Parameters	Control	*H. arenarium* (0.4% of feed weight)	*H. arenarium* (0.4%) compared to the control, %	*H. arenarium* (4.0% of feed weight)	*H. arenarium* (4.0%) compared to the control, %
Total protein, g/L	81.8 ± 3.0^{a}	85.8 ± 1.7^{a}	104.9	82.0 ± 2.3^{a}	100.3
Albumins, g/L	44.8 ± 0.9^{a}	45.5 ± 0.3^{a}	101.7	42.5 ± 1.0^{a}	95.0
Globulins, g/L	36.3 ± 2.3^{a}	41.0 ± 1.7^{a}	113.1	39.8 ± 1.3^{a}	109.7
Protein coefficient, U	1.23 ± 0.06^{a}	1.10 ± 0.06^{ab}	89.8	1.05 ± 0.03^{b}	85.7
Urea, mmol/L	3.30 ± 0.15^{a}	4.10 ± 0.49^{b}	124.2	3.98 ± 0.52^{b}	120.5
Blood urea nitrogen, mg/100 g	6.18 ± 0.34^{a}	8.23 ± 0.88^{b}	133.2	7.68 ± 0.97^{b}	124.3
Creatinine, µmol/L	52.0 ± 4.0^{a}	57.0 ± 7.4^{a}	109.6	51.7 ± 10.6^{a}	99.4
AST, U/L	75.5 ± 16.5^{a}	44.3 ± 7.6^{b}	58.6	54.5 ± 17.0^{ab}	72.2
ALT, U/L	74.8 ± 8.7^{a}	73.8 ± 7.3^{a}	98.7	133.5 ± 12.1^{b}	178.6
De Ritis ratio (AST/ALT), U	0.78 ± 0.38^{a}	0.60 ± 0.07^{a}	77.4	0.43 ± 0.16^{a}	54.8
Alkaline phosphatase, U/L	363 ± 30^{a}	454 ± 40^{ab}	125.1	572 ± 78^{b}	157.4
Alpha-amylase, U/L	1341 ± 143^{a}	1034 ± 43^{b}	77.1	1074 ± 33^{b}	80.1
Total bilirubin, µmol/L	6.13 ± 0.31^{a}	8.60 ± 1.44^{b}	140.4	7.48 ± 0.21^{b}	122.0
Glucose, mmol/L	5.70 ± 0.57^{a}	3.70 ± 0.23^{b}	64.9	4.13 ± 0.28^{b}	72.4
Total calcium, mmol/L	2.65 ± 0.13^{a}	2.60 ± 0.09^{a}	98.1	2.55 ± 0.06^{a}	96.2
Non-organic phosphorus, mmol/L	3.25 ± 0.18^{a}	4.10 ± 0.41^{b}	126.2	2.88 ± 0.39^{a}	88.5
Ca/P ratio	0.825 ± 0.025^{a}	0.425 ± 0.144^{b}	51.5	0.925 ± 0.103^{a}	112.1
Gamma-glutamyltransferase, U/L	3.63 ± 0.24^{a}	5.00 ± 0.71^{b}	137.9	4.50 ± 0.50^{b}	124.1
Cholesterol, mmol/L	1.575 ± 0.063^{a}	1.400 ± 0.041^{a}	88.9	1.575 ± 0.103^{a}	100.0

Note: see Table 1.

Table 5
Change in general analysis of blood and leukogram of male rats under effect of addition to *Helichrysum arenarium* (L.) Moench (x ± SE, n = 6, duration of experiment – 27 days)

Parameter	Control	*H. arenarium* (0.4% of feed weight)	*H. arenarium* (0.4%) compared to the control, %	*H. arenarium* (4.0% of feed weight)	*H. arenarium* (4.0%) compared to the control, %
Hemoglobin, g/L	135.3 ± 3.3^{a}	127.5 ± 3.9^{ab}	94.3	122.5 ± 3.3^{b}	90.6
Hematocrit, %	35.1 ± 1.0^{a}	31.5 ± 1.0^{b}	89.7	32.3 ± 1.8^{ab}	92.1
Erythrocytes, 10^{12}/L	6.87 ± 0.20^{a}	6.11 ± 0.16^{b}	88.9	6.46 ± 0.47^{ab}	94.0
MCV, $*10^{-15}$/L	51.1 ± 1.0^{a}	51.5 ± 0.9^{a}	100.8	50.2 ± 1.0^{a}	98.3
MCHC, $*10^{-12}$ г	19.7 ± 0.3^{a}	20.9 ± 0.7^{a}	106.1	19.2 ± 1.1^{a}	97.5
MCH, %	38.6 ± 0.2^{a}	40.7 ± 1.7^{a}	105.4	37.1 ± 2.0^{a}	96.3
Colour index, units	0.98 ± 0.02^{a}	1.05 ± 0.04^{a}	107.2	0.97 ± 0.05^{a}	99.0
Erythrocyte sedimentation rate (ESR), mm/h	1.00 ± 0.00^{a}	1.25 ± 0.25^{a}	125.0	1.50 ± 0.29^{a}	150.0
Thrombocytes, 10^{9}/L	505 ± 40^{a}	411 ± 46^{b}	81.4	399 ± 24^{b}	79.0
Leukocytes, 10^{9}/L	3.63 ± 0.35^{a}	3.63 ± 0.31^{a}	100.2	4.23 ± 0.77^{a}	116.6
Leukocytic formula					
Basophils, %	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0	0.0 ± 0.0^{a}	0.0
Eosinophils, %	0.50 ± 0.29^{a}	1.00 ± 0.00^{ab}	200.0	1.50 ± 0.29^{b}	300.0
Myelocytes, %	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0	0.0 ± 0.0^{a}	0.0
Neutrophils, %:	–	–	–	–	–
– young	0.0 ± 0.0^{a}	0.0 ± 0.0^{a}	0.0	0.0 ± 0.0^{a}	0.0
– band	1.25 ± 0.25^{a}	0.75 ± 0.48^{a}	60.0	1.00 ± 0.71^{a}	80.0
– with segmented nuclei	24.0 ± 2.9^{a}	20.8 ± 1.7^{a}	86.5	20.3 ± 2.3^{a}	84.4
Lymphocytes, %	74.0 ± 5.8^{a}	71.8 ± 1.4^{a}	97.0	71.8 ± 3.7^{a}	97.0
Monocytes, %	2.75 ± 0.85^{a}	5.75 ± 0.75^{b}	209.1	5.50 ± 0.65^{b}	200.0

Note: no statistically significant changes between samples were found.

Consumption of a high-fat diet with the addition of 20% fructose solution by rats caused a significant decline in the animals' emotional state by the end of the experiment (25–27th days), mainly evidenced by a sharp decrease in the number of grooming acts (Table 6). The addition of immortelle inflorescences to the diet at a dose of 0.4% of the feed mass caused a sharp intensification in the emotional state of the animals (mainly due to an increase in the number of grooming acts) both at the beginning and at the end of the experiment. Rats fed 4.0% immortelle inflorescences by feed weight also showed a higher emotional state at the beginning and end of the experiment, but this effect was significantly lower compared to the group that received a lower dose of this herb. There were no observations of significant changes in orientation activity in the open field test between the rats' groups with 0.4% and 4.0% *H. arenarium* inflorescences in the diet and the control group at the beginning and end of the experiment.

Discussion

An increased fat content in the diet causes weight gain, but both fat itself and fatty acids are very important in maintaining the normal functioning of the body (Murase et al., 2001). A number of substances that are elevated in obesity (free fatty acids, insulin, leptin, tumour necrosis factor alpha (TNF-α) play an important role in the development of insulin resistance due to obesity (Boden, 1997; Ruan & Lodish, 2003). Medicinal plants and nutraceuticals are increasingly used in the treatment and prevention of metabolic disorders, not only in traditional medicine in developing countries, but also in advanced economies (Ekor, 2014). Some herbs have already been shown to be effective against the manifestations of the metabolic syndrome in the treatment of dyslipidemia (Adel Mehraban et al., 2021) and insulin resistance (Tran et al., 2020). *Helichrysum arenarium* is known in folk medicine for its diuretic, choleretic and anti-inflammatory properties (Dănăilă-Guidea et al., 2022). Conducted by Kenig et al. (2022), a randomized, blind, comparative study of the use of *H. italicum* and *H. arenarium* infusions in patients with signs of metabolic syndrome showed significant efficacy of these plants. In patients on the background of 28-day use of these herbal infusions, physiological and biochemical parameters improved significantly.

To induce metabolic disorders in rats, we used a combined protocol in which, in addition to feeding them a high-fat diet, the animals were receiving a 20% fructose solution instead of water since it is the consumption of fructose that leads to weight gain, triggers lipogenesis and metabo-

lic disorders, compared with a simple high-fat diet and the use of sucrose (Ozkan & Yakan, 2019). The results of our studies indicate that *H. arenarium* significantly affected the body weight gain of laboratory animals. The addition of dry *H. arenarium* flowers to the diet (regardless of the dose) resulted in a decrease in the intensity of body weight gain in rats, while the amount of food consumed remained almost the same. At the same time, the addition of 4.0% vegetable raw materials to the diet showed a more pronounced effect.

Table 6

Changes in the behavioural characteristics of the three rat groups to whose diet *Helichrysum arenarium* (L.) Moench was added (x ± SE, n = 18, duration of the experiment was 27 days) during 2 minutes of the experiment

Characteristic	Control, 1–3th days	Control, 25–27th days	*H. arenarium* (0.4%), 1–3th days	*H. arenarium* (0.4%), 25–27th days	*H. arenarium* (4%), 1–3th days	*H. arenarium* (4%), 25–27th days
Number of visited peripheral squares	19.1 ± 2.4^{a}	19.1 ± 2.0^{a}	8.7 ± 2.7^{b}	8.5 ± 2.7^{b}	5.6 ± 2.1^{b}	4.6 ± 1.6^{b}
Number of visited central squares	0.06 ± 0.06^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.06 ± 0.06^{a}	0.00 ± 0.00^{a}
Number of racks in peripheral squares	4.22 ± 0.33^{a}	3.61 ± 0.28^{a}	3.17 ± 0.56^{a}	3.33 ± 0.58^{a}	3.72 ± 0.69^{a}	3.28 ± 0.44^{a}
Number of racks in central squares	1.11 ± 0.25^{a}	0.61 ± 0.18^{a}	0.50 ± 0.19^{a}	0.61 ± 0.26^{a}	0.72 ± 0.23^{a}	0.83 ± 0.32^{a}
Number of grooming acts	0.67 ± 0.21^{a}	0.11 ± 0.08^{b}	2.61 ± 0.57^{c}	2.17 ± 0.59^{c}	0.44 ± 0.17^{ab}	0.39 ± 0.18^{ab}
Number of faecal boluses	0.39 ± 0.18^{a}	0.44 ± 0.22^{ab}	0.83 ± 0.29^{ab}	1.06 ± 0.29^{ab}	1.28 ± 0.38^{b}	0.50 ± 0.19^{ab}
Number of acts of urination	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.06 ± 0.06^{a}	0.00 ± 0.00^{a}
Physical activity	19.1 ± 2.4^{a}	19.1 ± 2.0^{a}	8.7 ± 2.7^{b}	8.5 ± 2.7^{b}	5.6 ± 2.1^{b}	4.6 ± 1.6^{b}
Orientation activity	5.33 ± 0.35^{a}	4.22 ± 0.33^{a}	3.67 ± 0.72^{a}	3.94 ± 0.80^{a}	4.44 ± 0.84^{a}	4.11 ± 0.68^{a}
Emotional state	1.06 ± 0.22^{b}	0.56 ± 0.23^{a}	3.44 ± 0.74^{c}	3.22 ± 0.71^{c}	1.78 ± 0.45^{bc}	0.89 ± 0.28^{ab}

Notes: there were no significant differences between the groups in most of the studied parameters; differences in the number of peripheral visited squares are indicated by different Latin letters ($P < 0.05$) according to the results of the Tukey test with Bonferroni correction.

At the end of the experiment, no pathological changes in the internal organs were found in the studied animals. The absence of pathomorphological changes in animal organs under the influence of herbal preparations containing *H. arenarium* was also indicated by Savych & Mala (2021).

Animals fed a 20% fructose solution added to their diet showed significantly higher rates of body weight gain compared with animals fed a high-fat diet and animals fed glucose solution from the 3rd week of the experiment until the end of the study (13th week) (Ozkan & Yakan, 2019). In our experience, consumption of a high-fat diet with additional intake of 20% fructose solution did not cause hyperglucosemia. The glucose level was within the reference values, but the additional consumption of immortelle inflorescences in both dosages (0.4% and 4.0% of the feed weight) caused a significant decrease in blood glucose concentration compared to the control group. It should be noted that the animals' blood glucose did not go exceed the normal range (Abrashova et al., 2013).

The ratio of organ mass – the percentage of organ mass to body mass – is an integral indicator used in toxicology to assess the state of the internal organs. The results of our study showed that in rats fed a diet with the addition of dried *H. arenarium* inflorescences, the relative heart mass was significantly lower than in control rats. Liu & Lan (2022) showed that when cardiomyocytes were damaged by high glucose levels in diabetes mellitus, which was manifested by increased activity of creatine kinase (CK) and lactate dehydrogenase (LDH), the expression levels of apoptotic proteins (Bax and Bcl-2) and the expression levels of inflammatory factors (tumour necrosis factor alpha (TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6) and corresponding histological changes in the myocardium (interstitial edema and inflammatory cell infiltration), namely the use of *H. arenarium* flavonoid extract, reduced the manifestation of myocardial inflammation. Therefore, further studies of the effect of *H. arenarium* and its active substances on the heart and the cardiovascular system as a whole are promising not only in violation of carbohydrate, but also lipid metabolism. There was an increase in the mass of particular parts of the large intestine (caecum and colon). This can be explained by the activation of fiber digestion processes in these parts of rats' digestive system, since in our experiment we used dry crushed *H. arenarium* inflorescences, and not an extract. A decrease in the relative thymus weight in rats treated with 0.4% *H. arenarium* may indicate the activation of the age-related involution process of this organ.

Helichrysum arenarium is known to affect cholesterol metabolism: due to its choleretic effect, this herb is widely used in traditional medicine (Pljevljakušić et al., 2018). Some researchers consider the plant's ability to stimulate bile secretion to be the main mechanism for lowering cholesterol levels in the blood (Kenig et al., 2022). In our 27-day study, we found no effect of *H. arenarium* consumption on blood cholesterol levels of animals fed a high-fat diet. The choleretic effect of *H. arenarium* was manifested in the change of the total blood bilirubin concentration of experimental animals groups. Thus, this indicator in the control group of rats did not exceed the reference range, and eating *H. arenarium* caused a significant increase in the concentration of total bilirubin not only in comparison with the control group, but also in comparison with the normal values for this animal species (Abrashova et al., 2013).

Attention is drawn to the change in the enzymesactivity in the animals' blood. When dry *H. arenarium* inflorescences were added to the high-fat and high-carbohydrate diet, the activity of AST and alpha amylase decreased, but the activity of alkaline phosphatase and GGT increased, and the 4% dose also sharply increased the activity of ALT. This confirms the effect of *H. arenarium* active substances on the functional state of hepatocytes. Also, an increase in the activity of AP and the concentration of inorganic phosphorus, as well as a change in the Ca/P ratio in rats treated with 0.4% *H. arenarium* inflorescences, may indicate the effect of this plant on mineral metabolism, which requires further studies.

The addition of *H. arenarium* to the diet also caused a change in some indicators of general blood analysis. Additional supplementation with inflorescences caused a significant decrease in hematocrit, hemoglobin concentration, erythrocyte and platelet counts compared with the control group. However, comparing these indicators with the reference values for animals of this age group, we can say that *H. arenarium* contributed to the normalization of the state of the organism (Abrashova et al., 2013). The addition of *H. arenarium* inflorescences also caused changes in the leukocyte formula: in the blood of rats, the number of eosinophils and monocytes significantly increased, which may indicate the development of an allergic reaction of the body to certain active components of this plant.

In the available literature, we did not find studies of the effect of *H. arenarium* on the morphofunctional state of the nervous system and behavioural responses in animals. Our experiment showed that the consumption of a high-fat diet and 20% fructose solution by rats for 27 days did not cause changes in physical and orientation activity, but significantly affected the emotional state in the form of a decrease in the number of grooming acts. Adding dry *H. arenarium* inflorescences had a negative effect on the behavioural reactions of the animals: it sharply reduced motor activity starting from the 1st–3rd day of the experiment. Emotional state changed depending on the dose of the consumed plant. A low dose (0.4% of the feed weight) caused a heightening of the emotional state due to an increase in the number of grooming acts both at the beginning (days 1–3) and at the end of observation (days 25–27). The addition of 4% of the plant to the feed caused the same effect, but less pronounced compared to the dose of 0.4%.

In general, our previous studies on the use of various medicinal plants in model animals against the background of an increased fat content in the diet did not show a significant effect on motor and orientation activity in the open field test. Addition of *Rhodiola rosea* rhizomes and *Punica granatum* fruit peel (Lieshchova & Brygadyrenko, 2023a), dry grass of *Origanum vulgare* and *Scutellaria baicalensis* (Lieshchova & Brygadyrenko, 2022), *Salvia officinalis*, *S. sclarea* (Lieshchova et al., 2021), seeds

of *Echinacea purpurea* and *Sylibum marianum* (Lieshchova & Brygadyrenko, 2023b) showed a decline in their emotional state, while *Lavandula angustifolia*, *Melissa officinalis* and *Vitex angus-castus* (Lieshchova & Brygadyrenko, 2021) did not affect this parameter.

Conclusions

The addition of *H. arenarium* dry inflorescences to the rats' diet significantly affected the weight gain rate and caused strong changes in their body. Supplementing a high-fat diet with dry *H. arenarium* flowers with 20% fructose solution during a 27-day experiment caused a significant slowdown in the rate of body weight gain and reduced daily weight gain at the same feed intake by animals. We found a decrease in the heart and thymus relative mass when 0.4% of inflorescences were added to the diet and an increase in the relative mass of the colon with the addition of 0.4% of inflorescences and that of the colon and caecum with the addition of 4.0% of inflorescences by weight of the feed.

Supplementation with dried *H. arenarium* inflorescences (regardless of dose) caused an increase in urea, blood urea nitrogen, total bilirubin and decreased glucose and protein ratio, while a 0.4% dose of the plant increased the inorganic phosphorus concentration with a change in the Ca/P ratio. The addition of *H. arenarium* also significantly affected the blood enzymatic activity of experimental animals, with a significant increase in the activity of gamma-glutamyltransferase and alkaline phosphatase with a decrease in the activity of alpha-amylase in both groups. The consumption of inflorescences at a dose of 0.4% in the feed caused a decrease in AST activity, and 4.0% sharply increased blood ALT activity.

The addition of *H. arenarium* dry inflorescences to the diet caused changes in the general blood test (hematocrit, hemoglobin concentration, erythrocyte and platelet count decreased) and leukocyte count (increased eosinophil and monocyte count). The intake of the herb led to changes in the functional state of the nervous system (according to the results of the "open field" test). A decrease in physical activity and a heightening of emotional state were found in rats which consumed dry *H. arenarium* inflorescences, regardless of the dose, compared with the control group of animals. Significant changes in the animals' orientation activity were not observed.

In general, the obtained results indicate that the use of this herb in a diet with excess fat and carbohydrates does not cause pathological and toxic effects, but at the same time, it affects metabolic processes, which makes this herbal medicine attractive as a component of food supplements and multicomponent pharmaceuticals. Further studies are needed to assess the dose effect of this herbal preparation, as well as the effect of its chemical composition on the health state of model vertebrate animal species.

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