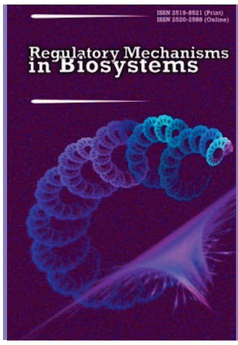

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Effect of *Bidens tripartita* leaf supplementation on the organism of rats fed a hypercaloric diet high in fat and fructose

M. A. Lieshchova*, V. V. Brygadyrenko*, **

**Dnipro State Agrarian and Economic University, Dnipro, Ukraine*
***Oles Honchar Dnipro National University, Dnipro, Ukraine*

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Dnipro State Agrarian and Economic University, Serhii Efremov st., 25, Dnipro, 49600, Ukraine. Tel.: +38-067-256-24-86. E-mail: lieshchova.m.o@dsau.dp.ua

Oles Honchar Dnipro National University, Gagarin av., 72, Dnipro, 49010, Ukraine. Tel.: +38-050-93-90-788. E-mail: brigad@ua.fm

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Herbs play an important role in folk medicine, and scientific research has confirmed the properties of their use as an alternative treatment, including the treatment and mild correction of metabolic disorders during disease. Trifid bur-marigold (*Bidens tripartita*) is a pharmacopoeial herbal raw material that is widely used in clinical practice as an external remedy for skin lesions and as an internal remedy for digestive and respiratory disorders. In this work, the general effect of dried leaves of *B. tripartita* on physiological activity and metabolic processes in model animals on a high-calorie diet was determined. For the experiment, three groups of 18 male white laboratory rats were formed and fed a hypercaloric diet (increased fat content and 20% fructose solution instead of water) for 27 days, in addition to 0.4% and 4.0% dried leaves of *B. tripartita*. The consumption of 0.4% and 4.0% of the medicinal plant resulted in a significant delay in the body weight gain and the average daily weight gain of the rats compared to the control group. Dried leaves of *B. tripartita* in the diet of rats decreased the relative weight of the thymus and increased the relative weight of the brain, and at a dose of 4.0%, increased the relative weight of the lungs and individual large intestines (cecum and colon). Dietary supplementation with *B. tripartita* caused an increase in globulin concentration and changes in protein coefficient. Blood parameters such as: urea, urea nitrogen, inorganic phosphorus, glucose and bilirubin levels changed depending on the dose. In the general blood test, consumption of dried leaves of *B. tripartita* caused a decrease in hematocrit, hemoglobin concentration and platelet count, but increased the number of eosinophils. *Bidens tripartita* at both concentrations significantly increased ALT activity with a corresponding change in the blood De Ritis ratio. The addition of 20 g of *B. tripartita* leaves to the diet increased alkaline phosphatase activity and decreased alpha-amylase activity, while 200 g increased blood gamma-glutamyltransferase activity. At the end of the experiment, the rats' orientation activity, determined in the open field, changed according to the herb dose consumed: 0.4% leaves caused an increase and 4.0% a decrease. Physical activity was reduced and emotional state increased, regardless of the dose of dried *B. tripartita* leaves, compared to the control group of animals. The results obtained show that the addition of *B. tripartita* dried leaves as a dietary supplement to a high-calorie diet is safe, does not cause pathological changes or side effects, and has a significant effect on metabolic processes. This provides theoretical support for the use of *B. tripartita* dried leaves in the manufacture of nutraceutical and pharmacological products for the correction of metabolic disorders in humans and animals. The doses and duration of their application require further studies.

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

Introduction

Herbal remedies and dietary supplements are very welcome in modern society. Medicinal plants in their natural form, their plant extracts or their phytocomponents are excellent candidates for products that modulate the body's metabolism, especially in the context of an unbalanced diet.

Bidens tripartita L. is a herbaceous plant of the Asteraceae family that is widely used in medicine. It is a traditional herbal remedy used in several countries (Alakbarov, 2001; Lopes et al., 2021). It is also widely used in folk medicine. In modern scientific medicine, the plant is official and can be purchased in the pharmacy network. The description and information on the therapeutic properties of *B. tripartita* are available in the State Pharmacopoeia of Ukraine 2.0. The raw material of the medicinal plant is the tops of stems and side branches not exceeding 15 cm in length, as well as large leaves - the herb of trifid bur-marigold (*Herba Bidentis*). The raw material is collected during the blossoming period at the beginning of the summer season. A wide range of biological activity of *B. tripartita* is associated with the presence of a complex of biologically active compounds, including flavonoids, polysaccharides and polyacetylenes. It is these bioactive compounds that determine the pharmacotherapeutic effect of drugs made from *B. tripartita* (Singh, et al., 2017). Phytochemical analysis by simple chemical tests of alcoholic extract of *B. tripartita* revealed the presence of various active compounds, particularly tannins, anthracene derivatives and triterpenes (Sandu et al., 2012). Substance 4, previously isolated from *B. tripartita*, was found to be (R-2)isookanin 7-O-β-D-glucopyranoside [(R-2)flavanomarein]. The isolated compound is new to the genus *Bidens* L. (Serbin et al., 1975). The raw material contains a significant amount of carotenoids (up to 60–70 mg%). The most important flavonoids are flavonols (rutin, quercetin, axillarin, 3,6,3'-trimethyl ester of quercetagetin), aurones (sulfuretin, 6,7,3',4'-tetrahydroxyauron), flavanones (flavanomarein (7-O-glucoside-isocyanine), naringenin-7-glucoside), catechins (epicatechin, (+,-)-catechin, catechin hydrate, epigallocatechin gallate), chalcones (3,2',4'-trihydroxy-4-methoxychalcone, 4'-O-β-D-glucopyranosyl-2',3-dihydroxy4-methoxychalcone, butein, okanin, okanin 4'-O-β-D-glucopyranoside, okanin 4'-O-(6''-O-acetyl-D-glucopyranoside), bidenoside G), flavones (flavone, diosmethine (O-methylated flavone), cynaroside (luteolin-7-O-glucoside), luteolin), phenylpropanoids (luteoside) (Serbin et al., 1975; Kaškoniene & Maruška, 2015). This medicinal plant contains significant amounts of phenolic compounds (butein, marein, isocoreopsin, flavanomarein, sulfuretin, sulfurein, marimetitin, luteolin, cynaroside), phenolic acids: gallic acid, gentisic acid, ellagic acid, salicylic acid, p-hydroxybenzoic acid, hydroxycinnamic acids (chlorogenic acid, caffeic acid, cichoric acid, rosmarinic acid, neochlorogenic acid, vanillic acid), 4-O-caffeoylquinic acid, syringic acid, coumaric acid, ferulic acid), tannins (tannins, catechins), macro and trace elements, fatty acids, coumarins and polyacetylene derivatives (2-β-D-glycopyrazyloxy-1-hydroxytrideca-3,5,7,9,11-pentaine; 3(R), 8(E)-8-decen-4,6-diin-3, 10-dihydroxy-1-O-βD-glucopyranoside) (Lv & Zhang, 2013; Oproshanskaya, 2015). The plant concentrates salts of Zn, Se, Mn and Cd. *Bidens tripartita* produces an essential oil in which the main compounds are acyclic monoterpenes (linalool, allozymene, (Z)-β-ocymene, p-cymen-9-ol), monocyclic monoterpenes (p-cymene, α-phellandrene), bicyclic monoterpenes (α-pinene), sesquiterpenes (β-bisabolene, β-elemene, sylfiperfol-6-ene, caryophyllene oxide, humulene II epoxide), aliphatic compounds (hexanal), furan derivatives (2-pentylfuran) α-pinene (3.7–12.1%), p-cymene (2.8–8.0%), β-ocimene (40.5–45.9%), β-elemene (9.9–15.6%), iso- and α-caryophyllenes (4.3–6.8% and 5.2–8.2%), and α-bergamotene (3.3–9.4%) (Tomczykowa et al., 2005; Kaškonienė et al., 2013). It has been found that the highest content and diversity of compounds in plant raw materials occurs during the period of full flowering, and their amount does not depend on the age of the plant (Kaškonienė et al., 2011). The content of flavonoids in the flower heads of *B. tripartita* is half that of the herb (Wolniak et al., 2007).

The plant is widely used in modern scientific medicine. Aqueous, 50% methanol and methanol extracts with pharmacological activity, as well as diethyl ether, ethyl acetate and butanol fractions were prepared from the herb *B. tripartita*, in which the relationship between the composition of phenolic compounds was determined (Uysal et al., 2018; Kotov et al., 2020; Mendel et al., 2020). The cytotoxic effect of *B. tripartita* extracts was demonstrated against three lines of cancer cells in a viability test using methylthiazolyl diphenyl tetrazolium bromide (MTT) (Uysal et al., 2018).

In the study of acute toxicity of the ethanolic extract of *B. tripartita* in mice, the LD_{50} value was 4038 mg/kg for a single intraperitoneal injection, and DL_{01} and DL_{99} were 2500 and 4950 mg/kg, respectively, which allows this drug to be classified as a low-toxic substance (Sandu et al., 2012). Based on folk medicine, infusions of *Bidens* species (Asteraceae) are successfully used in the treatment of acute and chronic enteritis. In addition, ethnopharmacological reports have demonstrated gastrointestinal, gastroprotective, anti-inflammatory, anti-ulcer and immunomodulatory activities of *B. tripartita*. The results showed a potent prokinetic effect of *B. tripartita* extracts and their flavonoids on jejunal smooth muscle. *In vivo* studies have shown that the extracts stimulate gastrointestinal peristaltics, which allows us to recommend their use to eliminate clinical symptoms (bloating and discomfort, constipation) in the treatment of irritable bowel syndrome, gastritis, gastroparesis and other gastrointestinal pathologies (Mendel et al., 2020). Wolniak et al. (2007) evaluated the antioxidant activity of the pure flavonoids, flavanomarein (isoocanine-7-O-glucoside), cynaroside (luteolin-7-O-glucoside) and luteolin isolated from the herb and flowers of *B. tripartita*. The flower extract was shown to have twice the antioxidant activity of the herb extract.

It is known that the plant is widely used in folk medicine thanks to cynaroside, the predominant luteolin derivative in the aerial parts of *B. tripartita* (Szekalska et al., 2020). In folk medicine, decoctions of the herb are drunk for rickets, liver and gallbladder diseases, as a diaphoretic, diuretic, blood purifying and hemostatic agent. Trifid bur-marigold is used for tuberculosis, headaches and spasms. Baths in a decoction of the herb are used for various itchy dermatoses, dandruff. Hot herbal infusions are also used to treat wounds. Decoctions of trifid bur-marigold root are taken internally as a diuretic for edema. Ingestion, hot infusion and decoction of trifid bur-marigold herb normalise metabolism, have antispasmodic, hemostatic effect. *Bidens tripartita* has diuretic, diaphoretic and choleretic properties that improve digestion. The anti-inflammatory, antibacterial and immunomodulating effects of *B. tripartita* have been reported (Lupușoru et al., 2016; Szekalska et al., 2020; Wahyuddin et al., 2020). İçöz et al. (2016) determined the antibacterial activity of aqueous and ethanolic extracts of several species of *B. tripartita* ground shoots. The ethanolic extracts of the tested species showed better antibacterial activity than the aqueous extracts. However, aqueous extracts of *B. frondosa* and *B. tripartita* showed antifungal activity against *Candida albicans* (İçöz et al., 2016).

A considerable amount of research has been devoted to the development of formulations from *B. tripartita* and its active compounds for internal or external use (Śliwa et al., 2016; Tomczykowa et al., 2018; Szekalska et al., 2020). In the treatment of allergic reactions, the herb trifid bur marigold has been used for a long time. The effect of polysaccharides and flavonoids from the trifid bur-marigold herb on the stabilization of mast cells *in vitro* has been investigated. Polysaccharides and flavonoids of trifid bur-marigold statistically significantly ($P < 0.05$) reduced the number of degranulated mast cells in comparison with that in the sample with allergen. This indicates the stabilizing effect of polysaccharides and flavonoids of trifid bur-marigold at the doses studied on mast cells. The local application of cynaroside from the herb *B. tripartita* in hydrogels as an anti-inflammatory and anti-allergenic agent was studied. It was found that 10% hydrogel containing cynaroside inhibited the release of anti-inflammatory mediators and reduced oxazolone-induced edema. Topical application of cynaroside was also shown to significantly reduce the number of T cells, mast cells and histiocytes in inflamed skin with atopic dermatitis (Szekalska et al., 2020). New gel formulations for topical essential oil carriers from *B. tripartita* were developed and characterized, while their bioadhesive properties were evaluated in an ex vivo model using hairless mouse skin (Tomczykowa et al., 2018). The therapeutic efficacy of topical *B. tripartita* formulations depends on the active phytocomponents and carrier properties. The antifungal properties of *B. tripartita* essential oil were also tested against *Candida* species, which allowed it to be considered as a promising topical anticandida agent (Tomczykowa et al., 2018). In an experiment on guinea pigs with allergic contact dermatitis induced by 2,4-dinitrochlorobenzene, the results of the efficacy of the ointment isolated from a new thick extract of the sum of flavonoids from local raw materials, including *B. tripartita*, on a hydrophobic base were studied. Study results showed that 5% ointment based on a thick extract of total flavonoids was more effective in reducing skin wrinkles than 1%, 3% ointment and antihistamine Psyllo-Balsam and glucocorticosteroid *Celestoderma* ointment (Khatamov et al., 2020).

This plant is known to improve metabolism. Orhan et al. (2016) evaluated the hypoglycemic and antidiabetic activity of *B. tripartita* extract. The total antioxidant capacity of extracts and sub-extracts was determined using the phosphomolybdenum assay method. It was shown that the antioxidant capacity of ethanolic extract was promising (352.51) and close to the antioxidant capacity of Trolox reference compound (382.5), while the highest total antioxidant capacity was found in chloroform sub-extract (1532.45) (Orhan et al., 2016). A large number of studies have been carried out to demonstrate the anti-diabetic activity of *B. tripartita* extracts from the leaves and the ground parts of the plant. These studies have shown that the extracts stimulate insulin secretion, reduce HbA1c and glucose levels in the blood, and increase sensitivity to insulin. In addition, they can prevent autoimmune diabetes through modulation of T-helper cell differentiation (Alarcon-Aguilar et al., 2002; Chang et al., 2004, 2005; Chien et al., 2009).

The herb of *B. tripartita* is used to make decoctions and infusions, as well as ointments for the treatment of various skin diseases (Śliwa et al., 2019; Khatamov et al., 2020; Mendel et al., 2020). The herb of *B. tripartita* is a part of the plant collections "Nephrophyte", "Phytocystol", "Detoxify", "Elekasol", "Elekasol", "Brusniver", "Brusnilan", "Elakosept", "Elekasol" and the elixir "Kliofit" (Donchenko et al., 2021; Oproshanska et al., 2021). The herb is used to make a dietary supplement "*Bidens* extract", which has anti-allergic, anti-inflammatory, diuretic and emollient effects and is recommended for use in allergies, inflammations, liver, gallbladder and all metabolic disorders, and biologically active liquid "Bidens" (Danicapharm) (Oproshanska et al., 2021).

Oproshanska et al. (2021) studied the effects of *B. tripartita* tincture on the state of cell membranes under conditions of spontaneous hemolysis of rat erythrocytes. In the experiment it was found that the tincture at a dose of 0.2 mL/kg had a pronounced membrane-stabilizing effect in the model of acute carrageenan foot edema in white rats. Aqueous and ethanolic extracts of *B. tripartita* improved short-term memory and had a mild anxiolytic effect (Lupușoru et al., 2017). The effects of two extracts (aqueous and ethanolic) of *B. tripartita* on somatic nociceptive reactivity in rats were investigated (Lupușoru et al., 2016).

Bidens tripartita tinctures are also successfully used in veterinary medicine to treat bacterial pathologies in sturgeon. The positive effect of *B. tripartita* was determined by evaluating the degree of wound healing on the body of fish, showing anti-inflammatory activity, stimulating effect on the development of granulation tissue and enhancing regeneration (Nurzhanova et al., 2021).

The aim of the study was to determine the effect of *B. tripartita* dried leaves on the body of rats consuming a hypercaloric diet.

Materials and methods

Animals and nutrition. The number of animals, the experimental design, the conditions of housing and feeding of the experimental animals were approved from the ethical point of view by the Local Animal Experimental Ethics Committee of Dnipro State Agrarian and Economic University (No. 3/22-23 of 16.09.2022). Three groups of 6 animals each were formed from adult male outbred rats. The experimental design consisted of feeding the animals a high-calorie, high-fat diet supplemented with a 20% fructose solution and adding dried, crushed *B. tripartita* leaves to their diet. The study lasted 27 days, the rats were housed in polycarbonate cages with steel grid lids and built-in feeders, room temperature was 20–22 °C, relative humidity was 50–65%, light regime was 12 h light/12 h dark. The animals received the combined diet and water *ad libitum*.

The high calorie ration was prepared on the basis of the basal diet consisting of 75% cereal mixture (corn, sunflower seeds, wheat, barley, soya), 8% root vegetables (carrots), 2% meat and bone meal, 2% mineral and vitamin supplements with the addition of 15% sunflower oil and 20% fructose solution instead of water. The first experimental group was fed 20 g of dried chopped leaves of *B. tripartita* and the second experimental group 200 g. Medicinal raw material in official dosage form (purchased from commercial pharmacy) was pre-crushed and added to the mixture of dry diet ingredients and further pelleted to a total of 5 kg per group of animals. Root vegetables in fresh form were given to the animals individually every day in the appropriate amount. The amount of food and water consumed by the animals of each group was recorded daily and for the whole duration of the experiment (27 days) (Lieshchova & Brygadyrenko, 2023).

Body weight, food and water consumption, euthanasia. Over the course of the study, we recorded changes in the body weight of each animal and also determined the amount of food and liquid consumed. We calculated the rats' total body weight gain and additional body weight gain. At the end of the study (day 27), the animals were euthanized by drug overdose (80 mg/kg ketamine and 12 mg/kg xylazine, intraperitoneally) and blood was sampled from the heart. During autopsy, internal organs (heart, liver, lungs, thymus, spleen, stomach, small and large intestine, kidneys) were examined for the presence of pathological changes, removed and weighed to an accuracy of 10 mg.

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

The number of erythrocytes and leukocytes, hematocrit and hemoglobin content were determined in the blood of rats after the addition of K3EDTA using a PCV-80 Vet automatic hematological analyzer. For the leukogram, Pappenheim's blood smears were prepared.

Behavioral parameters. The orientation-physical activity and emotional state of the experimental animals were studied in an open field test (Seibenhener & Wooten, 2015). A setup consisting of a 1 m^2 square area divided into 16 squares and bounded by a 20 cm high opaque wall was used. The experiment was conducted in complete silence with intense illumination of the field itself. An animal taken from a cage in a pre-darkened room was placed in the center of the field. The exposure time was 2 min. The animals were tested for four days at the beginning of the experiment (days 1–4) and for four days at the end of the experiment (days 26–30). The number of crossed squares was counted: peripheral and central squares – physical activity was assessed; peripheral (with support on the wall) and central (without support on the wall) stands – orientation activity; number of acts of grooming, defecation and urination – emotional status (Lieshchova et al., 2021).

Statistical analysis. Data were analyzed using Statistica 8.0 (StatSoft Inc., USA). Results are presented in tables as x ± SE (mean ± standard error). Differences between the values of the control and experimental groups were determined using the Tukey test (with Bonferroni correction), with differences considered significant at $P < 0.05$.

Results

During the experiment, the body weight of the animals in the three groups remained virtually unchanged for the first five days and increased from the sixth day at a dose of 200 g dried *B. tripartita* leaves, particularly in the control group. The opposite effect was observed in the group of rats that consumed the diet containing a smaller amount of dried *B. tripartita* leaves (20 g). Thus, from the sixth day of the experiment, the animals had a sharply reduced weight gain, no increase in body weight was observed throughout the study, and at the end of the experiment the average body weight was the same as at the beginning of the experiment (Figs. 1a, 1b, 1c). In the group of animals that received a high dose of the medicinal plant (200 g), we observed an increase in body weight from the sixth day onwards and a decrease at the end of the experiment. In general, rats fed a high-fat diet with added fructose gained up to 121% of their initial body weight. In groups of rats fed with dry leaves of *B. tripartita* at a dose of 200 g of the diet weight, the increase in body weight was insignificant (up to 102%), and at a dose of 20 g there was practically no change.

Adding dry leaves of *B. tripartita* to the diet of animals caused a decrease in body weight gain without a significant decrease in food and water intake. Thus, rats fed a high-fat diet with the addition of 0.4% dry leaves of *B. tripartita* had practically no increase in body weight, and with the addition of 4% – daily weight gain did not exceed 0.37 g/day, compared to the control group, where rats gained 2.38 g/day. It should be noted that the amount of food and water consumed by the rats in the experimental groups was lower than in the control group (Table 1).

The relative mass of the thymus was significantly decreased (to 80.5% of the control group level) and the relative mass of the brain increased (to 115.2% of the control group level) by the addition of dried *B. tripartita* leaves at a dose of 20 g. The relative mass of the lungs (up to 119.2% of the control group level), of the cecum (up to 126.3%), of the large intestine (up to 132.5%) and of the cerebrum (up to 110.3%) was increased, while the relative mass of the thymus was decreased when *B. tripartita* dried leaves were consumed at a dose of 200 g (Table 2).

Adding *B. tripartita* dried leaves to the high-calorie diet altered blood biochemical parameters (Table 3). The globulin level in the blood plasma of rats fed with dried leaves of *B. tripartita* increased significantly, which caused the protein coefficient to change. Thus, consuming 0.4% dry leaves of *B. tripartita* increased the level of globulins in the blood to 115.2% of the control group, and consuming 4.0% – to 118.6%. At the same time, while the levels of total protein and albumin remained unchanged, the protein coefficient decreased significantly in both groups compared to the control group. The dose of trifid bur marigold leaves affected the levels of urea and urea nitrogen in the blood plasma of the animals differently. For example, 0.4% trifid bur marigold leaves reduced the urea level by almost half, while 4.0% increased this indicator by 153.8% compared with the control. Similarly, the urea nitrogen indicator decreased by 51.8% at a dose of 0.4% *B. tripartita* leaves in the diet and increased by 156.7% at a dose of 4.0%. In both experimental groups, the level of inorganic phosphorus changed: at a dose of 0.4% of dry trifid bur marigold leaves, this indicator increased to 116.9% of the control, while at 4.0% it decreased (to 81.5%). The Ca/P ratio also changed: at a dose of 0.4% there was a significant decrease (87.9%) compared to the control group, and at a dose of 4.0% – an increase (112.1%). Enzymatic activity was affected by trifid bur marigold leaves with an increase in the activity of alanine aminotransferase with a corresponding change in the De Ritis ratio in both experimental groups. Consumption of dried *B. tripartita* leaves at a dose of 0.4% significantly increased alkaline phosphatase activity (154.8%) and decreased alpha-amylase activity (69.7%), whereas a dose of 4.0% *B. tripartita* leaves only increased gamma-glutamyltransferase activity (151.7%, Table 3). The addition of dried trifid bur marigold leaves to the diet of rats caused an increase in total plasma bilirubin (123.3%) at a dose of 0.4% and a decrease in glucose (57.9%) at a dose of 4.0%. Blood levels of creatinine, cholesterol and total calcium were not affected by trifid bur marigold leaves.

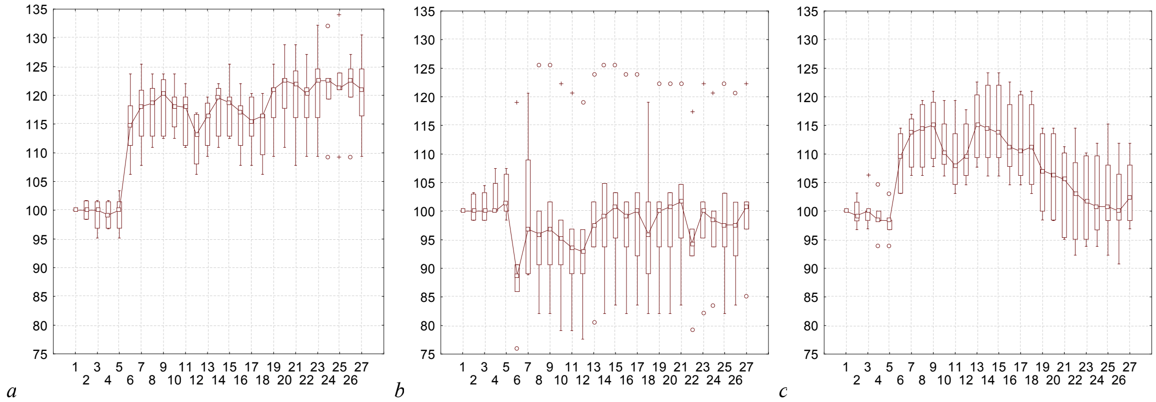

Fig. 1. Changes in body weight of rats in the control group (*a*) when 20 g (*b*) and 200 g (*c*) of *Bidens tripartita* L. inflorescences were added to their diet: abscissa axis – days of the experiment, ordinate – body weight of animals (% with respect to the initial body weight before the start of the experiment, taken as 100% for each of the experimental animals); small square – median, upper and lower borders of rectangle – 25% and 75% quartiles, vertical line – minimum and maximum values, circles – outliers; n = 6

Table 1
Changes in body weight and food consumption of young male rats in response to the addition of *Bidens tripartita* L. to their diet (x ± SE, n = 6, duration of experiment – 27 days)

Parameter	Control	*B. tripartita*, 0.4% of food weight	*B. tripartita* (0.4%) compared to the control, %	*B. tripartita*, 4.0% of food weight	*B. tripartita* (4.0%) compared to the control, %
Consumption of food by animals, g/day	29.3	28.3	96.5	28.6	97.5
Fructose solution consumption, g/day	36.5	33.1	90.6	34.0	93.2
Change in body weight, g/day	2.38 ± 0.32[a]	0.12 ± 0.58[b]	5.2	0.37 ± 0.26[b]	15.6
Change in body weight, %/day	0.759 ± 0.110[a]	0.045 ± 0.182[b]	6.0	0.123 ± 0.087[b]	16.2

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 2
Change in relative organ mass (%) in male rats under the influence of dietary supplementation with *Bidens tripartita* L. (x ± SE, n = 6, duration of experiment – 27 days)

Organ	Control	*B. tripartita*, 0.4% of food weight	*B. tripartita* (0.4%) compared to the control, %	*B. tripartita*, 4.0% of food weight	*B. tripartita* (4.0%) compared to the control, %
Heart	0.316 ± 0.017[a]	0.318 ± 0.011[a]	100.7	0.350 ± 0.041[a]	110.8
Liver	3.40 ± 0.21[a]	3.37 ± 0.12[a]	99.3	3.27 ± 0.15[a]	96.2
Lungs	0.646 ± 0.057[ab]	0.635 ± 0.031[a]	98.3	0.770 ± 0.069[b]	119.2
Thymus	0.262 ± 0.028[a]	0.211 ± 0.040[ab]	80.5	0.166 ± 0.006[b]	63.4
Spleen	0.184 ± 0.020[a]	0.208 ± 0.018[a]	113.2	0.201 ± 0.017[a]	109.6
Stomach	0.455 ± 0.017[a]	0.464 ± 0.038[a]	102.1	0.478 ± 0.027[a]	105.0
Small intestine	1.81 ± 0.11[a]	1.90 ± 0.06[a]	104.9	1.75 ± 0.10[a]	96.5
Cecum	0.209 ± 0.030[ab]	0.184 ± 0.028[a]	88.1	0.264 ± 0.020[b]	126.3
Colon	0.224 ± 0.013[a]	0.225 ± 0.016[a]	100.2	0.297 ± 0.049[b]	132.5
Rectum	0.188 ± 0.030[a]	0.215 ± 0.018[a]	114.2	0.195 ± 0.013[a]	103.5
Right kidney	0.288 ± 0.012[a]	0.299 ± 0.010[a]	103.8	0.289 ± 0.012[a]	100.4
Left kidney	0.281 ± 0.009[a]	0.283 ± 0.018[a]	100.8	0.294 ± 0.007[a]	104.6
Brain	0.476 ± 0.016[a]	0.548 ± 0.012[b]	115.2	0.524 ± 0.015[b]	110.3
Testicle	0.471 ± 0.032[a]	0.439 ± 0.025[a]	93.2	0.418 ± 0.019[a]	88.8

Note: see Table 1.

Changes in total blood count were observed under the influence of different doses of *B. tripartita* leaves in the rat diet (Table 4). Consumption of *B. tripartita* leaves caused a decrease in blood hemoglobin concentration, to 90.4% at a dose of 0.4% of the dietary weight, and to 89.6% of the control group at a dose of 4.0%. The hematocrit index also decreased in both groups, but significantly in the group consuming 4.0% of *B. tripartita* leaves (89.2% of the control group). Of the red blood indices, the consumption of *B. tripartita* leaves only had an effect on the color index, reducing it to 91.1% of the control group. *Bidens tripartita* leaf consumption reduced platelet counts in both groups, with a significant reduction at the 4.0% dose. Compared to the control group, the number of leukocytes did not change. The leukocyte formula was affected by a large increase in the number of eosinophils per unit volume of blood (up to 250% of the control group) after consumption of *B. tripartita* leaves at a dose of 4.0%.

Adding dried *B. tripartita* leaves to the hypercaloric diet affected the behavioral characteristics of the rats studied both at the beginning and at the end of the experiment (Table 5). The rats consuming 4.0% of *B. tripartita* leaves in the diet had significantly less physical activity (one and a half times less than the control group) during the first three days of the experiment. Orientation activity in the group of animals consuming 0.4%

of *B. tripartita* leaves was higher than in the control group (1.31 times), and in the group consuming 4.0% it was lower (1.65 times). Emotional state was increased in both groups of animals eating dried *B. tripartita* leaves.

At the end of the study (25–27 days), *B. tripartita* leaves eaten by rats during the experiment caused a decrease in physical activity in both experimental groups. At the same time, the rats that received 4.0% of the leaves showed a more significant decrease in this indicator than the rats that received 0.4%. It should be noted that a decrease in the number of peripheral fields visited was responsible for the decrease in physical activity at the end of the experiment. Orientation activity was assessed by the number of peripheral and central stands made by the rats. Orientation activity at the end of the experiment increased in the group of rats that consumed 0.4% trifid bur-marigold leaves and decreased in the group of rats that consumed 4.0% trifid bur-marigold leaves compared to the control group. The control group's emotional state was low at the end of the experiment. The addition of trifid bur-marigold leaves to the diet resulted in a significant increase in the emotional state of the animals (more due to an increase in the number of grooming actions and less due to an increase in the number of fecal boluses). At the same time, this effect was reduced in the group consuming a 4.0% dose of trifid bur marigold leaves compared to the group consuming a 0.4% dose of the medicinal plant.

Table 3
Changes in blood biochemical parameters of male rats under the effect of supplementation with *Bidens tripartita* L. (x ± SE, n = 6, duration of experiment – 27 days)

Parameters	Control	*B. tripartita*, 0.4% of food weight	*B. tripartita* (0.4%) compared to the control, %	*B. tripartita*, 4.0% of food weight	*B. tripartita* (4.0%) compared to the control, %
Total protein, g/L	81.8 ± 3.0^a	86.3 ± 1.4^a	105.5	84.5 ± 1.7^a	103.4
Albumins, g/L	44.8 ± 0.9^a	44.5 ± 0.6^a	99.4	42.0 ± 1.8^a	93.9
Globulins, g/L	36.3 ± 2.3^a	41.8 ± 1.7^{ab}	115.2	43.0 ± 2.0^b	118.6
Protein coefficient, U	1.23 ± 0.06^a	1.08 ± 0.05^{ab}	87.8	0.98 ± 0.09^b	79.6
Urea, mmol/L	3.30 ± 0.15^a	1.75 ± 0.16^b	53.0	5.08 ± 0.11^c	153.8
Blood urea nitrogen, mg/100 g	6.18 ± 0.34^a	3.20 ± 0.28^b	51.8	9.68 ± 0.20^c	156.7
Creatinine, µmol/L	52.0 ± 4.0^a	56.5 ± 4.6^a	108.7	56.5 ± 3.7^a	108.7
AST, U/L	75.5 ± 16.5^a	53.8 ± 12.7^a	71.2	63.0 ± 6.1^a	83.4
ALT, U/L	74.8 ± 8.7^a	116.5 ± 14.9^b	155.9	95.0 ± 7.3^b	127.1
De Ritis ratio (AST/ALT), U	0.78 ± 0.38^a	0.60 ± 0.07^{ab}	77.4	0.43 ± 0.16^b	54.8
Alkaline phosphatase, U/L	363 ± 30^a	562 ± 43^b	154.8	311 ± 49^a	85.8
Alpha-amylase, U/L	1341 ± 143^a	934 ± 34^b	69.7	1802 ± 463^a	134.4
Total bilirubin, µmol/L	6.13 ± 0.31^a	7.55 ± 0.34^b	123.3	7.35 ± 1.04^{ab}	120.0
Glucose, mmol/L	5.70 ± 0.57^a	5.03 ± 0.28^a	88.2	3.30 ± 0.09^b	57.9
Total calcium, mmol/L	2.65 ± 0.13^a	2.68 ± 0.06^a	100.9	2.48 ± 0.05^a	93.4
Non-organic phosphorus, mmol/L	3.25 ± 0.18^a	3.80 ± 0.15^b	116.9	2.65 ± 0.14^c	81.5
Ca/P ratio	0.825 ± 0.025^a	0.725 ± 0.025^b	87.9	0.925 ± 0.063^c	112.1
Gamma-glutamyltransferase, U/L	3.63 ± 0.24^a	3.25 ± 0.48^a	89.7	5.50 ± 1.32^b	151.7
Cholesterol, mmol/L	1.575 ± 0.063^a	1.600 ± 0.108^a	101.6	1.525 ± 0.048^a	96.8

Note: see Table 1.

Table 4
Changes in general blood analysis and leukogram of male rats under the effect of dietary supplementation with *Bidens tripartita* L. (x ± SE, n = 6, duration of experiment – 27 days)

Parameter	Control	*B. tripartita*, 0.4% of food weight	*B. tripartita* (0.4%) compared to the control, %	*B. tripartita*, 4.0% of food weight	*B. tripartita* (4.0%) compared to the control, %
Hemoglobin, g/L	135.3 ± 3.3^a	122.3 ± 4.8^{ab}	90.4	121.3 ± 1.4^b	89.6
Hematocrit, %	35.1 ± 1.0^a	33.1 ± 0.9^{ab}	94.3	31.3 ± 1.1^b	89.2
Erythrocytes, 10^{12}/L	6.87 ± 0.20^a	6.40 ± 0.13^a	93.2	6.40 ± 0.38^a	93.1
MCV, $*10^{-15}$/L	51.1 ± 1.0^a	51.7 ± 0.9^a	101.2	49.2 ± 1.3^a	96.2
MCHC, $*10^{-12}$ L	19.7 ± 0.3^a	19.1 ± 0.9^a	97.0	19.2 ± 1.3^a	97.4
MCH, %	38.6 ± 0.2^a	37.0 ± 1.3^a	95.9	38.9 ± 1.7^a	100.9
Colour index, units	0.98 ± 0.02^a	0.90 ± 0.04^b	91.9	0.94 ± 0.07^{ab}	95.4
Erythrocyte sedimentation rate (ESR), mm/h	1.00 ± 0.00^a	1.50 ± 0.29^a	150.0	1.25 ± 0.25^a	125.0
Thrombocytes, 10^9/L	505 ± 40^a	420 ± 3^a	83.1	395 ± 20^b	78.2
Leukocytes, 10^9/L	3.63 ± 0.35^a	4.40 ± 0.73^a	121.4	3.38 ± 0.44^a	93.1
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	0.75 ± 0.25^a	150.0	1.25 ± 0.25^b	250.0
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	1.50 ± 0.65^a	120.0	0.50 ± 0.50^a	40.0
– with segmented nuclei	24.0 ± 2.9^a	20.8 ± 3.8^a	86.5	18.0 ± 3.7^a	75.0
Lymphocytes, %	74.0 ± 5.8^a	73.5 ± 4.8^a	99.3	77.0 ± 4.0^a	104.1
Monocytes, %	2.75 ± 0.85^a	3.50 ± 0.29^a	127.3	3.25 ± 0.25^a	118.2

Note: see Table 1.

Discussion

In an experiment lasting 30 days, we studied the effect of the medicinal plant *B. tripartita* in two doses on the body of model animals that were fed a hypercaloric diet. *Bidens tripartita* is known to have a number of pharmacological effects, including antioxidant, antihypertensive, hepatoprotective, immunostimulant, antimicrobial and antifungal. Therefore, the aim of this study was to investigate the possibility of using *B. tripartita* plant material as a natural dietary supplement for mild metabolic correction in an unbalanced diet.

At the beginning of the study, during the first five days, we observed no changes in body weight despite the hypercaloric diet the rats received. As this was typical of all groups, it can be explained by the animals' adaptation to a high-fat diet and the replacement of water with a 20% fructose solution. At the end of the study, only the control group of rats showed an increase in body weight. This indicator remained almost unchanged in the experimental groups that also consumed dry leaves of *B. tripartita*. This fact suggests that *B. tripartita* slows down weight gain in hypercaloric diets. Other medicinal plants such as *Helichrysum arenarium*, *Salvia sclarea*, *Origanum vulgare*, *Inula helenium* and *Vitex angus-castus* have shown a similar effect in similar experiments with laboratory rats (Lieshchova & Brygadyrenko, 2021, 2022, 2023b, 2023c; Lieshchova et al., 2021). While the consumption of *Scutellaria baicalensis*, *Matricaria chamomilla*, *Lavandula angustifolia*, *Melissa officinalis*, *Salvia officinalis*, *Rhodiola rosea*, *Punica granatum* in addition to a high-fat diet showed the opposite effect (Lieshchova & Brygadyrenko, 2021, 2023a, 2023b; Lieshchova et al., 2021, 2023).

It is well known that the relative weight of internal organs is a reflection of the growth, development and general condition of the organism and is an important integral indicator in the assessment of the effects of various substances in toxicology and pharmacology. At the end of the experiment, when assessing the effect of a hypercaloric diet with the addition of dry leaves, no pathological changes in the internal organs were detected. It should be noted that when determining the toxicity of the alcoholic extract of trifid bur-marigold, even after a lethal dose, no macroscopic changes in the organs were detected, and histopathological changes were observed only in some organs (Sandu et al., 2012).

Table 5
Changes in the behavioral parameters of three groups of rats during 2 minutes of the experiment, to whose diet *Bidens tripartita* L. was added (x ± SE, n = 18, duration of the experiment was 27 days)

Parameter	Control, 1–3th days	Control, 25–27th days	*B. tripartita* (0.4%), 1–3th days	*B. tripartita* (0.4%), 25–27th days	*B. tripartita* (4%), 1–3th days	*B. tripartita* (4%), 25–27th days
Number of visited peripheral squares	19.1 ± 2.4^{a}	19.1 ± 2.0^{a}	17.4 ± 2.8^{a}	15.9 ± 2.2^{ab}	11.7 ± 1.9^{b}	12.4 ± 1.9^{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}	0.61 ± 0.24^{b}	0.28 ± 0.16^{ab}
Number of stands in peripheral squares	4.22 ± 0.33^{ab}	3.61 ± 0.28^{ab}	6.94 ± 0.88^{b}	5.67 ± 0.77^{b}	2.78 ± 0.50^{a}	2.83 ± 0.39^{a}
Number of stands in central squares	1.11 ± 0.25^{a}	0.61 ± 0.18^{b}	1.06 ± 0.31^{ab}	0.94 ± 0.24^{ab}	0.44 ± 0.20^{b}	0.17 ± 0.12^{bc}
Number of grooming acts	0.67 ± 0.21^{a}	0.11 ± 0.08^{b}	1.28 ± 0.29^{c}	1.56 ± 0.28^{c}	1.00 ± 0.27^{ac}	1.56 ± 0.37^{c}
Number of faecal boluses	0.39 ± 0.18^{a}	0.44 ± 0.22^{a}	0.61 ± 0.18^{a}	0.61 ± 0.26^{a}	0.50 ± 0.19^{a}	0.44 ± 0.22^{a}
Number of urination	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Physical activity	19.1 ± 2.4^{a}	19.1 ± 2.0^{a}	17.4 ± 2.8^{a}	15.9 ± 2.2^{ab}	12.3 ± 2.0^{b}	12.7 ± 2.0^{b}
Orientation activity	5.33 ± 0.35^{a}	4.22 ± 0.33^{b}	7.00 ± 1.13^{c}	6.61 ± 0.94^{ac}	3.22 ± 0.49^{b}	3.00 ± 0.43^{b}
Emotional state	1.06 ± 0.22^{a}	0.56 ± 0.23^{b}	1.89 ± 0.27^{c}	2.17 ± 0.31^{c}	1.50 ± 0.23^{ac}	2.00 ± 0.32^{c}

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

There were no changes in the relative weights of most internal organs, but both doses of *B. tripartita* caused an increase in the relative weight of the brain and a decrease in the weight of the thymus, which will require further histological and cytological studies. A high dose of dry leaves of *B. tripartita* (200 g) resulted in an increase in relative lung weight. As the plant material was dry leaves and not extracts or tinctures of the plant, the increase in the relative weight of the colon and cecum when rats received 4.0% of food weight of *B. tripartita* can be explained by the activation of fiber digestion in these parts of the intestine. It is also noteworthy that Mendel et al. (2020) reported a significant prokinetic effect of *B. tripartita* extracts and their flavonoids on intestinal smooth muscle.

When the indicators of protein metabolism were evaluated, it was found that the level of total blood protein in animals consuming a high-calorie diet was higher than the reference values, which may indicate blood thickening, since the drinking water was replaced by a 20% fructose solution. In rats supplemented with dried leaves of *B. tripartita*, the level of total blood protein was insignificantly higher than in the control group and correspondingly higher than the reference values (Shayakhmetova et al., 2020). Also, against the background of a high calorie diet in the control group of animals, the level of blood globulins was increased compared to the reference value, and the addition of dry leaves of *B. tripartita* to the diet contributed to an increase in this indicator with a corresponding protein coefficient disorder. Similarly, when the animals were fed a high-fat diet containing 20% fructose solution, blood urea and urea nitrogen levels were significantly reduced below the reference values. The addition of 0.4% dry *B. tripartita* herb to this diet further reduced these levels, almost halving them compared to the control group. At the same time, 4.0% *B. tripartita* increased blood urea and urea nitrogen to normal levels. Changes in blood urea may be an indicator of hepatocyte dysfunction, as urea is known to be synthesized in the liver as a by-product of the amino acid deamination reaction.

In folk medicine, trifid bur-marigold is used to treat diseases of the digestive system due to its chemical composition, in particular flavonoid compounds with antioxidant effects (Wolniak et al., 2007). It is known that flavonoids have a protective effect in various liver diseases, the pathogenesis of which is determined by increased lipid peroxidation of biomembranes. The total flavonoid complexes of the trifid bur-marigold, when administered therapeutically and prophylactically, can normalize hepatocyte cholestasis, carbohydrate, lipid, protein and pigment metabolism in the blood serum in conditions of acute toxic liver damage. Biochemical blood tests showed that the addition of *B. tripartita* dry leaves to hypercaloric diets affected the functional state of the liver. Thus, in the experimental groups of rats, there was an increase in total blood bilirubin levels when they consumed dry leaves of *B. tripartita* in comparison to the control group of animals on a hypercaloric diet only. It should be noted that this indicator exceeded the reference values in both experimental groups of rats (Shayakhmetova et al., 2020).

Experimental results of Orhan et al. (2016) showed that *B. tripartita* extract exerted potent hypoglycemic effects in normoglycemic and glucose-loaded rats, and promising antidiabetic effects in streptozotocin-induced diabetic rats acutely and subacutely. In our experiment, the consumption of a high-fat diet with the addition of 20% fructose solution did not induce hyperglycosemia. Glucose levels in the control and experimental groups were within reference values, but the supplementation of dried *B. tripartita* leaves at a dose of 4% of the diet weight caused an unreliable decrease in blood glucose levels compared to the control. One of the mechanisms of the hypoglycemic effect is the inhibition of the carbohydrate-degrading enzymes alpha-glucosidase and alpha-amylase by trifid bur-marigold. For example, in a study by Orhan et al. (2016), ethanolic extract of *B. tripartita* at a dose of 10.25 µg/mL showed moderate inhibition of α-glucosidase activity, while only the extract at high concentration (2.0 mg/kg) had an inhibitory effect on α-amylase activity.

In the present study, the addition of dried leaves of *B. tripartita* to the hypercaloric diet of rats resulted in an impairment of the enzyme activity in the blood plasma. The increase in blood ALT activity above reference values was caused by the hypercaloric diet, and the addition of the plant significantly and reliably increased this index. Similarly, a high-calorie diet and the addition of dried *B. tripartita* leaves to it affected alkaline phosphatase activity. This index in the control group of animals was almost twice the norm. The dose of 0.4% dry leaves of *B. tripartita* contributed to a significant and reliable increase in alkaline phosphatase activity, while 4.0% dry leaves, on the contrary, insignificantly decreased this index. The dose of dry leaves of *B. tripartita* in the diet had different effects on the activity of α-amylase in blood plasma. Thus, when rats consumed 0.4% of dry leaves, a strong significant decrease in the activity of blood alpha-amylase was observed, and when rats consumed 4.0% – an increase occurred compared to the control group and to rats consuming 0.4% of dry leaves. The activity of gamma-glutamyltransferase in animals of the control and experimental groups did not exceed normal values during the experiment, but 4.0% of dry leaves of *B. tripartita* caused a significant increase in this index compared to the control group.

Therefore, since the addition of dry leaves to the hypercaloric diet of rats causes significant changes in the biochemical parameters of the blood, which indicate the functional state of the liver and biliary tract (increased levels of total protein and globulins in the blood, changes in the protein coefficient, changes in urea and urea nitrogen, increased levels of total bilirubin, changes in the enzyme activity of the blood), this medicinal plant should only be used to remedy imbalances in the diet with strict control of the morphology and functional state of the liver.

In our experiment, adding dry *B. tripartita* leaves to the diet of rats at a dose of 4.0% slightly reduced hemoglobin and hematocrit levels. At the same time, the red blood parameters did not change, except for the color index, which slightly decreased under the influence of *B. tripartita* consumption. With regard to the total number of blood cells, the addition of this medicinal plant to the diet caused a decrease in the number of platelets. In the leukocyte blood formula, only the relative number of eosinophils changed. The addition of 4.0% dry leaves of *B. tripartita* to the diet caused a sharp and significant increase in the number of eosinophils by 2.5 times, indicating the possible development of an allergic reaction. Sandu et al. (2013) found that *B. tripartita* extracts, both alcoholic and aqueous, when administered to laboratory rats, did not cause any changes in the blood leukocyte count, as well as statistically significant differences in the phagocytic activity of blood neutrophils and the levels of complement activity in serum.

The effect of *B. tripartita* and its extracts on the functional state of the nervous system is not well described in the scientific literature. Lupuşoru et al. (2016) studied the effect of two *B. tripartita* extracts on somatic nociceptive reactivity in rats. During intraocular administration of aqueous and ethanolic extracts, nociceptive skin tests were performed using the tail flick assay to determine the potential temporal response to thermal noxious stimulation of the tail. It was shown that the ethanolic extract itself caused a statistically significant decrease in the latent response time in the tail flick test compared to the control, and demonstrated an analgesic effect. Intraperitoneal administration of aqueous and ethanolic extracts of *B. tripartita* for 1 month was investigated on spatial working memory in rats using the T-maze test. Both aqueous and ethanolic extracts of *B. tripartita* have been shown to facilitate spatial learning, improve short-term memory and exhibit weak anxiolytic effects in rats (Lupuşoru et al., 2017). Our experiment showed that the addition of dry *B. tripartita* leaves to a high-fat diet and 20% fructose solution affected the behavioral responses of model animals. At the beginning of the experiment, changes in physical activity, orientation activity and emotional state were observed compared to the control group. At the same time, the rats' emotional state was increased against a background of decreased physical activity, and orientation activity changed in a dose-dependent manner. At the end of the experiment (27 days), the tendency remained. Thus, the emotional state of the rats was high and the physical activity decreased in comparison to the control group after the consumption of dried leaves of *B. tripartita*.

Therefore, dried *B. tripartita* leaves should be used with caution alongside a hypercaloric diet for the purpose of mildly correcting metabolic processes. This medicinal plant in its natural form reduces weight gain, does not cause pathological changes in the internal organs, but at the same time causes changes in the functional state of the liver and affects behavioral reactions.

Conclusions

The addition of *B. tripartita* dry leaves to the diet of rats caused severe changes in their body and had an effect on the rate of body weight gain. When *B. tripartita* dry leaves were added to high-fat diets containing 20% fructose for 27 days, body weight gain slowed down and daily weight gain decreased when added at 4.0%, but not when added at 0.4%. At the end of the experiment, dry leaves of trifid bur-marigold caused a significant increase in the relative weight of the brain and a decrease in the relative weight of the thymus, and consumption of this herb at a dose of 0.4% also caused a significant increase in the relative weight of the lungs, colon and cecum.

Adding dry *B. tripartita* leaves to the diet, regardless of dose, resulted in an increase in blood globulin levels with a corresponding decrease in the protein coefficient. Depending on the dose of the medicinal plant, there were multidirectional changes in the levels of urea, urea nitrogen and inorganic phosphorus. Thus, 0.4% of the dried leaves of trifid bur-marigold caused a decrease in the level of urea and urea nitrogen and an increase in the level of inorganic phosphorus, and 4.0% of the medicinal plant caused an increase in the level of urea and urea nitrogen and a decrease in the level of inorganic phosphorus, with corresponding changes in the Ca/P ratio. The addition of dried trifid bur-marigold leaves to the diet of rats caused an increase in total plasma bilirubin (123.3%) at a dose of 0.4% and a decrease in glucose (57.9%) at a dose of 4.0%. Consumption of dry leaves of *B. tripartita* by rats significantly affected blood enzymatic activity, causing an increase in ALT activity with a corresponding change in the De Ritis ratio.

Consumption of dry leaves of *B. tripartita* at a dose of 0.4% in the diet increased alkaline phosphatase activity (154.8%) and decreased alpha-amylase activity (69.7%); a dose of 4.0% of *B. tripartita* leaves increased gamma-glutamyltransferase activity (151.7%). *Bidens tripartita* dried leaves in a hypercaloric diet caused changes in general blood count (reduced hemoglobin concentration, hematocrit, platelet count) and greatly increased eosinophil count.

The intake of *B. tripartita* affected the functional state of the nervous system, causing changes in physical activity, orientation activity and emotional state in the "open field" test. It was found that at the beginning of the experiment, compared to the control group, the animals of the experimental groups had an increased emotional state. Animals that consumed 0.4% of the dried leaves of trifid bur-marigold had increased orientation activity, and at a dose of 4.0% both orientation activity and physical activity decreased. At the end of the experiment, the consumption of dried leaves of trifid bur-marigold caused a decrease in physical activity and a sharp increase in emotional state. Orientation activity varied according to the dose, with a low dose (0.4%) of trifid bur-marigold causing an increase in orientation activity and a high dose (4.0%) causing a decrease.

In general, the results obtained indicate that the use of dry leaves of *B. tripartita* in a hypercaloric diet does not cause pathological and toxic effects, but does affect metabolic processes and slows down the weight gain of the animals. The results of the present study add to the growing body of literature on the multipharmacological activity of *B. tripartita*, which can be used to develop biological products for the treatment of common diseases.

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