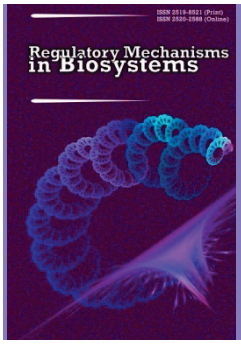

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Effect of *Viola tricolor* flower supplementation on body and intestinal microbiota in rats fed a high-fat diet

M. V. Bilan *, M. A. Lieshchova*, A. A. Bohomaz*, 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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The use of herbal medicines, due to their wide therapeutic spectrum and absence or minimal side effects, is an important area of therapy that is used in the treatment of diseases of various etiologies. Garden violet /wild pansy (*Viola tricolor* L.) is a medicinal plant of the violet family that is widely distributed in Ukraine. The herb of this plant (*Violae herba*) is used as the official raw material, which is sold in dry form as an independent remedy and as a part of herbal teas. In this study, we determined the overall effect of dry *V. tricolor* herb as part of a high-fat diet on body weight gain, metabolic processes in model animals, and the effect on their intestinal microbiota. For the experiment, 15 male white laboratory rats were divided into three groups and fed a high-fat diet (15% vegetable fat) supplemented with 0.5% and 2.0% dried *V. tricolor* herb for 30 days. Consumption of 2.0% of the herb resulted in increased weight gain compared to the control group. The dried herb *V. tricolor* at a dose of 0.5% in the diet of rats caused an increase in the relative weight of the brain and large intestine (caecum and colon) and a decrease in the weight of the thymus. At a dose of 2.0%, the relative weight of the thymus and caecum increased, but the relative weight of the colon decreased, and the length of the colon and rectum decreased compared to the control group. The diet supplemented with *V. tricolor* at a dose of 0.5% caused a decrease in globulin concentration and changes in protein ratio, and at a dose of 2.0% – an increase in total protein, albumin, albumin/globulin ratio and a decrease in globulin level. The addition of 0.5% *V. tricolor* dried herb resulted in an increase in high-density lipoprotein (HDL) cholesterol and a decrease in cholesterol at 2.0%. Regardless of the dose, *V. tricolor* contributed to a reduction in the plasma atherogenic index. Both doses of violet caused a sharp and significant increase in the De Ritis ratio and a decrease in alkaline phosphatase activity. Adding 0.5% and 2.0% violet herb to high-fat diet helps maintain the quantitative composition of the main intestinal microbiota of laboratory rats (*Bifidobacterium*, *Lactobacillus* and typical *Escherichia coli*). It was found that 2% of violet herb in the diet had a bacteriostatic effect on low-fermenting *Escherichia coli* and *Klebsiella* spp. and a bactericidal effect on *Enterococcus* spp., which can disrupt normal intestinal functions and cause diseases.

Keywords: relative organ mass; increase in body weight; garden violet; high fat diet; phytotherapy; obesity correction.

Introduction

Medicinal plants have been used since ancient times as a source of remedies for many diseases. In modern times, these plants have been scientifically analyzed to confirm their efficacy against human diseases. Most of these plants, due to the composition of their biologically active compounds, have been included in the pharmacopoeia of many countries and recognized by official medicine. The increasing role of traditional folk medicinal plants in the treatment of metabolic disorders is one of the trends in modern medicine (Saad et al., 2022). Herbal preparations used in treatment regimens for obesity, insulin resistance and diabetes mellitus are effective and less toxic than chemically synthesized ones (Wynn, & Fougère, 2007; Dimitrov et al., 2019).

Plants of the violet family include about 500 species in 20 genera. Different species of violets are ubiquitous and can be found in different geographical locations. Some of the best known are *Viola tricolor*, *V. canescens*, *V. biflora*, *V. odorata*, *V. cinerea*, *V. diffusa*, *V. patrinii* and others (Khare, 2008; Chandra et al., 2015; Bahadur et al., 2020; Batiha et al., 2020). *Viola tricolor* L. is a dark green annual or perennial plant of the genus *Violetaceae* (10–45 cm tall) with a weakly developed, sparsely glandular root. It grows in the Mediterranean, the Caucasus, Europe, Asia, America and Australia. Violets are grown as ornamentals in flower beds, vegetable gardens and pots at home. They have been used as medicinal plants since ancient times. They have an official place in scientific medicine. In medical practice, two members of the violet family are allowed to be used: *V. tricolor* and *V. arvensis*. They are the most studied and used in many countries around the world. Violets are very rich in various natural products, most of which have been isolated and identified in recent decades. Tricolor violets contain mucilage, tartaric acid salt, salicylic acid, vitamin C, beta-carotene, saponins, violoquercetin alkaloid, yellow pigment, phytoncides, catechins, gallic acid; flavonoid compounds: hyperoside, rutin, kaempferol, quercetin, apigenin; coumarin and dihydrocoumarin (Toiu et al., 2008). Flavonoids, coumarins, alkaloids, triterpenoids, saponins, anthocyanins, phenols, tannins, phytosterol terpenoids, lignans, sesquiterpenes, cyclotides and phenylpropanoids isolated from violet plant material have significant pharmacological activities (Zhang et al., 2015; Wang et al., 2022). Flavonoids are found in many species of violets, but they have only been studied in detail in a few members of the genus: *V. tricolor*, *V. arvensis*, *V. odorata*, *V. langsdorfii* and *V. bioflora*. *Viola tricolor* directly contains significant amounts of: p-coumaric acid, cyclotides, glucose, sterols, essential oils, aromatic acids and their derivatives (Batiha et al., 2020). Among the phenolic compounds in the terrestrial parts of *V. dalatensis* Gagnep, kaempferol was the most abundant with an average concentration of 16.2 mg/100 g dry weight (Tuan et al., 2023). In the flowers of *V. tricolor*, *V. mirabilis*, anthocyanin glycosides were found: delphinidin, peonidin; violanin, the latter consisting of delphinidin,

glucose, rhamnose and cinnamic acid; in the flowers of *V. biflora*, *V. elatior*, *V. mirabilis*, *V. stagnina* – leucoanthocyanidins: leucodelphinidin and leucocyanidin.

Essential oil, a compound with known antioxidant, antibacterial and antiseptic properties, is extracted from violet leaves. The essential oil is extracted from the plant by solvent hydrodistillation and further analyzed by gas chromatography and mass spectrometry. In the herbs *V. arvensis*, *V. odorata*, *V. tricolor*, the essential oil in the aerial parts of violets was found in negligible amounts (0.001% to 0.01%). The essential oil composition of these species is mainly represented by methyl ester of salicylic acid. Butyl-2-ethylhexyl phthalate and 5,6,7,7a-tetrahydro-4,4,7a-trimethyl-2(4H)-benzofuranone were detected in the essential oils of *Viola* species. Essential oils are widely used in the perfume industry (Akhbari et al., 2012). Analysis of the essential oil of *V. tricolor* (wild pansy) revealed a composition of secondary metabolites: 8 sesquiterpenes (59.3%), 17 aliphatics (29.8%), 6 shikimic acid derivatives (8.1%), and 4 monoterpenes (0.3%), oxide (43.3%), trans-β-farnesene (4.0%), and bisabolol oxide A and B (7.8% and 2.3%) (Anca et al., 2009; Rizwan et al., 2019; Batiha et al., 2020). The presence of bioactive compounds makes the garden violet a promising functional food. Mainly due to their different colors, shapes and flavors, the edible flowers of *Viola* species are used in cooking, desserts and drinks (Kaisoon et al., 2012).

Violets have long been known as medicinal plants. They have been used by folk healers to treat and control a variety of human diseases, including infectious diseases, diabetes, asthma, lung diseases, cough, fatigue and several other conditions (Batiha et al., 2020). Fragrant violets were mainly used in ancient medicine. Decoctions of sweet violet were used as a choleretic, to lower blood pressure, for fever, palpitations and unconsciousness. Topical compresses of violet were recommended to soften the skin, decoctions of violet were used to calm children, internally the plant was recommended for coughs, sore throats, pleurisy, inflammation of the lungs. Violets were believed to be useful in digestive and kidney disorders. Inhaling the scent of fresh flowers soothes headaches, calms and helps to combat insomnia. It was also believed that fresh flowers, when taken internally, acted as a sleeping pill and as an antidote for poisoning by various poisons. In ancient medicine, violet oil was very popular. It was recommended for use in the treatment of dry skin, injuries, hair loss, it is useful for nails. Violet oil was used to treat shingles and urticaria. Remarkably, some of these traditional uses have been confirmed. In modern folk medicine, violets are as popular as they were in ancient medicine. This plant has many pharmacological effects which allow it to be used in the treatment of fever, cancer, microbial infections, hypertension, inflammation, as a diuretic, etc. The stem, leaves, flowers, fruits and seeds are used for skin diseases, cystitis, rheumatism, bronchitis, inflammation, cough and as a diuretic (Anca et al., 2009). Decoctions of the herb are used in the treatment of rickets, rheumatism, gout, as a diaphoretic, mucolytic. Fragrant violets are used in the treatment of dysentery. A thick decoction of grass and violet roots is used as an emetic. Smaller concentrations are used for jaundice and epilepsy. Syrup of the herb is used for asthma, choking. Children are given 5–7 drops of fresh sap of the plant for chickenpox. Infusion of the herb is used externally for eye diseases. It is used internally and externally for various skin diseases (eczema, psoriasis), scrofula and tuberculosis of the skin. Herbal infusion (1:10) is prescribed for metabolic disorders, pustular skin diseases, eczema, rashes, diaphoretic. The aqueous infusion is also used as a diuretic. Violet aqueous infusion (1:10) is applied for treating pneumonia, tuberculosis. In dentistry – as an antiseptic and anti-inflammatory for toothache, treatment of oral mucosa, periodontitis.

Violets have expectorant, enveloping, antimicrobial, diuretic, anti-inflammatory, analgesic and antiallergic properties. The prophylactic effect of an aqueous extract of *V. odorata* on formalin-induced lung damage has been demonstrated experimentally in rats. In this case, the extract caused a significant reduction in inflammation, which made it possible to demonstrate antitussive, anti-asthmatic and bronchodilator effects (Koochek et al., 2003). Modern scientific medicine uses *V. tricolor* mainly as an expectorant in chronic respiratory diseases. The polysaccharides contained in violets increase the secretion of the bronchial glands, have an enveloping, anti-inflammatory effect, promote the liquefaction and separation of sputum, and relieve coughing. This plant has pronounced bactericidal properties. Experimental studies have shown the efficacy of violet extract in the treatment of acute staphylococcal pneumonia. Traditional Chinese phytotherapy has used it against *Helicobacter pylori* (Ma, 2010). Cyclotides isolated from *V. yedoensis* are macrocyclic plant peptides with potent anti-HIV activity, the most active being cycloviolacin Y5 (Wang et al., 2007; Conzelmann et al., 2022). Topical application of gel-formulated tricolour violet to treat microbial infections is attributed to the low pH, which prevents the release of pro-inflammatory cytokines (Prow et al., 2011). The insecticidal activity of *V. odorata* is attributed to cycloviolacins, and its essential oil also has repellent activity against mosquito strains (Amer & Mehlhorn, 2006). The presence of cycloviolacin in *V. odorata* has also been attributed to its antitumour and antifungal effects (Parsley et al., 2018). Violet extracts have been found to have diuretic properties. The diuretic effect of violet extracts is attributed to the presence of flavonoids in them. The aqueous extract has diuretic activity at 400 mg/kg, as evidenced by increased levels of potassium and sodium ions in urine products (Bose et al., 2007). Ethanolic extract of *V. canescens* leaves has a laxative effect in mice. Alcoholic and aqueous extracts at concentrations of 200 and 400 mg/kg respectively have significant laxative effects (Vishal et al., 2009). Violets have strong antioxidant properties. Antioxidants are generally known to be of plant origin. The 2,2-diphenyl-1-picrylhydrazyl radical isolated from the aqueous extract of *V. odorata* flower showed antioxidant potential (Stojković et al., 2011). Chloroform and methanol extracts of *V. odorata* showed antioxidant activity. Moreover, the antioxidant activity of *V. odorata* extracts (DCM, ethyl acetate, ethanolic and aqueous) tested by DPPH scavenging activity, metal chelating capacity, iron and phosphomolybdenum reducing antioxidant potential showed low to moderate activities (Erdogan Orhan et al., 2015). On the other hand, the essential oil did not show any antioxidant activity (Akhbari et al., 2012).

Viola yedoensis Makino was found to have anticoagulant activity. This species of violet is used in traditional Chinese medicine for the treatment of furuncles, carbuncles, as an anti-inflammatory agent and even against snake venom (Xia et al., 2010). The plant's mechanism of action as an anticoagulant has also been the subject of research. It is associated with new isolates of dicoumarins (dimeresculetin, euphorbetin, esculetin) identified from *V. yedoensis* Makino. This effect may be as a result of activation of partial thromboplastin time (aPTT), prothrombin time (PT) and thrombin time, potentiating (Zhou et al., 2009). The anti-inflammatory effect of violets has been attributed to the presence of prostaglandin-inhibiting components such as salicylic acid. Antipyretic activity has also been demonstrated in the nonpolar fraction of *V. odorata* (Khattak et al., 1985). Different extracts (n-hexane, chloroform, aqueous) from the leaves of this violet species were investigated for their antipyretic effect in an experiment on rabbits with yeast-induced fever (Mahboubi et al., 2018). The analgesic effects observed in violet extracts may be due to inhibition of pain response receptors or pathways leading to inflammation (Mahboubi et al., 2018). The anti-inflammatory properties of this plant have been confirmed when used both internally and externally. Violets are used to treat skin conditions, pain, inflammation and burns. The anti-inflammatory activity of garden pansies when used topically in a burn injury model has been attributed to antioxidant flavonoid compounds, particularly rutin (Vukics et al., 2008). Experimental studies have shown that a thick extract of violet has a pronounced anti-inflammatory effect in arthritis. In the acute phase of bone marrow inflammation, experimental studies have shown that violet extracts have anti-inflammatory effects. The hyposensitizing property of violets have been established and it is therefore included in the composition of formulations for the treatment of allergic diseases. Due to bioactive cyclotides, extracts of *V. tricolor* have a pronounced immunosuppressive effect (Hellinger et al., 2014). Cyclotides are known to block T-lymphocyte proliferation by acting as immunosuppressive peptides (Gründemann et al., 2013). Recently, antitumor and cytostatic properties of violets have been identified (Zhang et al., 2021; Rudzińska et al., 2023). The antitumor properties of violets have been attributed to the presence of cycloviolacin (Goransson et al., 2004). Vigno 5, a natural cyclopeptide, has been identified from *V. ignobilis* and has shown an inhibitory effect on growing cervical cancer cells (Hashempour et al., 2011). Studies have shown that alcoholic extracts of wild pansy stimulate apoptosis of cancer cells and inhibit angiogenesis. The leaves of *V. odorata* have antidyslipidaemic and hypotensive effects. When taken internally, the violet strengthens the walls of blood vessels due to the pres-

ence of rutin and reduces the amount of cholesterol in the blood (Siddiqi et al., 2012). The plant has gastroprotective, antihepatotoxic and antinephrotoxic effects (Elhassaneen et al., 2013).

Once ingested, medicinal plants are known to interact with the gut microbiota, modulating its composition and metabolism. Certain active components of plants (hesperidin, baicalin, glycyrrhizin, ginsenoside, daidin) have a therapeutic effect due to bioconversion mediated by the gut microbiota (Chen et al., 2016; Feng et al., 2019; Illiano et al., 2020). Understanding the interaction between the gut microbiota and medicinal plants added to the diet will help to develop treatment protocols for various diseases, including chronic ones, by normalizing impaired functions through substances of plant origin. In experiments with laboratory animals fed a high-fat diet containing 5% crushed dry young shoots of *Salvia officinalis* and *Lavandula angustifolia*, significant changes in the quantitative ratio of *Escherichia coli* with normal and altered enzymatic properties were found in the intestinal contents (Bilan et al., 2023a). No information was found in the available scientific literature on the effect of violet herb or its individual components on the quantitative or qualitative composition of the gut microbiota in humans or experimental animals.

Therefore, the aim of our study was to determine the effect of dry *V. tricolor* on body weight gain, changes in the weight of internal organs, and blood biochemical parameters in white laboratory rats, as well as qualitative and quantitative indicators of their gut microbiota during a high-fat diet.

Materials and methods

Animals and experimental design. The research was carried out in accordance with the 'European Convention for the Protection of Vertebrate Animals used for Experimental or other Scientific Purposes' (Strasbourg, France, 18 March 1986, ETS No. 123) and the Law of Ukraine 'On Protection of Animals from Cruelty' (Kyiv, 21 July 2006, No. 3447-IV). Bioethical expertise of the conducted research was performed and approved by the Local Animal Experimental Ethics Committee of the Dnipro State Agrarian and Economic University (Decision No. 2/23-24 of 18.09.2023). The following were reviewed and approved: the design of the study, the number of animals in the experiment, the conditions of housing and feeding, sampling, and the method of withdrawal of animals from the experiment. The animals used in this study (white male crossbreed rats, 200–220 g) were housed in the vivarium of the Veterinary Medicine Faculty of the Dnipro State Agrarian and Economic University in a controlled environment (22–24 ºC) with free access to food and water. Three groups of 5 rats each were formed: control and two experimental groups. All rats consumed a high fat synthetic diet for 30 days. The experimental groups were supplemented with chopped *V. tricolor* herb in addition to the high fat diet; the first experimental group received a dose of 0.5% of the diet weight and the second experimental group received 2.0%.

Nutrition and medicinal herb. The synthetic diet was prepared on the basis of a complete basic diet consisting of a mixture of cereals (maize, sunflower seeds, wheat, barley, soya) 75%, root vegetables (carrots) 8%, meat and bone meal 2%, mineral and vitamin supplements 2%. To increase the fat content of the diet, 15% sunflower oil was used and added to the mixture of ground dry feed components and further pelleted (Levchuk et al., 2021). For the experimental groups, dry chopped *V. tricolor* herb (*Violae herba*), purchased in officinal form from a commercial pharmacy, was added to the synthetic diet at the stage of grinding the dry ingredients.

Body weight, food and water intake, euthanasia. The animals received food and water ad libitum. For 30 days, the amount of food and water consumed by the animals in each group was counted daily and throughout the experimental period. The weight of each animal was determined during and at the end of the experiment using an analytic weight (Metrixo AB224, China). At the end of the study (day 30), animals were euthanized by overdose anesthesia (80 mg/kg ketamine and 12 mg/kg xylazine, intraperitoneally) and heart blood samples were taken. At autopsy, the internal organs (heart, liver, lungs, thymus, spleen, stomach, intestines, kidneys) were examined for the presence of pathological changes, selected and weighed to an accuracy of 10 mg.

Blood analysis. Blood samples collected at euthanasia were used for complete blood count (CBC) and biochemical analysis. 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 analyser (Italy) using reagent kits from High Technology (USA), PZ Cormay S.A. (Poland) and Spinreact S.A. (Spain). 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.

Microbiological studies. For the study of qualitative and quantitative indicators of the gut microbiota, fecal samples were collected in sterile weighing bottles immediately after euthanasia on day 30 of the experiment. The intestine was cut and the contents were removed according to the rules of asepsis. Following the rules of asepsis and antisepsis, 1 g of feces was placed in a sterile weighing bottle and 9 mL of sterile saline solution was added (tenfold dilution (10^{-1}) was obtained). To prepare the required dilutions, 9 mL of sterile saline was used and 1 mL of the contents of the previous tube was added. This was repeated until a 10^{-9} dilution was obtained (Bilan et al., 2023b). After all dilutions were made, 0.1 mL of solution was removed from each tube with a sterile pipette and added to a Petri dish containing the appropriate selective medium, increasing each dilution by a further 10-fold. Bifidus medium (HiMedia, India), *Lactobacillus* agar, *Enterococcus* agar and Endo, bismuth sulphite, Wilson-Blair, Byerd-Parker, Sabouraud dextrose agar (Farmaktiv LLC, Ukraine), 5% blood agar (Biomerieux, France) were used. Cultivation was performed in a thermostat at 24–37–43 °C for 24–72 h. Colonies were counted in all medium plates. CFU/g (colony forming units per 1 g of gut contents) were counted and multiplied by the appropriate dilution (Bilan et al., 2023a). Anaerobic conditions for bifidobacteria, lactobacilli and clostridia were established in anaerobic jars (7 L) using GENbox anaeropackages (Biomerieux, France). Anaerobiosis was monitored using Anaer Indicator (Biomerieux, France). The identification and differentiation of the various species of enterobacteria were carried out by determining their enzymatic properties on Hiss, Olkenitsky, Christensen, Simons and other media, tests API 20 REF 20 600, API Staph REF 10 20 500, API 20 E REF 20 100 / 20 160, API 20 NE REF 20 050, API Candida REF 10 500 (Biomerieux, France), taking into account their biological properties according to Bergey's definition (1986). The morphological and staining characteristics of the isolated microorganisms were studied after Gram staining of the smears and microscopy using a MICROmedXS-3330 microscope (Bilan et al., 2023b).

Statistical analysis. Data were analyzed using Statistica 12.0 (StatSoft Inc., USA). The results are presented in the tables as $x \pm SE$ (mean ± standard error). Differences between the values of the control and experimental groups were determined using the Tukey test (with Bonferroni correction), and differences were considered significant at $P < 0.05$.

Results

When analyzing the changes in body weight of the experimental animals, it was found that the intensity of body weight gain was different in

rats fed only a high-fat diet and rats fed additionally with dried herb of *V. tricolor*. In the control group of animals (high fat diet), an increase in average body weight was observed from the third day of the experiment. The rats gained weight rapidly from day 3 to day 24, after which no increase in body weight was observed until day 27. Average body weight then decreased for the remaining three days (Fig. 1). In rats given *V. tricolor* dried herb at a dose of 0.5% in addition to the high-fat diet, weight gain started from the first days to 6 days of the experiment, after which there was a decrease in weight up to 14 days, followed by a gradual increase up to 27 days of the experiment. By the end of the experiment, a significant decrease in body weight was observed in both experimental and control rats (Fig. 1). In the group of rats receiving 2.0% dry herb *V. tricolor*, a sharp increase in body weight was observed from the first days of the experiment up to 6 days, after which the average body weight remained practically unchanged until 20 days of the experiment. During the following days (20–24 days), the rats showed a slight decrease in this parameter, with a sharp increase at the end of the experiment, which was higher in comparison with the control and the rats receiving 0.5% of the plant (Fig. 1).

Rats fed a high-fat diet increased their body weight by up to 121% of their initial weight. In groups of rats given 0.5% dried *V. tricolor* herb in addition to the high-fat diet, the increase in body weight was up to 102%, and up to 110% in groups given 2.0% dried *V. tricolor* herb.

Compared to animals fed the high-fat diet alone, the addition of *V. tricolor* dry herb to the diet did not significantly affect the amount of feed consumed, but resulted in a slight reduction in daily water consumption (Table 1).

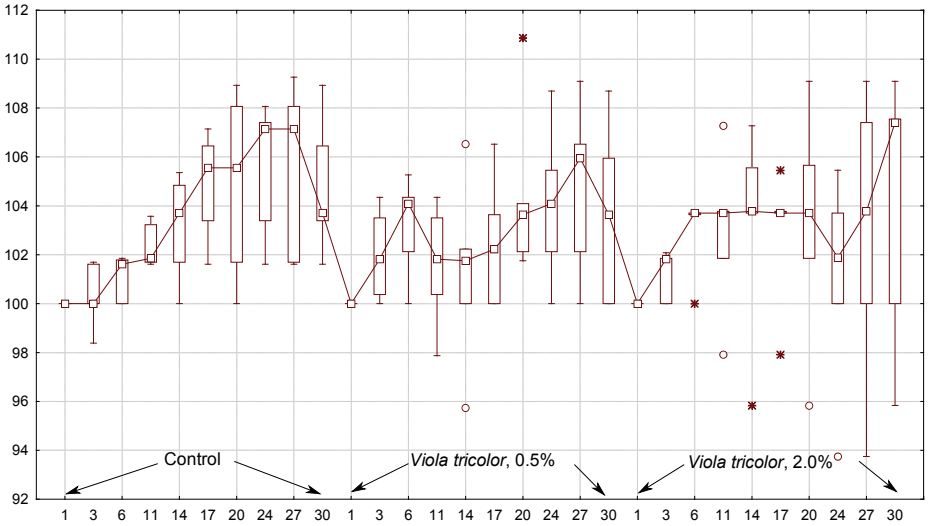

Fig. 1. Change in body weight of rats when crushed herb of *V. tricolor* was added to the animals' nutrition: abscissa – day of experiment, ordinate – body weight of animals (% relative to initial body weight of each animal, taken as 100% at the beginning of the experiment); small square – median, upper and lower borders of rectangle – 25% and 75% quartiles, vertical line – minimum and maximum values, circles and asterisks – outliers; n = 5

Table 1

Change in food consumption of young male rats under the influence of the supplementation of *Viola tricolor* to their diet (x ± SD, n = 5, duration of experiment – 30 days)

Parameter	Control	*V. tricolor, 0.5%*	*V. tricolor* compared to the control, %	*V. tricolor, 2.0%*	*V. tricolor* compared to the control, %
Consumption of food by animals, g/day	29.14	29.00	99.5	29.00	99.5
Consumption of water by animals, g/day	40.57	37.47	92.3	39.13	96.5

Note: different letters indicate values which reliably differed one from another (P < 0.05) within one line of the table according to the results of comparison using the Tukey test with Bonferroni correction.

The diet supplementation with 0.5% dry *V. tricolor* herb caused a significant decrease in the relative mass of the thymus (to 51.4% of the control group level), a sharp increase in the relative mass of the caecum and large intestine (to 173.6% and 117.8% of the control group level, respectively) and the brain (to 206.4% of the control group level). At the same time, the consumption of 2.0% dry *V. tricolor* herb resulted in an increase in the relative weight of the thymus and caecum (up to 183.1% and 270.7%, respectively) and a decrease in the relative weight of the colon (up to 71.3% of the control group level). It is noteworthy that the absolute length of the colon and rectum was significantly reduced in experimental rats consuming an additional 2.0% *V. tricolor* against the background of the reduction in body length (Table 2).

Supplementing the high-fat diet with dried *V. tricolor* resulted in altered blood biochemical parameters (Table 3). In the blood plasma of rats fed *V. tricolor* dried herb, total protein levels were significantly affected and the change was dose-dependent. Thus, 0.5% dried herb *V. tricolor* caused an insignificant decrease in the level of total protein (up to 96.2% of the level in the control group), whereas the dose of 2.0% caused an increase in this parameter by 7.3%. The increase in the level of total protein is attributable to the increase in the level of albumin in the rat's blood (24.7% increase) as a result of consuming 2.0% *V. tricolor*. At the same time, the dose of 0.5% *V. tricolor* of the diet weight caused a significant decrease in the level of blood globulins (up to 88.8% of the control group level). This was accompanied by a change in the albumin/globulin ratio in the experimental groups of rats. In rats receiving 0.5% *V. tricolor*, the albumin/globulin ratio increased by 17.6% compared to the control group, and in rats receiving 2.0% – by 32.4%. No changes were observed in the levels of urea and blood urea nitrogen, which are the end products of protein metabolism in the body. Evaluating the activity of blood enzymes, it was found that the addition of dry violet herb to the high-fat diet significantly reduced the activity of alkaline phosphatase, especially at a dose of 2.0% of the diet weight (up to 47% of the control group level). The activity of AST and ALT in the blood of the experimental animals did not change significantly compared with this parameter in the control group. However, it should be noted that the consumption of the medicinal plant caused an increase in the De Ritis ratio, especially at a dose of 2.0% *V. tricolor*. On the other hand, alpha-amylase and gamma-glutamyltransferase activities did not change (Table 3).

Consumption of dried *V. tricolor* by rats induced an alteration in lipid metabolism. This was manifested by a slight decrease in blood cholesterol levels in rats consuming 2.0% *V. tricolor*, while 0.5% *V. tricolor* significantly increased high-density lipoprotein (HDL) cholesterol levels by more than twice. Irrespective of the dose, violet herb contributed to the reduction of the plasma atherogenic index. Violet in high-fat diet did not affect creatinine, total bilirubin, glucose levels. Total calcium, inorganic phosphorus and their ratio did not change either.

Table 2

Change in relative organ mass (%) of male rats under the influence of the supplementation of their diet with *Viola tricolor* (x ± SD, n = 5, duration of experiment – 30 days)

Organ	Control	*V. tricolor*, 0.5%	*V. tricolor* compared to the control, %	*V. tricolor*, 2.0%	*V. tricolor* compared to the control, %
Heart	0.342 ± 0.050	0.299 ± 0.011	87.6	0.385 ± 0.061	112.7
Liver	2.57 ± 0.15	2.76 ± 0.17	107.4	2.68 ± 0.28	104.2
Lungs	0.714 ± 0.059	0.614 ± 0.162	86.0	0.708 ± 0.122	99.2
Thymus	0.094 ± 0.034	0.048 ± 0.022*	51.4	0.172 ± 0.060*	183.1
Spleen	0.208 ± 0.026	0.220 ± 0.035	105.7	0.241 ± 0.062	116.0
Stomach	0.532 ± 0.020	0.547 ± 0.116	102.8	0.587 ± 0.127	110.3
Small intestine	1.55 ± 0.33	1.87 ± 0.49	120.5	1.56 ± 0.09	100.7
Caecum	0.238 ± 0.039	0.413 ± 0.095**	173.6	0.644 ± 0.223***	270.7
Colon	0.471 ± 0.021	0.555 ± 0.080**	117.8	0.336 ± 0.069**	71.3
Right kidney	0.309 ± 0.017	0.289 ± 0.033	93.4	0.313 ± 0.067	101.3
Left kidney	0.316 ± 0.029	0.277 ± 0.020	87.5	0.309 ± 0.055	97.6
Brain	0.585 ± 0.030	1.207 ± 0.157***	206.4	0.646 ± 0.109	110.5
Length of small intestine, cm	122.6 ± 14.3	125.4 ± 6.9	102.3	118.0 ± 9.7	96.2
Length of colon and rectum, cm	20.2 ± 0.8	20.0 ± 2.2	99.0	14.2 ± 6.0*	70.3
Body length, cm	20.8 ± 0.8	19.0 ± 1.0	91.3	19.4 ± 0.5*	93.3

Note: * – P < 0.05, ** – P < 0.01, *** – P < 0.001.

Table 3

Changes in blood biochemical parameters of male rats under the effect of *Viola tricolor* supplementation (x ± SD, n = 5, experiment duration – 30 days)

Parameters	Control	*V. tricolor*, 0.5%	*V. tricolor* compared to the control, %	*V. tricolor*, 2.0%	*V. tricolor* compared to the control, %
Total protein, g/L	74.4 ± 2.6^{ab}	71.6 ± 1.7^{a}	96.2	79.8 ± 4.3^{b}	107.3
Albumins, g/L	30.0 ± 2.4^{a}	32.0 ± 1.0^{a}	106.7	37.4 ± 1.5^{b}	124.7
Globulins, g/L	44.6 ± 2.6^{a}	39.6 ± 1.3^{b}	88.8	42.6 ± 3.6^{ab}	95.5
Albumin/Globulin ratio, U	0.68 ± 0.08^{a}	0.80 ± 0.00^{b}	117.6	0.90 ± 0.10^{b}	132.4
Urea, mmol/L	5.16 ± 1.10^{a}	4.80 ± 0.99^{a}	93.0	5.68 ± 2.11^{a}	110.1
Blood urea nitrogen, mg/100 g	9.8 ± 2.1^{a}	9.2 ± 1.9^{a}	93.7	10.8 ± 4.0^{a}	110.4
Creatinine, µmol/L	46.8 ± 5.2^{a}	40.4 ± 6.3^{a}	86.3	43.8 ± 8.7^{a}	93.6
AST, U/L	154 ± 16^{a}	206 ± 31^{a}	133.2	248 ± 90^{a}	160.6
ALT, U/L	61.6 ± 11.9^{a}	42.8 ± 4.5^{a}	69.5	49.4 ± 16.5^{a}	80.2
De Ritis ratio (AST/ALT), U	2.60 ± 0.52^{a}	4.82 ± 0.64^{b}	185.4	5.02 ± 0.63^{b}	193.1
Alkaline phosphatase, U/L	386 ± 183^{a}	221 ± 68^{ab}	57.3	181 ± 53^{b}	47.0
Alpha-amylase, U/L	1458 ± 220^{a}	1656 ± 395^{a}	113.6	1476 ± 121^{a}	101.3
Total bilirubin, µmol/L	2.98 ± 0.34^{a}	3.12 ± 0.28^{a}	104.7	2.96 ± 0.45^{a}	99.3
Direct bilirubin, µmol/L	0.72 ± 0.13^{a}	0.80 ± 0.21^{a}	111.1	1.06 ± 0.28^{a}	147.2
Indirect bilirubin, µmol/L	2.40 ± 0.12^{a}	2.34 ± 0.27^{a}	97.5	1.92 ± 0.45^{a}	80.0
Glucose, mmol/L	5.02 ± 0.63^{a}	4.24 ± 0.65^{a}	84.5	5.02 ± 0.64^{a}	100.0
Total calcium, mmol/L	2.52 ± 0.08^{ab}	2.38 ± 0.11^{a}	94.4	2.60 ± 0.07^{b}	103.2
Non-organic phosphorus, mmol/L	2.16 ± 0.22^{a}	1.72 ± 0.28^{a}	79.6	1.74 ± 0.50^{a}	80.6
Ca/P	1.18 ± 0.15^{a}	1.42 ± 0.19^{a}	120.3	1.58 ± 0.44^{a}	133.9
Gamma-glutamyltransferase, U/L	3.0 ± 1.2^{a}	1.4 ± 0.5^{a}	46.7	3.4 ± 2.7^{a}	113.3
Cholesterol, mmol/L	1.96 ± 0.17^{a}	1.92 ± 0.12^{a}	96.9	1.65 ± 0.15^{a}	86.7
High-density lipoprotein (HDL) cholesterol, mmol/L	0.65 ± 0.14^{a}	1.45 ± 0.38^{b}	223.1	0.84 ± 0.15^{a}	129.2
Low-density lipoprotein (LDL) cholesterol, mmol/L	0.91 ± 0.56^{a}	0.57 ± 0.15^{a}	62.6	0.62 ± 0.09^{a}	68.1
Atherogenic index of plasma	1.98 ± 1.08^{a}	1.23 ± 0.87^{a}	62.1	1.34 ± 0.38^{a}	67.7

Note: see Table 1.

It was found that the absolute numbers of bacteria of the genera *Bifidobactrium*, *Lactobacillus* and typical *Escherichia coli* per unit weight of intestinal contents of all experimental animals were not affected by the addition of dry violet herb to the high-fat diet. In all groups of rats, representatives of these genera formed the basis of the isolated microorganisms. The quantitative composition of these bacteria was within the reference values of faecal biopsy samples from laboratory rats: the number of bifidobacteria in the experimental groups reached lg 8–9, *Lactobacilli* – lg 7–8 and typical *Escherichia coli* within lg 6 CFU/g (Table 5). Opportunistic enterobacteria of the genus *Citrobacter* and haemolytic strains of *Escherichia coli* were not detected in animals of either experimental or control groups.

Table 4

Number of microorganisms (lg KOE/g faeces) in the group of white rats fed a high-fat diet supplemented with *V. tricolor* (x ± SD, n = 5, 30-day experiment)

Gut microbiota	Reference range	Control (high-fat diet)	High-fat diet+ 0.5% *V. tricolor*	High-fat diet + 2.0% *V. tricolor*
Bifidobacterium spp.	10^7–10^9	9.25 ± 0.19^{a}	9.20 ± 0.37^{a}	8.60 ± 0.24^{a}
Lactobacillus spp.	10^5–10^8	7.77 ± 0.23^{a}	7.86 ± 0.21^{a}	7.78 ± 0.06^{a}
Escherichia coli typical	10^6–10^8	6.28 ± 0.14^{a}	6.34 ± 0.48^{a}	5.91 ± 0.23^{a}
Escherichia coli weakly fermentative	<25%	4.39 ± 0.13^{a}	3.54 ± 0.41^{a}	3.35 ± 0.06^{b}
Escherichia coli lactose-negative	10^2	1.90 ± 0.49^{a}	3.12 ± 0.19^{a}	2.55 ± 0.22^{a}
E. coli (haemolytic)	0	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Enterococcus spp.	10^5–10^7	5.33 ± 0.10^{a}	1.56 ± 0.98^{b}	0.00 ± 0.00^{c}
Enterobacter spp.	10^2–10^4	3.29 ± 0.12^{a}	2.62 ± 0.74^{a}	2.28 ± 0.58^{a}
Citrobacter spp.	10^2–10^4	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}	0.00 ± 0.00^{a}
Klebsiella spp.	10^2–10^4	4.31 ± 0.03^{a}	5.69 ± 0.31^{b}	1.42 ± 0.59^{c}
Proteus spp.	10^2–10^4	4.47 ± 0.02^{a}	4.32 ± 0.04^{a}	4.32 ± 0.04^{a}
Staphylococcus epidermidis	10^2–10^4	3.81 ± 0.13^{a}	5.37 ± 0.19^{b}	5.65 ± 0.16^{b}
Staphylococcus aureus	10^3–10^5	4.17 ± 0.09^{a}	4.63 ± 0.11^{ab}	5.05 ± 0.10^{b}
Candida spp.	10^2–10^5	4.36 ± 0.02^{a}	5.21 ± 0.24^{b}	4.61 ± 0.14^{ab}
Clostridium spp.	10^2	1.95 ± 0.03^{a}	4.53 ± 0.01^{b}	0.71 ± 0.44^{a}

Note: see Table 1.

Based on this, it was found that the addition of 0.5% violet herb to the diet led to an increase in the number of *Klebsiella* spp., *Staphylococcus epidermidis* and *S. aureus* ($P < 0.05$), and 2% of the herb resulted in a decrease in the number of weak-fermenting *Escherichia coli* and *Klebsiella* spp. ($P < 0.05$), but had no effect on *Staphylococcus epidermidis* and *S. aureus* ($P < 0.05$). Compared to animals consuming only the high-fat diet, the number of weak-fermenting form of *E. coli* decreased by 1.30 times. The number of *Enterococcus* spp. decreased sharply in the presence of 0.5% violet herb in the high-fat diet and disappeared at 2% herb concentration ($P < 0.05$). After an increase in the number of *Candida* spp. and *Clostridium* spp. in the faecal biopsy of the group fed 0.5% dried violet herb, a non-significant decrease in the number of these microorganisms was observed in the group fed 2% of this herb in the diet.

Discussion

The problem of obesity is one of the most important issues of our time, with numerous adverse consequences, including atherosclerosis and cardiovascular disease, type 2 diabetes and nervous system disorders (Parkinson's disease, etc.). The main cause of obesity is an unbalanced diet, in particular an excess of fats and carbohydrates with a lack of proteins. Experimental studies in recent years have shown that the pathogenesis of the metabolic syndrome, even in the context of a high-fat diet, is always accompanied or largely determined by changes in the gut microbiome. According to some authors, restoring the normal intestinal microbiome in metabolic syndrome is one of the most promising areas of prevention and treatment of obesity, both from the point of view of one of the main causes of this medical problem and from the point of view of correcting the complex of metabolic disorders consequences (Koleva et al., 2015; Al-Muzafar, & Amin, 2017). 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 dyslipidaemia (Adel Mehraban et al., 2021) and insulin resistance (Tran et al., 2020). Species used in pharmacopoeia: *V. arvensis* and *V. tricolor* are mainly used as expectorant and diuretic. These properties are due to polysaccharides, phenolic compounds (flavonoids, phenolic acids), saponins. Recently, however, the dyslipidaemia effect of violet has been reported in the scientific literature. Siddiqi et al. (2012) observed a reduction in total cholesterol and high-density lipoprotein levels in diet-induced atherogenic rats treated with a hydromethanol extract of violet leaves. In our experiment, in rats fed a high-fat diet for 30 days, cholesterol levels were within the reference range for healthy animals, and the addition of *V. tricolor* dry herb to the diet did not cause a significant change in this parameter (Ihedioha et al., 2011). However, analysis of other indices of lipid metabolism showed an increase in high-density lipoprotein (HDL) cholesterol and a decrease in low-density lipoprotein (LDL) cholesterol in the blood of rats fed the herb compared to rats fed the high-fat diet alone. This led to a decrease and atherogenic index in rats on the background of eating dried *V. tricolor* herb. The atherogenicity index is an important index that describes the risk of coronary heart disease (Khosravi et al., 2022). A decrease in the atherogenicity index was observed with the administration of violet plant extract, based on the results, the pharmacological use of *V. odorata* in the treatment of lipid disorders and hypertensive conditions (Batiha et al., 2023). Inhibition of pancreatic lipase activity by saponins has been reported to reduce dietary fat absorption and increase fat disposal in mice fed a high-fat diet (Han et al., 2002).

High-fat diets are mainly used to model obesity. Excessive fat consumption leads to increased liver stress. In animals fed a high-fat diet (from 15 to 65%) for a long period of time, and especially when carbohydrates are added, the liver shows accumulation of steatosis, inflammation, fibrosis and cirrhosis. Morphological abnormalities are preceded by changes in functional parameters, primarily an increase in plasma ALT activity (Levchuk et al., 2021). In our experiment, we did not observe a significant increase in body weight or relative liver weight in rats fed a high-fat diet (15% vegetable fat of diet weight) for 30 days (Table 2). At the same time, the excess fat in the diet caused changes in functional blood parameters used to assess liver function. For example, AST, ALT, alkaline phosphatase and alpha-amylase activities in the blood of rats consuming a high-fat diet were greatly increased compared to the reference values for this type of animal (Shayakhmetova et al., 2020). Despite the existence of scientific data on the antihepatotoxic effect of violets, in our experiment, the addition of the dry herb *V. tricolor* to the high-fat diet did not improve these parameters. Thus, against the background of consumption of dry herb *V. tricolor* as part of a high-fat diet, there was a sharp increase in blood AST activity compared to the control group, but at the same time there was a decrease in ALT activity. *Viola tricolor* also caused a less pronounced increase in alkaline phosphatase activity, and alpha-amylase activity increased at the dose of 0.5% dry herb. The change in the De Ritis ratio (AST/ ALT) in the blood of experimental rats attracts attention. It is known that the AST/ALT ratio is the ratio of the concentrations of the two enzymes aspartate transaminase (AST) and alanine transaminase, it is used as one of several tests of liver function and is measured by blood tests. It is sometimes useful in the medical diagnosis of elevated transaminases to differentiate causes of liver damage or hepatotoxicity (Anchinmane & Sankhe, 2023). Most causes of liver cell damage are associated with a more pronounced elevation of ALT than AST, but an AST/ALT ratio of 2:1 or higher indicates liver disease, especially in the setting of elevated gamma-glutamyltransferase levels. In our experiment, we observed a significant increase in the De Ritis ratio (AST/ALT) in the blood of rats treated with dried violet herb. The decrease of Gamma-glutamyltransferase activity practically by two times against the background of 0.5% dry violet herb consumption compared to the control group of animals also attracts attention. It is well known that an elevated AST/ALT ratio may indicate liver disease, in particular non-alcoholic steatohepatitis. But also a condition where AST is higher than ALT can be considered as a muscle source of these enzymes (Anchinmane & Sankhe, 2023). It has been reported that animal diet supplemented with flower powder of *V. odorata* (dose 0.2–1.6 g/100 g) caused a decrease in serum AST, alkaline phosphatase and ALT activities (Elhassaneen et al., 2013).

Several publications have reported antinephrotoxic effects of violets (Batiha et al., 2023). In an experiment conducted on rats with kidney damage by administration of carbon tetrachloride (CCl_4), a decrease in blood concentrations of some kidney markers such as urea and creatinine was observed (Elhassaneen et al., 2013). The decrease in blood creatinine and uric acid concentrations on the background of *V. odorata* consumption may be due to the high content of phytochemicals, especially polyphenols. There are reports explaining that flavanone protects and maintains renal function by reducing serum urea and creatinine concentrations, excessive urination leading to loss of sodium ions, and improving body weight (Badary et al., 2005; Batiha et al., 2023). In the present study, relative kidney weight and blood creatinine levels were not significantly different or outside the reference range in rats consuming the high fat diet and dried *V. tricolor* herb. At the same time, the concentration of urea in the blood of the animals was also not significantly different between the experimental and control groups, but it should be noted that in rats receiving 0.5% of the plant, this index was slightly lower than the reference values. Blood urea nitrogen was also elevated in rats fed a high-fat diet and in rats given *V. tricolor* dried herb in addition to the reference levels, which requires further investigation.

In previous studies on the effect of medicinal plants on body weight gain, we found that *Helichrysum arenarium*, *Salvia sclarea*, *Origanum vulgare*, *Inula helenium* and *Vitex angus-castus* added to a high-fat diet promoted more active body weight gain in animals (Lieshchova & Brygadyrenko, 2021, 2022, 2023b, 2023c; Lieshchova et al., 2021, 2023). Meanwhile, consumption of *Scutellaria baicalensis*, *Matricaria chamomilla*, *Lavandula angustifolia*, *Melissa officinalis*, *Salvia officinalis*, *Rhodiola rosea*, *Punica granatum* by rats in addition to high-fat diet reduced body weight gain (Lieshchchova & Brygadyrenko, 2021, 2023a, 2023b; Lieshchova et al., 2021, 2023). In the present experiment, the supplementation with dry *V. tricolor* herb at a dose of 0.5% of the diet weight did not affect the intensity of body weight gain in the experimental animals, whereas 2.0% of dry herb by 30 days promoted a strong increase in body weight (Fig. 1).

In our opinion, the change in the relative mass of the thymus in rats consuming *V. tricolor* dried herb as part of a high-fat diet should be considered as a manifestation of the violet immunomodulatory activity, which

is associated with the presence of cyclotides (natural cyclic peptides) in this plant (Ireland et al., 2006; Hashempour et al., 2013; Aslam et al., 2021). Recently, cyclotides have been reported to block T-lymphocyte proliferation by acting as immunosuppressive peptides (Gründemann et al., 2013). Similarly, the cytotoxic effect of *V. tricolor* is associated with the presence of the three major cyclotides (Tang et al., 2010). At the same time, the changes in the relative mass of the colon sections should be considered in relation to the state of the intestinal microbiome.

The antibacterial effect of plant extracts of *Violae herba* is supported by the results of research carried out by scientists from all over the world. Witkowska-Banaszczak et al. (2005) found antimicrobial activity of infusion, decoction and alcoholic extract of *V. tricolor* (Violaceae) with significant inhibitory effect against *Staphylococcus aureus*, *Bacillus cereus*, *Staphylococcus epidermidis* and *Candida albicans* and moderate activity against *Pseudomonas aeruginosa*, *Enterococcus faecalis*, *Escherichia coli* and *Klebsiella pneumoniae*. Pränting, et al. (2007) reported the bactericidal activity of the cyclotide cycloviolacin O_2 isolated from *V. odorata* against gram-negative *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*. Tobyn et al. (2011) found that *V. yedoensis* is used in China for the treatment of acute skin infections. Zarrabi (2013) found that 1.6 mg/mL of semi-purified *V. odorata* cyclotides was effective against *Staphylococcus aureus*. According to Panni & Bakht (2018), *Xanthomonas campestris*, *Bacillus subtilis* and *S. aureus* were most susceptible to the butanol-extracted fraction of *Viola pilosa* root and the ethyl acetate-extracted extract at a concentration of 2.0 mg/disc was effective against *Pseudomonas aeruginosa* and *Escherichia coli*. Yousuf et al. (2021) investigated the effect of *Viola patrinii* essential oil against some gram-positive and gram-negative bacteria. The authors found that the oil had an inhibitory effect of about 6.62 mm against *S. aureus* at a concentration of 250 µg/mL and about 16.37 mm against *E. coli* at a concentration of 1000 µg/mL. Studies by Ali et al. (2023) show that the ethanolic extract of *V. odorata* flowers showed an inhibition zone of 18 mm against *P. aeruginosa* and 7 mm against *B. subtilis*; the ethanolic extract of *V. odorata* leaves inhibited the growth of *B. subtilis* by only 5 mm and *P. aeruginosa* by 4 mm, but did not inhibit *E. coli*. In addition, only the aqueous extract of *V. odorata* flowers showed antibacterial activity against *B. subtilis* with an inhibition zone of 3 mm. Lian et al. (2024) found the minimum inhibitory concentrations (MICs) of *Viola japonica* extract against *Acinetobacter baumannii* and *Bacillus subtilis* to be 4.2 and 2.1 µM, respectively. This study found that the addition of 2% *Viola tricolor* L. to a high-fat diet was most effective against intestinal pathogens: weak-fermenting *E. coli*, *Klebsiella* spp.

Conclusions

Consumption of *V. tricolor* dried herb as part of a high-fat diet for 30 days influenced the intensity of body weight gain, metabolic parameters and caused qualitative and quantitative changes in the intestinal microbiome of model animals. Consumption of 2.0% dried *V. tricolor* increased the rate of body-weight gain, and at the end of the experiment the relative weight of the thymus and caecum increased, but the relative weight of the colon and the length of the colon and rectum decreased. A dose of 0.5% dry herb of *V. tricolor* caused a significant increase in the relative weight of the brain, caecum and colon, while the relative weight of the thymus decreased compared to the control group.

The introduction of dried *V. tricolor* herb into the diet caused a dose-dependent change in blood biochemical parameters. In protein metabolism, 0.5% dried herb caused a decrease in total protein (by 3.8%), globulin level (by 11.2%) and an increase in albumin/globulin ratio (by 17.6%), and 2.0% dried herb caused an increase in total protein (by 7.3%), albumin level (by 24.7%), albumin/globulin ratio (by 32.4%) and a decrease in globulin level (by 4.5%) compared to rats fed a high fat diet. In terms of lipid metabolism, the addition of 0.5% dry herb of *V. tricolor* increased high-density lipoprotein (HDL) cholesterol and 2.0% dry herb of *V. tricolor* decreased cholesterol levels, and both doses of violet contributed to a decrease in the atherogenic index of plasma. In addition, both doses of violet caused a strong and significant increase in the De Ritis ratio and a decrease in the activity of alkaline phosphatase.

The addition of 0.5% and 2.0% of violet herb to the high-fat diet favours the support of the quantitative composition of the main intestinal microflora of laboratory rats (bacteria of the genus *Bifidobacterium*, *Lactobacillus* and *Escherichia coli*). It was found that 2.0% violet herb in the diet had a bacteriostatic effect on weakly fermentative *Escherichia coli* and *Klebsiella* spp. and a bactericidal effect on *Enterococcus* spp. which are capable of disrupting normal intestinal functions and causing disease.

The results presented in this study add to the growing body of literature on the multipharmacological activity of *V. tricolor*, which can be applied to the development of biological products for the treatment of metabolic diseases.

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