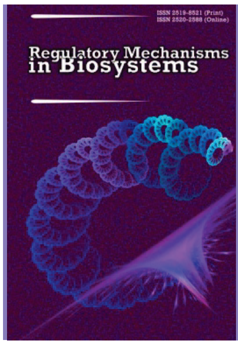

Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.,
2024, 15(4), 992–999
doi: 10.15421/0224146

Effect of *Sambucus nigra* inflorescence supplementation on the organism of rats fed a high-fat diet

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Article info

Received 04.09.2024
Received in revised form 01.10.2024
Accepted 16.10.2024

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Lieshchova, M. A., & Brygadyrenko, V. V. (2024). Effect of Sambucus nigra inflorescence supplementation on the organism of rats fed a high-fat diet. Regulatory Mechanisms in Biosystems, 15(4), 992–999. doi:10.15421/0224146

Medicinal plants are interesting as mild and effective therapeutic agents for treating and correcting metabolic disorders caused by an unbalanced diet. *Sambucus nigra* has antioxidant and anti-inflammatory properties, which are used to alleviate cardiovascular, neurodegenerative and inflammatory diseases. It also has antidiabetic, anticancer, antiviral and immunostimulant properties. The effect of adding crushed *S. nigra* inflorescences to the diet of rats was modelled under experimental laboratory conditions. For this purpose, three groups of 5 animals each were formed from white male laboratory rats that were fed a high-fat diet (15% vegetable fat) for 35 days, with the addition of 0.5% and 2.0% dry crushed *S. nigra* inflorescences. The overall effect of introducing the plant in the high-fat diet on body weight gain, the relative weight of some internal organs and the state of metabolic processes in the model animals was assessed. In the high-fat diet group, body weight increased to 108% of baseline at the end of the experiment. Supplementing the diet with *S. nigra* inflorescences at a dose of 0.5% promoted an increase in body weight gain (up to 112% of the initial weight), and a dose of 2.0% caused a slowdown and even a decrease in body weight at the end of the experiment. A high-fat diet supplemented with 2.0% of *S. nigra* inflorescences significantly increased the relative weights of liver, lung and spleen, whereas 0.5% of the plant did not cause significant changes in these indices. The addition of 2.0% of *S. nigra* inflorescences to the diet of animals significantly reduced blood glucose concentration. Both doses reduced the level of low-density lipoprotein (LDL) cholesterol in the blood, and the 2.0% dose also increased the level of high-density lipoprotein (HDL) cholesterol, while the amount of total cholesterol in the blood did not change and the atherogenicity index decreased. The addition of *S. nigra* inflorescences to the high-fat diet did not cause changes in protein and mineral metabolism, but did affect bilirubin metabolism, especially the levels of direct and indirect bilirubin. Both doses of elderflower caused a significant decrease in alpha-amylase activity, ALT activity and a strong significant increase in the De Ritis ratio. The results show the possibility of using dried *S. nigra* inflorescences as a dietary supplement in unbalanced diets to correct possible metabolic disorders.

Keywords: sambuci nigrae flores; relative organ mass; increase in the body weight; trifid bur-marigold; high-fat diet; phytotherapy; obesity correction.

Introduction

Metabolic disorders such as dyslipidemia and diabetes are multifactorial, caused by a combination of genetic predisposition and environmental factors such as diet and lifestyle. Obesity, which occurs as a result of metabolic changes at the organ and tissue level, leads to an imbalance between energy intake and energy expenditure, resulting in increased lipid accumulation in adipose tissue. Over time, these metabolic abnormalities can cause vascular complications leading to dysfunction of vital organs with fatal outcome or significant reduction in quality of life. Medicinal plants and their derivatives have always been used to treat diseases caused by metabolic disorders. They are usually less toxic, less expensive than synthetic drugs and, importantly, environmentally friendly (Dimitrov et al., 2019; Saad et al., 2022).

The genus *Sambucus* L. belongs to the family Adoxaceae and comprises 25 species distributed in temperate and subtropical regions of both hemispheres. The most studied and popular species are the black elderberry (*Sambucus nigra* L.), the red elderberry (*Sambucus racemosa* L.) and the dwarf elderberry (*Sambucus ebulus* L.). The black elderberry is a shrub or small tree whose fruits, flowers and roots are used for medicinal purposes. The height of the plant reaches 3–10 m, the diameter of the trunk up to 30 cm. Inflorescences of *S. nigra* L. have a flattened shape, with an apex and five ray flowers, milky-white in color, with five yellow stamens, with a weak, aromatic fragrance and a slightly bitter flavor (Zawiślak et al., 2022). The ripe berries are spherical, purple-black in color and contain 2 to 4 brown seeds. Various parts of the plant are used as medicinal raw materials. However, the fruits and flowers of the black elderberry are the most widely used as a source of medicinal raw materials and in the food industry. Black elderberry flowers are listed in the pharmacopoeia and their chemical composition consists mainly of polyphenolic compounds such as flavonoids, hydroxycinnamic acids and triterpenes (Młynarczyk et al., 2020). Less commonly, elder bark, young branches and leaves are used in the pharmaceutical and food industries. *Sambucus nigra* L. is rich in nutrients such as proteins, carbohydrates, fats, fatty and organic acids, essential oils, vitamins and minerals (Młynarczyk et al., 2018; Imenšek et al., 2021; Osman et al., 2023). In Europe, elderberry has been intensively used for centuries, both in the food industry for the production of pies, jellies, jams, ice cream, yoghurts and various alcoholic beverages, and in folk medicine for the treatment of various diseases (Senica et al., 2016; Kolesarova et al., 2022; Gentscheva et al., 2022).

The fruits of the black elderberry contain a variety of biologically active compounds, such as anthocyanins, flavonols, oxycinnamic acids, proanthocyanidins, vitamin C, terpenes and lectins (Papagrigoriou et al., 2023). The medicinal properties of black elderberry are largely attributed to the presence of phenolic compounds in its fruits, flowers and leaves, which have high antioxidant activity (Dawidowicz et al., 2006; Haş et al., 2023). Polyphenols and lectins give elderberry fruits the ability to inhibit coronaviruses, which is a topic of great interest nowadays (Mocanu & Amariei, 2022).

Elderflower infusions were experimentally found to contain more phenolic compounds than elderberry infusions. Among the quantified plant metabolites, Q and M were found in the highest concentrations in teas prepared from elderberry and elderflower, respectively (Viapiana & Wesolowski, 2017). In the aqueous extract of elderberry inflorescences obtained at 90 °C, 46 compounds were identified as quercetin and chlorogenic acid derivatives, representing 86% of the total phenolic compounds in the hydrophilic fraction, while naringenin (27.2%) was the major compound present in the lipophilic fraction (Ferreira-Santos et al., 2021). The predominant phenolic acids in elderberry fruit extracts were chlorogenic acid, sinapic acid and t-cinnamic acid, and the mean contents of rutin and quercetin were 1105.39 and 306.6 mg/g extract, respectively (Przybylska-Balcerek et al., 2021). In elderberry (*Sambucus nigra* L.), more than 20 ribosome-inactivating proteins (RIPs) and related lectins have been isolated and characterized from flowers, seeds, fruits and bark (Iglesias et al., 2022).

A limiting factor in the use of elderberry is the high content of the toxic glycoside sambunigrin in its bark, leaves, roots, fruits and especially seeds, which breaks down into benzaldehyde and hydrogen cyanide when metabolized in the human body. Elderberry leaves contain more sambunigrin than the flowers, and the smallest amount of this compound is found in the berries. Elderberry and elder flowers are also widely used in folk medicine (Uncini Manganelli et al., 2005). Consumption of elderberry juice may be beneficial in the prevention of a number of diseases such as cancer, inflammatory and cardiovascular diseases and diabetes (Fazio et al., 2013; Vujanović et al., 2020).

The antioxidant potential of the elder infusions, assessed by DPPH and FRAP assays, showed that the teas prepared from flowers had higher mean DPPH and FRAP activities than those prepared from berries, which was confirmed by the significant relationships obtained between the antioxidant properties and TPCs and TFCs (Viapiana & Wesolowski, 2017). The flowers of *S. nigra* L. contain the highest amount of phenolic compounds compared to fruits and leaves, which accounts for the highest antioxidant activity among all parts of this plant. Phenolic acids, especially p-coumaric acid (p-CouA), which is derived from cinnamic acid, have shown high biological activity, antioxidant properties, ability to neutralize free radicals (reactive oxygen species) and anti-inflammatory properties (Mozaffari Godarzi et al., 2020). Anthocyanins and polyphenols are also responsible for the antioxidant properties of black elderberry fruit extracts. These compounds increase the activity of enzymes in the small intestine, liver and lung, including peroxidase, S-transferase, glutathione reductase and catalase, as shown in in vitro studies (Pascariu & Israel-Roming, 2022). The biological effects and molecular mechanisms of black elderberry in neurodegeneration were investigated in a human neuroblastoma cell line (SH-SY5Y) using various *in vitro* approaches. *Sambucus nigra* flowers have been shown to regulate mTORC1 signalling activity and reduce oxidative stress through activation of autophagy/mitophagy flux (Palomino et al., 2021). Elderberry extract also possesses nephroprotective activity against lipoperoxidation, as shown in a study by Ungur et al. (2022) in a gentamicin model. Rutin, caffeic acid and ferulic acid from elderberry extract were shown to be responsible for reducing lipid peroxidation.

A study of *S. nigra* fruit/flower extracts and various polyphenols on lipopolysaccharide (LPS)-stimulated RAW 264.7 macrophages showed high anti-inflammatory activity. Both elderberry juice and extract inhibited the secretion of pro-inflammatory factors by macrophages (Ho et al., 2017). Quercetin isolated from elderberry showed high anti-inflammatory potential. It inhibits the secretion of pro-inflammatory cytokines (TNF-α and interleukins (IL-1β, IL-6), promotes the reduction of cyclooxygenase and lipoxygenase activity (Ahmed et al., 2018). The inhibitory effect of *S. nigra* fruit extracts on proinflammatory release was assessed using LPS-stimulated macrophages (RAW 264.7), confirming anti-inflammatory activity (Ferreira et al., 2022). Oral administration of elderberry inflorescence extracts at different doses was studied in a mouse model of carrageenan-induced inflammation. Inhibition of neutrophil migration, reduced pro-inflammatory cytokines (TNF, IL-1β and IL-6), reduced neutrophil reactive nitrogen species, as well as increased anti-inflammatory IL-10 and neutralized CD62L and CD18 expression were demonstrated. *Sambucus nigra* flower extracts have been shown to exert anti-inflammatory activity by modifying macrophage and neutrophil proinflammatory cytokine secretion (Santin et al., 2022). Rutin, which has antioxidant properties (reducing levels of the pro-inflammatory markers tumor necrosis factor-α, interleukin (IL)-6, cyclooxygenase-2 and IL-1β), plays an important role in the anti-inflammatory activity of elderberry (Muvhulawa et al., 2022). One of the pathways of anti-inflammatory activity is the presence of kaempferol in elderberry, which is able to inhibit the activity of COX1 and COX2 enzymes, thus preventing the development of inflammation (Lee, & Kim, 2010). The anti-inflammatory effect of kaempferol is also due to the inhibition of nitric oxide synthesis and the inclusion of kaempferol in the treatment regimen of diabetic neuropathy decreased nitric oxide levels and the amount of IL-1B, TNF-α (Abo-Salem, 2014; Sharma et al., 2019). The antioxidant, anti-inflammatory and protective effects of black elderberry fruit extract and its enzyme obtained by fermentation with kombucha tea mushroom were evaluated in an experiment to assess the viability and metabolism of skin fibroblast and keratinocyte cells. Both *S. nigra* extract and its kombucha enzyme were shown to be effective in preventing free radical-induced cell damage and to have a positive effect on skin cell health (Wójciak et al., 2023). Polyphenolic components (flavonoids and phenolic acids) of black elderberry flower and fruit extracts showed marked and dose-dependent anti-inflammatory activity *in vivo*, as assessed by the cotton pellet induced granuloma test (Seymenska et al., 2024).

Numerous scientific publications have investigated the anti-tumor activity of this medicinal plant, as the flowers and fruits of *S. nigra* are rich in biologically active compounds, primarily antioxidants (Chowdhury et al., 2017; Khorsandi et al., 2017; Khan et al., 2021; Tezerji et al., 2022; Stępień et al., 2023). Flavonoids identified by chemical analysis from the butane fraction of flower extracts of *S. nigra* showed cytotoxic effects on human bladder carcinoma T24 cells (Periera et al., 2020). An alcoholic extract of elderberry flowers affected the proliferation of a breast cancer cell line (MCF7) (Schröder et al., 2016). Among the active ingredients of elder, kaempferol has gained the most interest for its anti-cancer properties. *In vitro*, the effect of kaempferol on human pancreatic cancer cell lines (MIA PaCa-2 and PANC-1) was investigated and its anticancer properties were shown to be due to its ability to induce cancer cell apoptosis in a manner dependent on regulating reactive oxygen species levels (Wang et al., 2021). Further studies showed that incubating cancer cells with kaempferol very strongly reduced their viability as well as inducing them to apoptosis and inhibiting their migration. This was shown in several cancer cell lines (ovarian (A2780), lung (H460), skin (A431), pancreatic (MIA PaCa-2), prostate (DU145), colorectal adenocarcinoma (HT29), breast (MCF-7), neuroblastoma (BE2-C) and glioblastoma (U87) (Pham et al., 2018; Stępień et al., 2023). In addition to quercetin, rutin also has cytotoxic activity, as shown on the growth of leukemia cells (HL-60) (Araújo et al., 2013). Rutin also has antioxidant, antibacterial, anti-inflammatory and UV-filtering effects on the skin. Triterpenoid compounds, especially ursolic and oleanolic acids, are abundant in *S. nigra* flowers. Due to their antioxidant properties, they have various pharmacological effects and influence on the immune system. Both acids reduce the viability of human liver cancer cells (HepG2, Hep3B, Huh7 and HA22T) by inducing their apoptosis (Yan et al., 2010). *In vitro*, ursolic acid exhibits anticancer properties against breast cancer cells (T47D, MCF-7 and MDA-MB-231) (Luo et al., 2017). p-Cumaric acid (p-CouA) also exhibits anticancer activity, namely the ability to induce apoptosis and block the cell cycle, suppressing cell growth of many cancer cell lines (Pei et al., 2016; Tehami et al., 2023). Chlorogenic acid, which is also present in significant amounts in elderberry extracts, affects proliferation and suppresses angiogenesis and metastatic growth of cancer cells. Its cytotoxic activity against human hepatocarcinoma cells (HepG2), colon cancer cells (HT-29), breast cancer cells (MCF-7), human lung cancer cells (A549) and human leukemia cells (U9370) has been demonstrated (Wang et al., 2020; Zeng et al., 2020). The anticancer activity of *S. nigra* fruits is mainly attributed to anthocyanins and polyphenols. The ability of anthocyanins to neutralize free radicals and reduce oxidative stress influences their anti-inflammatory and anticancer properties. *In vitro* studies have shown that cyanidin-3-glucoside induces apoptosis of HER2-positive breast cancer cells, as well as a significant reduction in tumor size and volume (Liu et al., 2013). Ferreira-Santos et al. (2021) evaluated the antiproliferative effect of an aqueous extract of *S. nigra* inflorescences on the colon cancer cell lines RKO, HCT-116, Caco-2, and the antigenotoxic potential of the extract was assessed using the Comet assay in RKO cells (Ferreira-Santos et al., 2021).

Elderberry extracts were shown to have antimicrobial activity. Of the compounds analyzed, apigenin, kaempferol and ferulic, protocatechuic and p-coumarin acids had the greatest influence on the high antibacterial activity of the elderberry extracts. *Micrococcus luteus*, *Proteus mirabilis*, *Pseudomonas fragi* and *Escherichia coli* were most sensitive to the extracts. And extracts at concentrations of 0.5-0.05% were highly active (Przybylska-Balcerek et al., 2021). An aqueous extract of elderflower showed antimicrobial activity against gram-positive bacteria, especially *Staphylococcus aureus* and *S. epidermidis* (Ferreira-Santos et al., 2021). Elderberry also has antiviral properties due to its ability to modulate inflamematory cytokines. While elderberry is suitable for the prevention and initial treatment of viral diseases, its use is limited due to its ability to overstimulate the immune system and increase the risk of cytokine storm (Asgary & Pouramini, 2022). *Sambucus nigra* extract suppresses infectious bronchitis virus at an early replication stage, as demonstrated in an experiment in which cells and virus were pre-treated with the extracts and then infected in the presence of the extract. Viral cytopathic effect was assessed visually after 24 h incubation. Treatment with *S. nigra* extracts reduced virus titers by four orders of magnitude at a multiplicity of infection (MOI) of 1 in a dose-dependent manner (Chen et al., 2014). In an experiment on dendritic cells (DC), which are essential cells for the induction of potent T-cell responses, polysaccharides from the aqueous fraction of elderberry showed immunomodulatory effects, providing the basis for a strong immune-mediated response to viruses, including influenza (Stich et al., 2022).

The ability of elderberry extract and its individual components (rutin, anthocyanins, agglutinin) to stimulate steroid hormones and their receptors in the ovaries and to influence embryogenesis in general has been reported. These findings indicate the potential usefulness of black elderberry extract as a bio stimulant of female reproductive processes, as well as its potential applicability in the treatment of ovarian cancer and other reproductive disorders (Kolesarova et al., 2022). The study by Baldovska et al. (2021) demonstrated the potential of elderberry extract to regulate steroidogenesis in ovarian cells.

Elderberry flowers and fruits are thought to have analgesic, antiviral, antipyretic, expectorant, antifungal and antidiuretic properties. Many pharmacological effects are attributed to the presence of anthocyanins, a class of polyphenols belonging to the flavonoid family. They are dietary bioactive compounds whose consumption is inversely associated with inflammation and insulin resistance in humans (Jennings et al., 2014; Raafat & El-Lakany, 2015). Cyanidin-3-glucoside, a major natural anthocyanin, improves adipocyte function and protects adipocytes from metabolic stress in vitro by increasing PPARγ activity and inhibiting forkhead box O1 (Scazzocchio et al., 2011). In an obese mouse model, black elderberry extract reduced liver weight after 16 weeks, produced markers of reduced fatty acid synthesis in the liver, indicating decreased lipid synthesis in general, and lowered blood insulin levels. The authors suggest that the extract studied improves metabolic disorders in an obese mouse model, mainly by reducing serum TAG levels, markers of inflammation and insulin resistance (Farrell et al., 2015).

Due to the fact that black elderberry has antiviral, antibacterial and anti-diabetic properties, antitumour potential, antioxidant, antidepressant and immunostimulant properties, as well as a certain effect on obesity and metabolic disorders (Krawitz et al., 2011; Mocanu & Amariei, 2022), the aim of our study was to determine the effect of elderberry inflorescences in a high-fat diet on changes in body weight, internal organ weight and blood biochemical parameters in white laboratory rats.

Materials and methods

All procedures involving animals and the research protocol were conducted 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 the Protection of Animals from Cruelty' (Kyiv, 21 July 2006, No. 3447-IV) and were approved by the Local Animal Bioethics Committee of the Dnipro State Agrarian and Economic University, Dnipro, Ukraine (decision number: No. 2/23-24 of 18.09.2023). The number of animals used and their suffering were minimized.

Adult male crossbreed rats (200–220 g) were kept in the vivarium of the Faculty of Veterinary Medicine of the Dnipro State Agrarian and Economic University under controlled environmental conditions (21 ± 2 °C) and humidity (55–60%) with a 12 h/12 h day/night cycle throughout the experiment. Three groups of 5 animals each were formed from 15 rats: control and two experimental groups. All rats consumed a synthetic diet with increased fat content for 35 days. The synthetic diet was based on a complete basic diet consisting of a mixture of cereals (corn, 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, which was added to the mixture of ground dry feed components and further pelleted (Levchuk et al., 2021). The experimental groups were supplemented with chopped dried *S. nigra* inflorescences in addition to the high-fat diet, the first experimental group at a dose of 0.5% of the diet weight and the second one at a dose of 2.0%. The experiment was conducted using the official form of the medicinal plant (*sambuci nigrae flores*), which was purchased from a commercial pharmacy.

The experiment lasted 35 days and the animals received food and water ad libitum. The amount of food and water consumed by the animals each day was recorded. In addition, the animals' body weight changes were determined periodically by weighing on an analytical balance (Metrixo AB224, China). On the 30th day, animals from all three groups were removed from the experiment (anesthetic overdose – 80 mg/kg of ketamine and 12 mg/kg of xylazine, intraperitoneally). Blood samples were taken for biochemical analyses. Possible changes in the mass of the internal organs were determined during the dissection of the animals. The heart, liver, lungs, thymus, spleen, stomach, intestines and kidneys were examined for the presence of pathological changes and measured to the nearest 10 mg. Organ mass was calculated in relation to body weight.

Blood serum was obtained by maintaining blood for some time and its centrifugation on a CM-3M.01 MICROmed centrifuge (200×g, 5 min; MICROmed, Shenzhen, China). Biochemical parameters were determined on an automatic analyzer Miura 200 (Italy) using reagent kits from High Technology (USA), PZ Cormay S.A. (Poland) and Spinreact S.A. (Spain). Parameters of protein metabolism were evaluated by biochemical indices: total protein was determined by the biuret method; albumin concentration – by reaction with bromcresol green; globulins and protein coefficient – by calculation. Total bilirubin concentration was determined by enzymatic method. Urea was measured enzymatically. Blood urea nitrogen was determined simultaneously. Creatinine was determined kinetically using the Jaffe reaction with picric acid. Serum creatinine reacts with picric acid in acidic solution to form a yellow-red complex. The carbohydrate metabolism was assessed by the level of glucose, which was determined by the glucose oxidase method (Chawla, 2014). Lipid metabolism was described by indicators of total cholesterol concentration, determined enzymatically using cholesterol oxidase; triglycerides – after cleavage by lipoprotein lipase with detection by the Trinder reaction; HDL and LDL – using selective detergents with subsequent staining of the enzymatic reaction products; the atherogenicity index was also calculated. Indicators of mineral metabolism such as total calcium and inorganic phosphorus were determined using a spectrophotometric method. Calcium in the sample reacted with arsenazo III (Cormay Diagnostics, Warsaw, Poland) to form a colored complex, and phosphorus reacted with ammonium molybdenum. The Ca/P ratio is the ratio of total calcium to inorganic phosphorus. Changes in enzymatic activity in blood plasma were monitored in relation to aspartate aminotransferase (AST) and alanine aminotransferase (ALT) activities. For this purpose, we applied the kinetic method based on the Warburg test. The activities of AST and ALT were determined according to the rates of NADH (nicotinamide adenine dinucleotide) and the absorbance was measured spectrophotometrically at $\lambda = 340$ nm. The De Ritis ratio indicator was identified by the ratio of aspartate aminotransferase activity to alanine aminotransferase activity. Alkaline phosphatase activity (U/L) was determined enzymatically from the rates of formation of 4-nitrophenol, the absorbance of which was quantified at $\lambda = 405$ nm. The rates of 4-

nitrophenol formation are directly proportional to the activity of alkaline phosphatase. The activity of γ-glutamyltransferase was assessed kinetically according to the breakdown of L-γ-glutamyl-3-carboxy-4-nitroanilide with the formation of 5-amino-2-nitrobenzoate. The rates of its formation were determined spectrophotometrically; the absorbance at λ = 365–405 nm is directly proportional to the activity of γ-glutamyltransferase.

Data were analyzed using Statistica 12.0 (StatSoft Inc., USA). The results are presented in the 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), and differences were considered significant at $P < 0.05$.

Results

Rats fed a high-fat diet for 35 days reached 109% of their initial weight. By observing the changes in body weight gain in rats fed a high-fat diet with the addition of dried inflorescences of *S. nigra*, it was found that this indicator varied depending on the dose of the medicinal plant. For example, in the group of animals that received 0.5% dried *S. nigra* inflorescences in addition to a high-fat diet, a gradual increase in body weight was observed throughout the study period. Rats in this group increased their body weight to 112% of their initial weight. In the group of rats receiving 2% dry *S. nigra* inflorescences in addition to the high-fat diet, body weight gain was observed only during the first 10 days, after which the body weight of the animals gradually decreased until the end of the experiment (Fig. 1). The average body weight of the rats in the high-fat diet group at the end of the experiment was 319 ± 20 g. In the group of animals that consumed an additional 0.5% of *S. nigra* inflorescences, body weight was only 3.4% higher, and in the group that consumed 2.0% of *S. nigra* inflorescences, body weight was 4.4% lower (Table 2).

Adding *S. nigra* dried inflorescences to the high-fat diet did not cause significant changes in feed and water consumption (Table 1).

Only at a dose of 2% of the diet did *S. nigra* inflorescences significantly affect the morphometric parameters of the rats' internal organs. For example, consumption of 2% *S. nigra* inflorescences significantly increased liver weight by almost 15% compared to rats fed only a high-fat diet. There was also a significant increase, almost doubling, in the relative weights of the lungs (by 60.3%) and spleen. The relative weights and lengths of individual internal organs of rats were not affected by the addition of 0.5% *S. nigra* inflorescences to the high-fat diet (Table 2).

The supplementation of the high-fat diet with *S. nigra* inflorescences resulted in changes in some biochemical parameters of the animals' blood (Table 3). The most significant effect was on bilirubin metabolism. Thus, in rats supplemented with *S. nigra* inflorescences, the level of direct bilirubin decreased sharply and significantly, at a dose of 0.5% – by 13.9%, and at a dose of 2.0% – by 44.4%. At the same time, at a dose of 0.5% of *S. nigra* inflorescences, the level of indirect bilirubin decreased significantly (by 19.2%). However, the level of indirect bilirubin in blood plasma did not change significantly with the addition of *S. nigra* inflorescences in the amount of 2.0% of the diet volume compared to the group that consumed only a high-fat diet.

Carbohydrate metabolism was affected by the addition of *S. nigra* inflorescences to the high-fat diet for 35 days. For example, rats treated with 2.0% of the medicinal herb showed a significant 24.3% decrease in blood glucose levels, and the 0.5% dose did not have a significant effect. Consumption of *S. nigra* inflorescences by rats did not cause any significant changes in protein metabolism, nor did it affect the level of total calcium and inorganic phosphorus and their ratio in blood plasma.

When analysing the indicators of lipid metabolism, it was found that the inflorescences of *S. nigra* as a part of a high-fat diet did not cause any changes in the content of cholesterol in the blood. However, depending on the dose of the herb, the ratio of lipoproteins of different density changed. Thus, *S. nigra* inflorescences caused a significant decrease in the level of low-density lipoprotein (LDL) cholesterol by 24.5% at the dose of 0.5% and by 40.7% at the dose of 2.0%. At the same time, *S. nigra* inflorescences at a higher dose (2.0%) simultaneously increased the level of high-density lipoprotein (HDL) cholesterol by 13.8% compared to the index of rats receiving a diet with excess fat. In this regard, the atherogenicity index of the rats in the high-fat diet group was close to 2, and the addition of *S. nigra* inflorescences contributed to a slight decrease in this index.

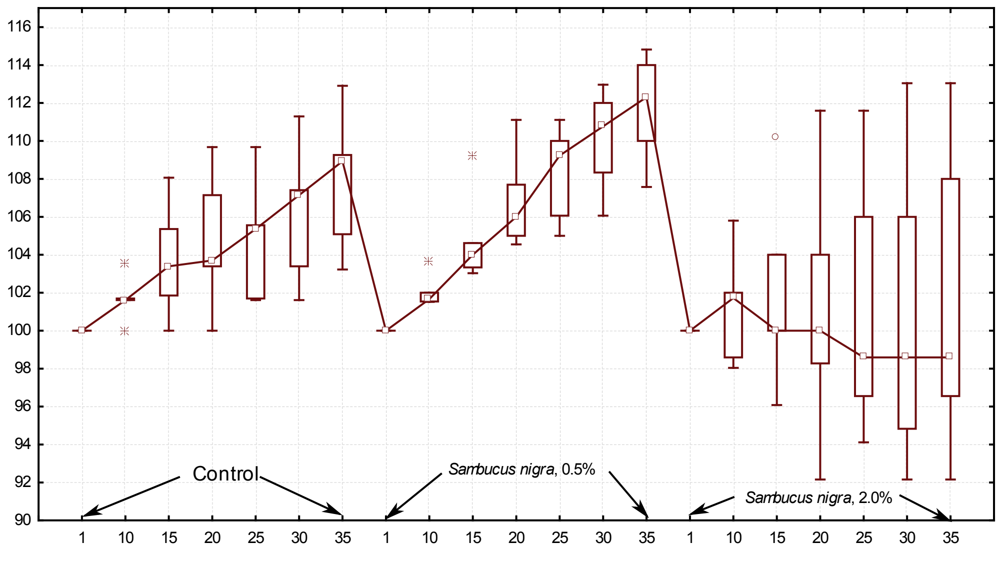

Fig. 1. Change in body weight of rats when crushed *Sambucus nigra* inflorescences were added to the animals' diet: abscissa – day of experiment, ordinate – body weight of animals (% relative to initial body weight of each animal, taken as 100% at the start of the experiment); small square – median, upper and lower limits 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 supplementation with *Sambucus nigra* in their diet (x ± SD, n = 5, experiment duration – 35 days)

Parameter	Control	*S. nigra, 0.5%*	*S. nigra* compared to the control, %	*S. nigra, 2.0%*	*S. nigra* compared to the control, %
Food consumption by animals, g/day	29.14	28.29	97.1	27.43	94.1
Water consumption by animals, g/day	40.57	42.40	104.5	40.00	98.6

Table 2
Changes in morphometry of internal organs of young male rats under the influence of *Sambucus nigra* supplementation (x ± SD, n = 5, experiment duration – 35 days)

Organ	Control	*S. nigra*, 0.5%	*S. nigra* compared to the control, %	*S. nigra*, 2.0%	*S. nigra* compared to the control, %
Heart	0.342 ± 0.050	0.382 ± 0.064	111.7	0.462 ± 0.138	135.1
Liver	2.57 ± 0.15	2.55 ± 0.11	99.4	2.95 ± 0.35*	114.9
Lungs	0.714 ± 0.059	0.706 ± 0.109	98.9	1.144 ± 0.326**	160.3
Thymus	0.094 ± 0.034	0.095 ± 0.017	101.5	0.090 ± 0.051	96.1
Spleen	0.208 ± 0.026	0.245 ± 0.054	117.7	0.401 ± 0.151*	192.8
Stomach	0.532 ± 0.020	0.498 ± 0.054	93.5	0.590 ± 0.203	110.9
Small intestine	1.55 ± 0.33	1.43 ± 0.17	92.4	1.46 ± 0.41	94.0
Caecum	0.238 ± 0.039	0.303 ± 0.061	127.4	0.405 ± 0.162	170.3
Colon	0.471 ± 0.021	0.519 ± 0.041	110.1	0.554 ± 0.087	117.6
Right kidney	0.309 ± 0.017	0.302 ± 0.063	97.5	0.338 ± 0.055	109.4
Left kidney	0.316 ± 0.029	0.298 ± 0.053	94.2	0.347 ± 0.057	109.8
Testicle	0.421 ± 0.035	0.454 ± 0.065	107.9	0.452 ± 0.066	107.5
Brain	0.585 ± 0.030	0.589 ± 0.056	100.7	0.616 ± 0.109	105.4
Body weight, g	319 ± 20	330 ± 37	103.4	295 ± 65	92.5
Length of small intestine, cm	123 ± 14	127 ± 13	103.6	117 ± 15	95.6
Length of colon and rectum, cm	20.2 ± 0.8	21.2 ± 1.9	105.0	20.2 ± 1.5	100.0
Body length, cm	20.8 ± 0.8	20.9 ± 0.7	100.5	21.2 ± 0.8	101.9

Note: * – P < 0.05, ** – P < 0.01 compared to Control.

When blood enzymatic activity was evaluated, it was found that the addition of *S. nigra* inflorescences to the high-fat diet significantly decreesed ALT activity by 34.4% at a dose of 0.5% and by 44.5% at a dose of 2.0%. At the same time, the De Ritis ratio also changed. Its sharp increase up to 169.2% compared to the control group was observed at the consumption of 2.0% of *S. nigra* inflorescences, and up to 183.1% at the consumption of 0.5%. The addition of the studied medicinal plant also affected the activity of alpha-amylase. Thus, when 2.0% of *S. nigra* inflorescences were added to the high-fat diet, alpha-amylase activity decreased by 19.0%, and 0.5% decreased by 29.7%. At the same time, AST, alkaline phosphatase and gamma-glutamyltransferase activities did not change (Table 3).

Table 3
Changes in blood biochemical parameters of male rats under the effect of *Sambucus nigra* supplementation (x ± SD, n = 5, experiment duration – 35 days)

Parameters	Control	*S. nigra*, 0.5%	*S. nigra* compared to the control, %	*S. nigra*, 2.0%	*S. nigra* compared to the control, %
Total protein, g/L	74.4 ± 2.6[a]	71.2 ± 3.2[a]	95.7	73.0 ± 7.2[a]	98.1
Albumins, g/L	30.0 ± 2.4[a]	29.6 ± 2.9[a]	98.7	28.0 ± 2.5[a]	93.3
Globulins, g/L	44.6 ± 2.6[a]	41.6 ± 4.2[a]	93.3	45.0 ± 5.4[a]	100.9
Albumin/Globulin ratio, U	0.68 ± 0.08[a]	0.74 ± 0.11[a]	108.8	0.62 ± 0.04[a]	91.2
Urea, mmol/L	5.2 ± 1.1[a]	4.6 ± 0.8[a]	88.8	5.2 ± 0.6[a]	101.6
Blood urea nitrogen, mg/100 g	9.8 ± 2.1[a]	8.7 ± 1.5[a]	89.2	10.0 ± 1.1[a]	102.7
Creatinine, µmol/L	46.8 ± 5.2[a]	40.2 ± 3.5[a]	85.9	41.4 ± 3.6[a]	88.5
AST, U/L	154 ± 16[a]	189 ± 67[a]	122.2	144 ± 8[a]	93.3
ALT, U/L	62 ± 12[a]	40 ± 16[ab]	65.6	34 ± 8[b]	55.5
De Ritis ratio (AST/ALT), U	2.60 ± 0.52[a]	4.76 ± 0.84[b]	183.1	4.40 ± 1.01[b]	169.2
Alkaline phosphatase, U/L	386 ± 183[a]	310 ± 91[a]	80.3	346 ± 121[a]	89.6
Alpha-amylase, U/L	1458 ± 220[a]	1024 ± 222[b]	70.2	1181 ± 138[ab]	81.0
Total bilirubin, µmol/L	2.98 ± 0.34[a]	2.56 ± 0.42[a]	85.9	2.76 ± 0.23[a]	92.6
Direct bilirubin, µmol/L	0.72 ± 0.13[a]	0.62 ± 0.13[ab]	86.1	0.40 ± 0.12[b]	55.6
Indirect bilirubin, µmol/L	2.40 ± 0.12[a]	1.94 ± 0.34[b]	80.8	2.36 ± 0.13[ab]	98.3
Glucose, mmol/L	5.02 ± 0.63[a]	4.80 ± 0.25[a]	95.6	3.80 ± 0.58[b]	75.7
Total calcium, mmol/L	2.52 ± 0.08[a]	2.44 ± 0.15[a]	96.8	2.46 ± 0.09[a]	97.6
Non-organic phosphorus, mmol/L	2.16 ± 0.22[a]	2.00 ± 0.35[a]	92.6	2.02 ± 0.11[a]	93.5
Ca/P	1.18 ± 0.15[a]	1.22 ± 0.20[a]	103.4	1.24 ± 0.11[a]	105.1
Gamma-glutamyltransferase, U/L	3.0 ± 1.2[a]	3.0 ± 1.7[a]	100.0	3.8 ± 1.5[a]	126.7
Cholesterol, mmol/L	1.96 ± 0.17[a]	1.92 ± 0.12[a]	98.0	1.74 ± 0.42[a]	88.8
High-density lipoprotein (HDL) cholesterol, mmol/L	0.65 ± 0.14[a]	0.68 ± 0.21[a]	104.6	0.74 ± 0.07[b]	113.8
Low-density lipoprotein (LDL) cholesterol, mmol/L	0.91 ± 0.56[a]	0.66 ± 0.12[b]	75.5	0.54 ± 0.04[b]	59.3
Atherogenic index of plasma	1.98 ± 1.08[a]	1.81 ± 0.34[a]	91.4	1.35 ± 0.15[a]	68.2

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.

Discussion

An unbalanced diet, especially with an excess of fat and carbohydrates against a background of insufficient protein, is the main cause of obesity and, as a consequence, the development of many diseases. Medicinal plants are of great interest to scientists as alternative medicines for treating and gently correcting metabolic disorders caused by unbalanced nutrition. At the same time, various pharmaceutical forms are used for therapeutic purposes – extracts, decoctions, tinctures, in the form of dry ingredients. In our experiment, we used dried inflorescences of *S. nigra*, which were added in crushed form to a high-fat diet. *Sambucus nigra* is a traditional medicinal plant widely used for therapeutic and dietary purposes (Badescu et al., 2012; Tiralongo et al., 2016). Black elderberry flowers contain the glycosides samunigrin and rutin, essential oil, tannins, and ascorbic acid. They are also a natural source of organic acids such as malic acid, valeric acid, chlorogenic acid and caffeic acid. In the scientific literature, the antioxidant, anti-inflammatory, antimicrobial and especially anticancer effects of this plant are the most widely reported (Mota et al., 2020; Ferreira et al., 2022; Mocanu & Amariei, 2022). In our experiment, animals fed a high-fat diet for 35 days did not show any significant changes in lipid metabolism. Thus, the rats increased their average body weight to 109% of the initial one, and the biochemical parameters of blood (cholesterol, high-density lipoprotein (HDL) cholesterol, low-density lipoprotein (LDL) cholesterol) did not exceed the reference values of the norm (Shayakhmetova et al., 2020). There were different effects on the body weight of animals when *S. nigra* inflorescences were added to a high-fat diet. Thus, *S. nigra* inflorescences at a dose of 0.5% contributed to an increase in body weight gain (up to 112% of the initial value). On the contrary, a dose of 2.0% caused a slowdown in body weight gain. Our previous studies have reported the effect of some medicinal plants on the rate of weight gain in the context of an unbalanced diet. Thus, the addition of 5% of the dry and ground components of *Salvia sclarea*, *Origanum vulgare*, *Sylibum marianum*, *Inula helenium* to a high-fat diet (15% fat) caused a decrease in the average daily weight gain in rats, and *Melissa officinalis*, *Lavandula angustifolia*, *Salvia officinalis*, *Punica granatum*, *Matricaria chamomilla*, *Scutellaria baicalensis*, *Echinacea purpurea*, *Rhodiola rosea*, *Vitex angus-castus*, on the contrary, contributed to an increase in the average daily bodyweight gain compared to animals consuming a diet without the addition of medicinal plants (Lieshchova & Brygadyrenko, 2021, 2022, 2023b, 2023c; Lieshchova et al., 2021, 2023). Also, the addition of 0.4% and 4.0% dry leaves of *Bidens tripartita* significantly slowed body weight gain and average daily weight gain in rats in an experiment with a high-fat diet (15% fat) in which water was replaced by a 20% fructose solution (Lieshchova & Brygadyrenko, 2024). *Helichrysum arenaria* inflorescences had the same effect with the same food intake in the control group (Lieshchova & Brygadyrenko, 2023). In an experiment with white male rats fed a high-fat diet (15% vegetable fat) with the addition of dried *Viola tricolor* herb for 30 days, consumption of 2.0% of the medicinal plant caused an increase in body weight gain compared to the control group (Bilan et al., 2024).

Elderberry hypoglycaemic activity has been repeatedly reported in scientific literature (Ciocoiu et al., 2009; Salvador et al., 2016). In this study, *S. nigra* inflorescences at a dose of 2.0% caused a decrease in blood glucose levels, but it should be noted that this indicator did not exceed the reference values throughout the experiment, both in rats on a high-fat diet and in the experimental groups (Shayakhmetova et al., 2020). Elderberry extracts have been studied as a hypoglycaemic agent in diabetes mellitus. Thus, in the model of streptozotocin-induced diabetes mellitus, elderberry methanolic extract showed a decrease in blood glucose levels and an improvement in lipid metabolism (lower cholesterol and triacylglycerols) (Ciocoiu et al., 2009). In the obese mouse model, the effect of black elderberry extract on metabolic processes has also been demonstrated, in particular reducing blood triacylglycerols, inflammatory markers and insulin levels (Farrell et al., 2015). The modulatory effect of elderberry extracts on carbohydrate metabolism was also demonstrated in experiments on rats with type 2 diabetes mellitus fed a high-fat diet. Polar elderberry extract led to a decrease in fasting blood glucose, while lipophilic extract reduced insulin levels. Furthermore, both extracts reduced insulin resistance without notable changes in haematological indices, serum lipid patterns, and trace element (Zn, Fe, Cu) homeostasis from serum and tissues (Salvador et al., 2016).

The present study demonstrated the effect of dry *S. nigra* inflorescences as part of a high-fat diet on lipid metabolism. Thus, in rats fed a high-fat diet for 35 days, the level of cholesterol was within the reference value for healthy animals, and the addition of *S. nigra* to the diet did not cause a significant change in this indicator (Ihedioha et al., 2011). However, the levels of cholesterol of different densities changed. In rats, a high-fat diet increased the level of high-density lipoprotein (HDL) cholesterol to 0.65 ± 0.14 mmol/L and low-density lipoprotein (LDL) cholesterol to 0.91 ± 0.56 mmol/L, which was beyond the reference values of the norm. Moreover, additional consumption of *S. nigra* inflorescences increased the level of high-density lipoprotein (HDL) cholesterol, especially at a dose of 2.0%, but at the same time contributed to a significant decrease in low-density lipoprotein (LDL) cholesterol, which in general even led to a slight decrease in the atherogenic index of plasma – an important indicator used to assess the risk of developing atherosclerosis and coronary heart disease (Khosravi et al., 2022).

Analysing the effect of *S. nigra* inflorescence supplementation on the body of rats, a significant increase in the relative weight of the liver in rats at a dose of 2.0% was found compared to the group receiving only a high-fat diet. In a study of elderberry fruit extract against obesity caused by a high-fat diet, the opposite trend was found. Liver weights were approximately 13% lower in both 0.25% and 1.25% anthocyanin-rich (BEE) groups compared to the HFD control group (Farrell et al., 2015). In the same study, it was shown that a high-fat diet caused hepatic steatosis, manifested by the accumulation of lipid droplets in liver cells, and feeding animals a diet with elderberry extracts attenuated this condition. The ability of elderberry active substances (cyanidin 3-glucoside (C3G) to reduce the manifestation of liver steatosis in an obesity model was also indicated in the studies of Guo et al. (2012).

In vitro studies suggest that black elderberry fruit extracts have antioxidant properties. Anthocyanins and polyphenols are able to increase the activity of enzymes (catalase, peroxidase, glutathione reductase, S-transferase) in the small intestine, liver and lung (Pascariu, & Israel-Roming, 2022). In our experiment in the blood of rats consuming a high-fat diet, we observed a sharp significant decrease in ALT activity, almost to normal levels (Shayakhmetova et al., 2020). Blood AST activity remained high both in the group of animals consuming a high-fat diet and when elderberry inflorescences were added, while the De Ritis ratio in the groups receiving medicinal raw materials was almost twice as high as in the control group. The increased activity of alpha-amylase in animals of all three groups compared with reference values for this age group is also noteworthy (Shayakhmetova et al., 2020). Alkaline phosphatase activity remained high in rats fed a high-fat diet, and the addition of *S. nigra* inflorescences caused a slight decrease in this index, especially at a dose of 0.5%.

An increase in the relative weight of liver and spleen in animals fed a high-fat diet supplemented with *S. nigra* inflorescences, together with a violation of the ratio of direct to indirect bilirubin in the blood, may indicate increased breakdown of blood erythrocytes and increased liver stress. Increased activity of blood enzymes, especially ALT, and increased De Ritis ratio may also indicate increased liver load. It is also known that excessive consumption of fats causes increased liver stress. In animals fed a high-fat diet, accumulation of steatosis, inflammation, fibrosis and cirrhosis are seen in the liver. Morphological abnormalities are preceded by changes in functional parameters, primarily an increase in plasma ALT activity (Levchuk et al., 2021). Steinbauer et al. (2024) showed that elderflower and leaf extracts increased macrophage ABCA1 expression and reduced foam cell formation without affecting hepatic lipogenesis. This is the basis for our further studies in this area and requires more detailed examination of liver tissue, including at the microscopic level.

Conclusions

Consumption of *S. nigra* inflorescences with a high-fat diet for 35 days affected body weight gain, relative weight of internal organs and metabolic parameters. The intake of *S. nigra* inflorescences caused a dose-dependent change in the body weight of rats at the end of the study. A dose of 0.5% of the medicinal plant gradually increased the body weight to 112% of the initial body weight, whereas a dose of 2.0% decreased it. The relative weights of liver, lung and spleen increased at the end of the experiment following the consumption of 2.0% *S. nigra* inflorescences, whereas 0.5% of the plant caused no significant changes in these parameters. The supplementation with dried *S. nigra* inflorescences of a high-fat diet caused changes in blood biochemical parameters. The blood glucose level of the animals decreased significantly (by 24.3%) under the influence of *S. nigra* inflorescences, especially at the 2.0% dose. *Sambucus nigra* inflorescences also influenced lipid metabolism indices. Thus, in the experimental groups of rats, the amount of low-density lipoprotein (LDL) cholesterol in the blood decreased, and the dose of 2.0% increased the level of high-density lipoprotein (HDL) cholesterol, while the amount of total cholesterol in the blood did not change. The atherogenic index of plasma was reduced at both doses of elderberry. The addition of *S. nigra* inflorescences also caused changes in bilirubin metabolism. A dose of 0.5% of the medicinal plant caused a decrease in direct bilirubin by 13.9% and in indirect bilirubin by 19.2%; a dose of 2.0% decreased the level of direct bilirubin by 44.4% without causing changes in the level of indirect bilirubin. There was also a significant decrease in alpha-amylase activity, ALT activity and a large significant increase in the De Ritis ratio with both doses of elderberry.

The results presented in this study contribute to the data on the antioxidant activity of *S. nigra* and its modelling effect on metabolic processes, which may be useful in developing new biological products and drugs based on *S. nigra* to treat and prevent metabolic diseases.

This research was funded by the Ministry of Education and Science of Ukraine under the topic "Modelling of metabolic processes and immune status of animals by drugs based on medicinal herbs with high calorie diet", grant number 0122U000975.

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