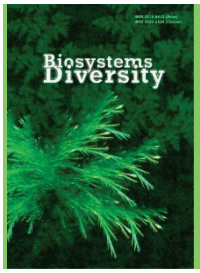

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Effect of *Echinacea purpurea* and *Silybum marianum* seeds on the body of rats with an excessive fat diet

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The impact of excess fat and high-calorie intake on the human body is an acute problem for many economically developed countries. Modelling the effects on the health of rats of supplementing their diet with crushed seeds of *Echinacea purpurea* (L.) Moench and *Sylibum marianum* (L.) Gaertn was carried out in a laboratory experiment. In the control group of animals, body weight increased by 700 mg/day, with the addition of *E. purpurea* seeds – by 1394 mg/day and with the addition of *S. marianum* seeds – by only 155 mg/day. A hypercaloric diet supplemented with *E. purpurea* caused a significant decrease in the relative weight of the liver, thymus, spleen, stomach, and brain. The supplementation with *S. marianum* seeds to the diet of animals significantly reduced only the relative weight of the thymus. Adding *E. purpurea* to the diet caused a strong increase in blood alkaline phosphatase activity, an increase in the cholesterol content, and a sharp increase in the atherogenic index. The seeds of *S. marianum* increased the alkaline phosphatase activity, reduced the glucose concentration, and triglycerides, significantly reduced the atherogenic index and lowered the C-reactive protein concentration in the rats' blood when compared with the control group. The seeds of *E. purpurea* contributed to an increase in the erythrocyte and lymphocyte number in the blood, and the seeds of *S. marianum* – to a decrease in the thrombocyte concentration. The research results show the possibility of wider use of *S. marianum* fruits as a dietary supplement in the diet of patients with hypertension and impaired liver function.

Keywords: relative mass of the organs; increase in the body weight; high-fat diet; milk thistle.

Introduction

According to the World Health Organization, coronary heart disease, stroke and liver cirrhosis are ranked third, fourth and tenth among all causes of human death in 2019, respectively. And according to the same organization, coronary heart disease and stroke are the first and second leading causes of death in humans in 2019, respectively, in upper-middle-income countries. One of the reasons for the development of these diseases is the increased calorie intake and high-fat content in the human daily diet (Pinto Júnior & Seraphim, 2012). Increasing the role of medicinal plants that are traditional in folk medicine for the treatment of metabolic diseases is one of the trends in modern medicine (Wilson et al., 2006; Yamada et al., 2011; Saad et al., 2017; Zazharskyi et al., 2020). Despite the widespread use of distinct medicinal plants, their potential has not been fully explored. In particular, this applies to widespread species from the Asteraceae family – *Echinacea purpurea* and *Silybum marianum*. Currently, products containing them are very common: they can be purchased in pharmacies, food stores and health stores – hundreds of different commercial preparations are available (Saini et al., 2020).

Purple coneflower (*Echinacea purpurea* (L.) Moench) is a perennial decorative and medicinal plant with large, up to 15 cm in diameter, purple-pink inflorescences-baskets. A long flowering period (up to two months), its high decorativeness and unpretentiousness to certain soil and hydrological conditions contribute to the spread of this species in gardens, and flowerbeds on most continents, although the birthplace of this plant is the eastern part of the United States. *Echinacea purpurea* is a medicinal plant with a long history of use in medicine, especially for infections (Kligler, 2003; Sharifi-Rad et al., 2018; Zazharskyi et al., 2019). It contains many active substances, but the main active component of this plant is a mixture of hydroxycinnamic acids: cichoric, ferulic, coumaric and caffeic. In addition, the plant contains polysaccharides (heteroxylans, arabinoramnogalactans), essential oils (0.15–0.50%), flavonoids, tannins, saponins, polyamines, echinacin (polyunsaturated acid amide), echinolone (unsaturated keto-alcohol), echinacoside (glycoside containing caffeic acid and catechol), organic acids, resins, phytosterols and other components. The plant is rich in macro- (potassium, calcium) and microelements (selenium, cobalt, silver, molybdenum, zinc, manganese, etc.) (Barnes et al., 2005).

As a medicinal plant, *E. purpurea* is safe and does not have significant toxicity, does not show consequential interactions with other medicinal plants and synthetic drugs, and has practically no contraindications and unwanted side effects (Izzo & Ernst, 2001; Block & Mead, 2003). It has been widely studied in laboratory animals (Skaudickas et al., 2004; Jiang et al., 2014; Boyko & Brygadyrenko, 2016; Cadiz et al., 2019; Gutyj et al., 2022), and is also being continuously studied in humans for its potential clinical applications (Šutovská et al., 2015; Stevenson et al., 2016). *Echinacea purpurea* is used for various diseases: depression, mental and physical fatigue, tonsillitis, inflammatory diseases of internal organs, acute infectious diseases, wounds, ulcers and burns (Barrett, 2003; Barnes et al., 2005; Ali, 2008; Arafa, 2010; Hudson, 2012). Regardless of the extract type or preparation, *E. purpurea* is considered an immune response enhancer, and its main indications are the prevention and treatment of cold, flu, and infections of the upper respiratory tract or lower urinary tract (Sperber et al., 2004; De Rosa et al., 2019; Cheng et al., 2020). *Echinacea* polysaccharides are used as antioxidant agents (Hou et al., 2020). *In vitro*, *E. purpurea* has shown direct antiviral activity against enveloped viruses such as coronavirus, yellow fever virus, herpes simplex virus, and parainfluenza virus (Aucoin et al., 2020; Bokelmann, 2022).

Echinacea purpurea is acknowledged to activate the immune system by stimulating T-cell production, lymphocytic activity, phagocytosis, cellular respiration, and suppressing the secretion of the hyaluronidase enzy-

me. Only four main groups of compounds in echinacea preparations have been determined to stimulate the immune response: the phenylpropanoids related to caffeic acid derivatives (caftaric acid, cynarine, cichoric acid and echinacoside), the alkylamides (2-ene and 2,4-diene), glycoproteins and polysaccharides (Bauer, 1999; Dobrange et al., 2019). Among the described mechanisms of the immunomodulatory effect, the evidence is the activation of macrophages, polymorphonuclear leukocytes and natural killers, the ability to change the number and activity of T- and B-lymphocytes (Gurbuz et al., 2002; Barrett, 2003). The ability of *E. purpurea* to improve the immune function of the organism due to the activation of innate immune responses and the possibility of enhancing humoral immunity has also been studied (Hall et al., 2007). Enhancement of cellular immunity occurs with a 4-day administration of *E. purpurea* extract at a dose of 0.6 mL/kg/day, which indicates the effect of the drugs only when they are urgently used (Freier et al., 2003).

Despite numerous studies, the therapeutic effect of *E. purpurea* is difficult to unequivocally attribute to any particular class of components. In medical practice, as an immunostimulant, ethyl alcohol tincture of various concentrations, water decoction and echinacea extract are used. In the treatment of cold, *E. purpurea* can reduce the duration of the disease and alleviate its course (Schulten et al., 2001; Sperber et al., 2004; David & Cunningham, 2019), but this effect is pronounced at the onset of symptoms; its long-term use has also been explored (Block & Mead, 2003). However, according to the review by Karsch-Völk et al. (2014), this plant is not effective in preventing or treating the common cold.

The use of *E. purpurea* had a significant radioprotective and radio recovery effect on the levels of lymphocytes, neutrophils, and monocytes in blood during an experiment in gamma-irradiated mice (Abouelella et al., 2007; Ramasahayam et al., 2011). *In vitro* studies have shown that the main component of echinacea, chicoric acid, inhibits the proliferation of human colon cancer cells in a dose- and time-dependent manner. It reduces telomerase activity and induces apoptosis in colon cancer cells. In mice with leukaemia, it exerts a suppressive effect on leukemic cells through IFN-γ activity (Yao et al., 2019; Bokelmann, 2022).

A number of scientific works are devoted to the study of the biological effect of echinacea on the farm animals organism (Bashchenko et al., 2020; Hashem et al., 2020; Awad et al., 2021). In poultry farming, chicoric acid, the main active component of echinacea, is used as a growth stimulant with antioxidant, anti-inflammatory, antibacterial, hypoglycaemic and hepatoprotective effects. Chicoric acid can be used as an alternative to antibiotics and can improve meat quality and health in broiler chickens (Saeed et al., 2018). *Echinacea purpurea* extract positively affects the production of antibodies against Newcastle disease during vaccination and significantly reduces the mortality rate of chicks (Bozorgmehrifard et al., 2010). The use of chicoric acid also increases the antibody titers against Newcastle disease, but the echinacea extract itself, added to the diet of chickens, does not have a positive effect on growth rates and intestinal histology during the growth period (Gurbuz et al., 2010). The effect of the inclusion of dried *E. purpurea* grass as a feed additive in the diets of sows, piglets and fattening pigs on their growth rates, blood composition dynamics, plasma enzymes, including lymphocyte proliferation, antibody status, and the content of proteins and immune globulins in colostrum was studied. Maass et al. (2005) concluded that *E. purpurea* could be used as a feed additive to achieve immunostimulatory efficacy in pig production and improve feed conversion. The use of *Echinacea* juice as a feed additive in the diet of laying hens and pigs has shown that repeated two-day stimulation is sufficient to enhance the immune response. Also, the use of juice did not affect the productivity of chickens, but caused a significant change in the number of blood lymphocytes, the rate of phagocytosis and the antibody titers to Newcastle disease (Böhmer et al., 2009). *Echinacea purpurea* extract at a dosage of 1.5 g per head per day has a positive effect on the composition and physicochemical parameters of rabbit meat quality and meat productivity (Voroshilin et al., 2020). The effect of echinacea over significant periods of time on the relative mass of animal organs has not been studied, there are only fragmentary studies of the effect of echinacea on biochemical parameters and the blood cell composition. Comprehensive assessments of the effects of any *Echinacea* preparation in mammals under conditions of excess caloric intake or high-fat diets are not available.

Milk thistle (*Sylibum marianum* (L.) Gaertn.) is an annual or biennial herbaceous plant, up to 2.5 m high. The leaves are large, up to 80 cm long, with yellow spikes along the edge of the leaf and along the veins, the leaf blade is shiny with bright white spots. The flowers are pink, collected in cephalic inflorescence. Milk thistle is native to the Mediterranean (Egypt, Israel, Turkey, Italy, Greece, France, Bulgaria, Albania, countries of the former Yugoslavia, Spain and Portugal). The species is widely distributed throughout the world: Europe, Africa, North and South America, Australia, Central and East Asia. It grows in weedy places, is bred in gardens, orchards. It is classified as an aggressive weed. It is cultivated for medicinal raw materials and as a honey plant. For a long time, milk thistle was not considered as a raw material for the production of pharmacological preparations, however, it was found that the fruits contain a group of flavonolignans, the components of which are silibinin, silidianin and silichristin, and also raw materials such as isosilibinin, silidianin, isosilichristin, silimonin, silandrin. The chemical composition of the fruits includes proteins, saponins, fatty oil (up to 25%), alkaloids, vitamin K, macro- and microelements, tyramine, histamine, resins, mucus.

The main active ingredients of milk thistle are flavonoids and flavonolignans under the general name silymarin. Silymarin is a complex mixture of polyphenolic molecules, including seven closely related flavonolignans (silibinin A, silibinin B, isosilibinin A, isosilibinin B, silichristin, isosilichristin, silidianin) and one flavonoid (taxifolin). Silibinin, a semipurified fraction of silymarin, is primarily a mixture of two diastereoisomers, silibinin A and silibinin B, in a roughly 1:1 ratio. Silibinin is the component with the highest degree of biological activity and makes up from 50% to 70% of silymarin. Silymarin is found throughout the plant, but its highest concentration is in the seeds. Silymarin acts as an antioxidant, reducing the free radical formation and lipid peroxidation, has an antifibrotic effect, and can act as a toxin blocker by inhibiting the toxins binding to hepatocyte cell membrane receptors (Wesołowska et al., 2007). Silymarin has been used to treat alcoholic liver disease, acute and chronic viral hepatitis, and toxin-induced liver diseases (Abenavoli et al., 2010; Kostek et al., 2012). Research also shows that silymarin and its active ingredient, silibinin, work as antioxidants, scavenging free radicals and inhibiting lipid peroxidation. They protect against genome damage, increase protein synthesis in hepatocytes, decrease tumour promoter activity, stabilize mast cells, chelate iron, and slow down calcium metabolism (Flora et al., 1998). Neha et al. (2014) assessed the association between high-fat diet-induced obesity and dementia, stating that silymarin may play an important role in improving cognitive status in post-obesity dementia through anti-inflammatory and antioxidant effects. According to scientific research, the mechanisms by which silymarin exerts its influence on the prevention and treatment of metabolic syndrome can be divided into several groups. In diabetes mellitus, silymarin reduces plasma glucose levels by reducing insulin resistance and restoring the beta cell of pancreatic insular islets (Yao et al., 2013). Silymarin regulates vascular tone and prevents platelet aggregation, which regulates blood pressure in hypertensive conditions. In diabetes and hypertension, silymarin prevents urinary excretion of albumin, thereby reducing kidney damage (Fallahzadeh et al., 2012). The last two mechanisms focus on non-alcoholic fatty liver disease and hyperlipidaemia, by which silymarin regulates the lipid profile by reducing hepatic steatosis and improves liver function through its antioxidant and cytoprotective effects (Detaille et al., 2008; Salomone et al., 2012, 2015, 2017). Studies conducted on patients with type 2 diabetes mellitus with severe hyperlipidaemia have shown the clinical efficacy of silymarin in combination with berberine. Their use may improve fasting blood sugar, total cholesterol, LDL, triglycerides, and hepatic transaminases (Di Pierro et al., 2013, 2015; Derosa et al., 2015). A study on the effect of silymarin on diabetic patients showed a significant increase in the body's total antioxidant capacity (Ebrahimpour Koujan et al., 2015).

2,3-Dehydrosilybin, which exhibits pronounced antioxidant properties, has been isolated from the seeds of *S. marianum*. Milk thistle meal contains 5% silymarin (the sum of milk thistle flavonoids and flavonolignans). A valuable effect of flavonolignans is the ability to neutralize the action of poisons in hepatocytes. The mechanism of action is direct interaction with free radicals, which slows down the intensity of free radical reactions, due to which one can observe a pronounced effect of slowing down the effect of toxic substances and their derivatives on the

structural components of hepatocytes (Torres et al., 2004). Scientists have created hepatoprotective drugs based on the seeds of milk thistle, which have antioxidant, and immunomodulatory properties, including a slight choleretic effect. Produced drugs "Bonjigar", "Silibor", "Legalon", "Karsil", "Gepabene", "Zdravushka", "Gepasil" contain a number of flavonoids and flavonolignans. This plant is used to treat diseases of the liver (hepatitis, cirrhosis, toxic lesions), spleen, gallstones, jaundice, chronic cough and others.

In medicine, milk thistle oil, squeezed from seeds, alcohol and water extracts of milk thistle, milk thistle syrup are used. The fatty oil of the fruit is characterized by regenerating and wound healing properties (Meddeb et al., 2018). Milk thistle is used in dietary nutrition: young shoots and leaves are recommended to be soaked in cold water to remove bitterness and added to salads or boiled like spinach dishes. Dried leaves and flowers are crushed and added to meat dishes as a seasoning. Pharmacies sell ground milk thistle fruits and fruit meal, which can be added to meat and vegetable dishes or consumed in powder form. Tea bags with ground milk thistle seeds are also on sale. Milk thistle seeds are also used in cooking for the manufacture of functional foods. Biscuits with the addition of milk thistle seeds as a source of fibre and an antioxidant ingredient at a dose of 5% reduce the calorie content, firmness and volume of the biscuit, but have a higher fluidity (Bortlíková et al., 2019; Krystyjan et al., 2022).

The objective of this study was to determine the general effect of adding crushed *E. purpurea* and *S. marianum* seeds to the diet of male rats with excess calories and high fat content.

Materials and methods

The choice of animals for the experiment, research protocols, and withdrawal of animals from the experiment was approved by the local ethical committee of Dnipro State Agrarian and Economic University (Dnipro, Ukraine). The maintenance, nutrition, care of animals and their withdrawal from the experiment were carried out in accordance with the principles set forth in the "European Convention for the Protection of Vertebrate Animals used for Experimental or other Scientific Purposes" (Strasbourg, France, March 18, 1986, ETS No. 123) and in Law of Ukraine "On Protection of Animals from Cruel Treatment" (Kyiv, February 21, 2006, No. 3447-IV).

For the experiment, three groups of outbred laboratory 1.5-month-old male rats with an average weight of 200 ± 10 g were used. The control group (n = 8) was kept on a high-fat diet with free access to water and food for 30 days. Two experimental groups, consisting of 8 animals, also received a high-fat diet for 30 days. The first experimental group was fed with 5% crushed dry seeds of *E. purpurea*, and the second group – dry crushed seeds of *S. marianum*. A high-fat diet was made on the basis of a standard diet (75% grain mixture (corn, sunflower grain, wheat, barley), 8% root crops (potatoes, carrots), 2% meat and bone meal, 2% mineral-vitamin complex) with the addition of 15% sunflower oil (Levchuk et al., 2021). First, grain, meat and bone meal, mineral-vitamin complex were ground in a mill, vegetable oil was added to the mixture, and granules were made on a granulator (Feed granulator GKM-100, Tekhnomashstroy, Cherkasy, Ukraine, 2020) for each group at the amount of 4200 g per 30-day period. For experimental groups, crushed plant seeds were introduced into the mixture before granulation. Fresh root crops were given every day in the appropriate amount. Animals had free access to food and water throughout the day, the amount of food and water consumed was taken into account daily and at the end of the experiment. Animals were kept in polycarbonate cages, 4 individuals per cage, indoors (temperature 20–22 °C, relative air humidity 50–65%, light regime 12 hours of light and 12 hours of darkness). Mature fruits of *Echinacea purpurea* and milk thistle grown and collected from the Botanical Garden of the Oles Honchar Dnipro National University (48.4355° N, 35.0431° E) were used as medicinal raw materials.

Every day, each animal was observed and weighed, the daily weight gain and the total weight gain of the rats fed the high-fat diet and supplemented with the test plants were calculated. Live weight and abdominal volume were determined on the first and 30th day of the experiment (Lieshchova et al., 2018, 2019, 2020). The functional state of the nervous system of the experimental animals was determined by determining their orientation-physical activity and their emotional status in the "open field" test at the beginning (days 1–4) and at the end of the experiment (days 26–30). A square setup (1 m^2) consisting of 16 squares was used. For 2 minutes, the number of crossed squares from (peripheral and central) was determined - physical activity was assessed; peripheral (with support on the wall) and central (without support on the wall) racks – orientation activity; number of acts of grooming, defecation and urination – to determine emotional status (Lieshchova & Brygadyrenko, 2021).

Euthanasia of animals was performed on the 30th day of the experiment under anesthesia (80 mg/kg ketamine and 12 mg/kg xylazine, intraperitoneally) by total bloodletting from the heart. After autopsy, the condition of the internal organs was visually assessed for the presence of pathological changes. Selection of organs and tissues (heart, liver, lungs, thymus, spleen, stomach, small and large intestines, kidneys) was performed with surgical instruments. The mass of internal organs was determined with an accuracy of ± 10 mg.

Blood sampling was performed during the euthanasia of rats. After anesthesia, blood was taken directly from the heart of the animals. Whole blood (1.0–1.5 mL) was collected in one tube to obtain serum and further conduct biochemical studies. 0.5–1.0 mL of blood was collected into a disposable microtube with K2 EDTA anticoagulant (Chengdu Rich Science Industry Co., Ltd, China) for further automatic blood cell counting and preparing blood smears for the leukogram.

Blood serum was obtained by keeping the blood for a certain time and then centrifuging it in a CM-3M.01 MICROmed centrifuge (3000 rpm, 5 minutes; MICROmed, Shenzhen, China). To evaluate the parameters of the protein and mineral metabolism in the obtained blood serum, the following were determined: total protein and its individual fractions, albumin/globulin ratio, urea and blood urea nitrogen, creatinine, total bilirubin, total calcium, non-organic phosphorus, ratio of Ca/P, C-reactive protein. Carbohydrate metabolism was assessed by the level of glucose (mmol/L). The state of lipid metabolism was assessed by the following parameters: cholesterol, blood triglycerides, high density lipoprotein (HDL) cholesterol (mmol/L) and low density lipoprotein (LDL) cholesterol, atherogenic index of plasma. Changes in the enzymatic activity in the blood plasma were determined by the activities of aspartate aminotransferase (AST, U/L) and alanine aminotransferase (ALT, U/L), alkaline phosphatase (U/L), gamma-glutamyltransferase (U/L). The De Ritis ratio (U) was obtained as the ratio of the aspartate aminotransferase activity to the alanine aminotransferase activity.

All studies of blood biochemical parameters were performed on a Miura 200 automated analyzer (I.S.E. Srl, Rome, Italy). When determining biochemical parameters, commercial reagent kits manufactured by High Technology kits of reagents (High Technology Inc, North Attleborough, MA, USA), PZ Cormay S. A. were used according to the instructions for use (Cormay Diagnostics, Lublin, Poland) and Spinreact S. A. (Spinreact, Girona, Spain).

The numbers of red and white blood cells in the rats' stabilized blood were determined using an automatic hematology analyzer MicroCC-20Plus (High Technology Inc, North Attleborough, MA, USA). For the leukogram, blood smears were prepared according to Pappenheim with their further staining according to Romanovsky-Giemsa, following generally accepted methods.

The tables show results as x ± SD (mean ± standard deviation). Differences between the control and experimental group values (calculated in the program Statistica 7.1, StatSoft Inc., USA) were identified using the Tukey test (with consideration of Bonferroni's correction), where the differences were considered as significant at $P < 0.05$.

Results

In control, the median body weight of rats fed a high-fat diet increased to 110.2% relative to the initial weight of animals on the 11th day of the experiment and did not exceed 112.0% until the 30th day of the experiment (Fig. 1a). The seeds of *E. purpurea* and *S. marianum* caused multidirectional changes in the body weight of the experimental animals relative to the control group. Male rats fed on *E. purpurea* seeds did not exceed 110.0% until the 13th day of the experiment, and by the 30th day of the experiment they reached 118.2% of the initial animal weight

(Fig. 1b). The seeds of *S. marianum* caused a slower increase in body weight than in the control (Fig. 1c): the median body weight of male rats reached a maximum on the 29th day of the experiment – 107.3% relative to the initial body weight of male rats.

Male rats supplemented with *E. purpurea* seeds drank 21.4% more water (Table 1) and gained 99.2% more body weight compared to control animals. This resulted in an increase in abdominal circumference of 17.7% compared to control animals.

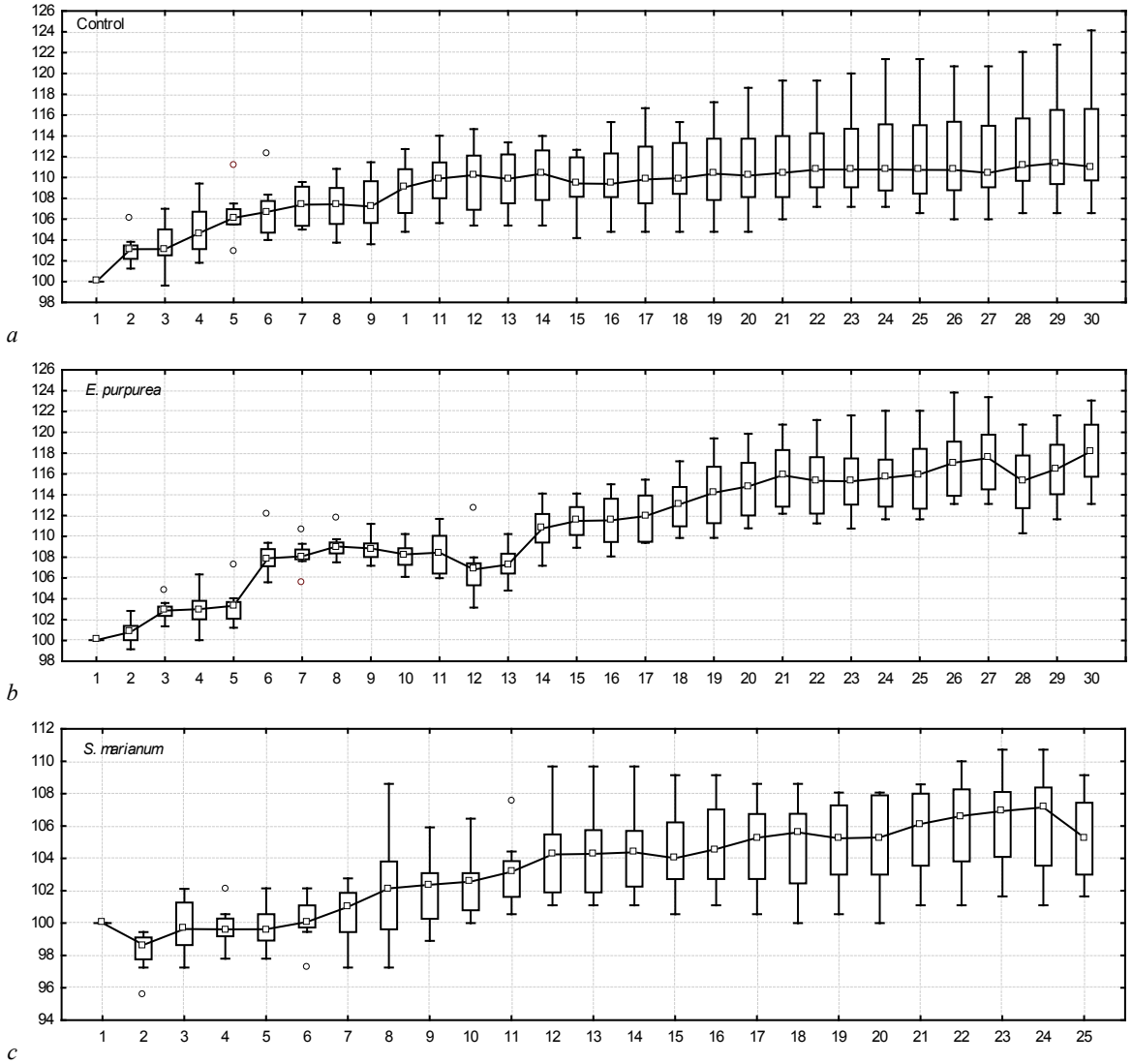

Fig. 1. Changes in body weight of the control variant (*a*) and when ground seeds of *Echinacea purpurea* (L.) Moench (*b*) and *Sylibum marianum* (L.) Gaertn were added to the animal diet (*c*): on the abscissa axis – the day of the experiment; on the ordinate axis – body weight of the animals (%) relative to the initial body weight before the start of the experiment, taken as 100% for each of the experimental animals; small square – median, the upper and lower boundary of the rectangle – 75% and 25% of the quartile, vertical line – minimum and maximum values, circles – outliers; n = 8

The addition of *S. marianum* seeds to the diet of rats did not cause a significant increase in water intake, but contributed to a decrease in the amount of food consumed by 15.6% compared with the control group (Table 1). This caused a decrease in daily body weight gain from 700 mg/day in control to 310 mg/day when the rats were fed a diet supplemented with milk thistle; the abdominal circumference of this group did not significantly differ from the control group.

Consuming *E. purpurea* (Table 2) significantly reduced the relative weight of the thymus (to 58.7% of the control group), brain (73.9%), stomach (77.7%), spleen (81.8%) and liver (to 89.1%). Eating seeds of *S. marianum* did not cause a significant change in the relative mass of organs, with the exception of the thymus relative mass (to 58.7% of the control group).

Seeds of *E. purpurea* in the animals' diet contributed to a significant increase in protein content (by 13.2% compared with the control group, Table 3). Excessive consumption of calories and fats in the composition of the feed led to excesses beyond the physiological norm for both the experimental and control groups. The concentration of albumin in all three groups of rats was within the normal range and did not change under the influence of medicinal plants. The use of *E. purpurea* increased the concentration globulins in relation to the physiological norm against the background of a high-fat diet.

The protein coefficient and urea content, did not go beyond the physiological norm (Table 5). The use of *E. purpurea* in the feed reduced the concentration of urea nitrogen below the physiological norm (to 81.3% of the concentration in the control group), and caused an increase in the creatinine concentration above the physiological norm (up to 118.0% of the control group).

A high-fat diet caused an increase in the activity of aspartate and alanine aminotransferases several times higher than the physiological norm in all three experimental groups of animals (Table 3). It should be noted that the activity of alanine aminotransferase significantly decreased to 60.9% of the values of this parameter in the control group of animals. The De Ritis ratio of the three experimental groups was within the normal range only in the group which was consuming *S. marianum* seeds. Alkaline phosphatase activity significantly increased in the groups that consumed *E. purpurea* seeds (up to 240.6% relative to the control group) and *S. marianum* (up to 271% relative to the control group).

The blood total bilirubin concentration (Table 3) went beyond the physiological norm when eating seeds *S. marianum*, but there were no

statistically significant differences from the control group. The glucose concentration was within the physiological norm in the three experimental groups, although, in the group that consumed the seeds of *S. marianum*, it decreased to 75.2% of the physiological norm. The concentration of blood calcium in animals did not differ from the control group and the physiological norm (Table 3). The concentration of phosphorus with the use of *S. marianum* seeds decreased to 74.0% of the control group levels, and correspondingly, the Ca/P ratio increased to 137.3% of the control group.

Table 1

Change in the body weight, food and water consumption of young male rats under the influence of *Echinacea purpurea* (L.) Moench and *Sylibum marianum* (L.) Gaertn. addition to their ration (x ± SD, n = 8, duration of experiment – 30 days)

Parameter	Control	*E. purpurea*	*E. purpurea* compared to the control, %	*S. marianum*	*S. marianum* compared to the control, %
Consumption of food by animals, g/day	20.09	21.94	109.2	16.96	84.4
Consumption of water by animals, g/day	18.42	22.36	121.4	19.20	104.2
Change in body weight, µg/day	700 ± 271	1394 ± 340***	199.2	310 ± 155**	44.2
Change in body weight, %/day	13.6 ± 5.9	18.1 ± 3.7	133.2	5.3 ± 2.6***	38.7
Abdominal volume, cm	14.0 ± 0.5	16.5 ± 0.8*	117.7	14.2 ± 1.3	101.5

Note: * – P < 0.05, ** – P < 0.01, *** – P < 0.001, significant differences inside one line of the table according to the results of ANOVA with Bonferroni correction.

Table 2

Change in relative mass of the organs (%) of male rats under the influence of addition to their ration of *Echinacea purpurea* (L.) Moench and *Sylibum marianum* (L.) Gaertn. (x ± SD, n = 8, duration of experiment – 30 days)

Organ	Control	*E. purpurea*	*E. purpurea* compared to the control, %	*S. marianum*	*S. marianum* compared to the control, %
Brain	0.867 ± 0.052	0.640 ± 0.049***	73.9	0.826 ± 0.160	95.3
Thymus	0.285 ± 0.046	0.167 ± 0.046***	58.7	0.167 ± 0.071**	58.7
Spleen	0.370 ± 0.036	0.303 ± 0.043*	81.8	0.382 ± 0.071	103.2
Heart	0.352 ± 0.023	0.330 ± 0.032	93.7	0.382 ± 0.035	108.6
Lungs	0.979 ± 0.169	0.838 ± 0.231	85.6	0.964 ± 0.151	98.5
Right kidney	0.358 ± 0.031	0.306 ± 0.032	85.4	0.382 ± 0.055	106.7
Left kidney	0.372 ± 0.040	0.297 ± 0.026	80.0	0.371 ± 0.039	99.9
Liver	4.08 ± 0.17	3.63 ± 0.18*	89.1	3.95 ± 0.22	96.8
Stomach	0.699 ± 0.060	0.543 ± 0.038**	77.7	0.765 ± 0.176	109.5
Small intestine	2.58 ± 0.52	2.17 ± 0.36	83.9	1.82 ± 0.33	70.5
Cecum	0.509 ± 0.176	0.439 ± 0.121	86.2	0.444 ± 0.130	87.2
Colon	0.374 ± 0.085	0.375 ± 0.064	100.4	0.442 ± 0.238	118.5
Rectum	0.398 ± 0.073	0.413 ± 0.178	103.6	0.361 ± 0.223	90.7

Note: see Table 1.

The addition of *E. purpurea* seeds to the diet almost halved the activity of gamma-glutamyltransferase relative to the control group.

Particular attention should be paid to the increase in the concentration of cholesterol in the blood to the level of 160.1% of the control group in the animals consuming the *E. purpurea* seeds. Both plant species caused a significant decrease in blood triglyceride concentrations in the animals: to 46.2% of the control group levels with *E. purpurea* and to 34.2% with *S. marianum* seeds.

Drastic changes were noted in the absolute concentration of high-density lipoproteins (decrease to 25.6% from *E. purpurea* and increase to 240.0% from *S. marianum*) and low-density lipoproteins (increase to 281.0% from *E. purpurea* and no significant changes from *S. marianum*). This was reflected in a sharp increase in the atherogenic index to 1723.6% compared to the control group in animals consuming *E. purpurea* seeds, and a corresponding decrease to 23.3% of the control group level in the animals consuming *S. marianum* seeds (Table 3).

Less pronounced changes were noted in the cellular composition of the blood (Table 4). Feeding with *E. purpurea* seeds led to a significant increase in the content of erythrocytes in the blood up to 112.2% of the control, and feeding with *S. marianum* seeds led to a significant decrease in the concentration of platelets to 71.2% of the control (Table 4). *Echinacea purpurea* seeds also contributed to an increase in the concentration of lymphocytes in the blood (up to 113.3% of the control group). There were no significant changes in the open field test between groups and within groups for the beginning and end of the experiment (Table 5). Significant changes in physical activity (Fig. 2a) and orienting activity (Fig. 2b) were not observed during the experiment. There was a tendency (without statistically significant changes in animals at the beginning and at the end of the experiment) to a decrease in the emotional status in the animals' groups fed on the seeds of *E. purpurea* and *S. marianum* (Fig. 2c).

Discussion

In most economically developed countries, obesity has become one of the most pressing social problems. Even a moderate degree of obesity increases the risk of cardiovascular disease, diabetes, and dyslipidemia (a component of the metabolic syndrome). Some progress has been made in the treatment and prevention of obesity, and a number of schemes have been proposed, including non-drug, drug and surgical methods. Thus, among the medical methods widely used synthetic drugs that normalize the hormonal background, suppress appetite, reduce digestion and more. An alternative to synthetic drugs is medicinal plants, which often have a similar mechanism of action, but are less toxic, have a relatively low cost and do not harm the environment (Thyagarajan et al., 2002).

Metabolic syndrome is one of the growing global health and medical concerns due to several clinical complications it can cause, such as an increased risk of myocardial infarction and hypertension. On the other hand, much attention has been directed to the value of herbal medicines. For example, a well-known remedy with many scientific publications confirming the preventive and therapeutic effects of silymarin, extracted from the dried seeds of *S. marianum*, on various components of the metabolic syndrome. It has been shown to have protective effects such as reducing insulin resistance, regulating blood pressure and lipid profile, as well as antioxidant and cytoprotective effects (De Avelar et al., 2017; Tajmohammadi et al., 2018; Camini & Costa, 2020).

In order to select the most effective medicinal plants that can significantly affect the normalization of metabolic processes, while not causing morphological and functional disorders in the body, we conducted studies of some medicinal plants – *Origanum vulgare* and *Scutellaria baicalensis* (Lieshchova & Brygadyrenko, 2022), *Salvia officinalis* and *S. sclarea* (Lieshchova et al., 2021), *Lavandula angustifolia*, *Melissa officinalis* and *Vitex angus-castus* (Lieshchova & Brygadyrenko, 2021), as well as the official spirit tincture *Aralia elata* (Brygadyrenko et al., 2019; Lieshchova et al., 2022). In this study, we determined the effect of *E. purpurea* and *S. marianum* seeds on the growth and development of laboratory animals on a high-fat diet.

The results of studies indicated that these medicinal plants affect the body weight gain of laboratory animals, and the addition of *E. purpurea* seeds to the diet stimulated an increase in body weight up to 1394 mg/day, and the consumption of *S. marianum* seeds significantly slowed down to

155 mg/day compared with the control group, which received only a high-fat diet (weight gain 700 mg/day). The addition of medicinal plant seeds also changed the amount of feed and water consumed. Thus, eating the seeds of *E. purpurea* increased the amount of water drunk by animals, and *S. marianum* reduced the amount of food eaten. Since the addition of *E. purpurea* seeds sharply increased the intensity of body weight gain, and by the end of the experiment, it was the rats of this group that had the largest weight and abdominal volume, this is probably why the relative weight of some internal organs was reduced comparatively to the parameters of the control group animals.

Table 3

Change in blood biochemical parameters of male rats under effect of addition to their diet *Echinacea purpurea* (L.) Moench and *Sylibum marianum* (L.) Gaertn (x ± SD, n = 8, duration of experiment – 30 days)

Parameters	Control	*E. purpurea*	*E. purpurea* compared to the control, %	*S. marianum*	*S. marianum* compared to the control, %
Parameters of the protein and mineral metabolism					
Total protein, g/L	77.0 ± 4.9	87.2 ± 2.0**	113.2	79.9 ± 4.2	103.7
Albumins, g/L	39.6 ± 2.6	44.8 ± 2.0	113.3	42.9 ± 2.5	108.3
Globulins, g/L	37.4 ± 3.9	42.3 ± 2.4	113.1	37.0 ± 2.4	98.9
Albumin/Globulin ratio, U	1.10 ± 0.15	0.92 ± 0.42	83.3	1.16 ± 0.09	105.2
Urea, mmol/L	6.84 ± 1.02	5.57 ± 0.50	81.4	6.00 ± 0.61	87.7
Blood urea nitrogen, mg/100 g	13.1 ± 2.0	10.6 ± 1.0	81.3	11.5 ± 1.2	87.9
Creatinine, μmol/L	63.0 ± 4.4	74.3 ± 9.9	118.0	68.3 ± 6.9	108.4
Total bilirubin, μmol/L	6.07 ± 1.67	4.48 ± 1.79	73.8	8.49 ± 1.92	139.8
C-reactive protein, mg/L	12.5 ± 5.4	12.4 ± 3.1	99.0	8.0 ± 1.3*	63.6
Total calcium, mmol/L	2.53 ± 0.09	2.38 ± 0.18	94.3	2.57 ± 0.13	101.7
Non-organic phosphorus, mmol/L	3.07 ± 0.58	3.03 ± 0.52	98.8	2.27 ± 0.32*	74.0
Ratio of Ca/P	0.84 ± 0.13	0.82 ± 0.16	96.9	1.16 ± 0.16**	137.3
Parameters of enzymatic activity in the blood					
AST, U/L	186 ± 61	191 ± 32	102.3	179 ± 36	96.3
ALT, U/L	131 ± 41	173 ± 45	132.6	80 ± 13*	60.9
De Ritis ratio (AST/ALT), U	1.63 ± 0.78	1.25 ± 0.30	76.8	2.29 ± 0.44	140.4
Alkaline phosphatase, U/L	129 ± 64	310 ± 107***	240.6	350 ± 63***	271.0
Gamma-glutamyltransferase, U/L	9.3 ± 2.6	5.0 ± 1.2***	53.8	8.6 ± 4.1	92.3
Parameters of lipid and carbohydrate metabolism					
Cholesterol, mmol/L	1.27 ± 0.14	2.04 ± 0.17***	160.1	1.57 ± 0.22	123.6
Blood triglycerides, mmol/L	2.13 ± 0.55	0.98 ± 0.13***	46.2	0.73 ± 0.21***	34.2
High-density lipoprotein (HDL) cholesterol, mmol/L	0.65 ± 0.13	0.17 ± 0.16***	25.6	1.56 ± 0.15***	240.0
Low-density lipoprotein (LDL) cholesterol, mmol/L	0.52 ± 0.29	1.45 ± 0.35***	281.0	0.33 ± 0.10	63.3
Atherogenic index of plasma, U	1.04 ± 0.45	17.97 ± 8.84***	1723.6	0.24 ± 0.13***	23.3
Glucose, mmol/L	7.39 ± 1.04	5.87 ± 1.22	79.4	5.56 ± 0.34***	75.2

Note: see Table 1.

Table 4

Change in blood cell count and leukogram of male rats under effect of *Echinacea purpurea* (L.) Moench and *Sylibum marianum* (L.) Gaertn. addition to their diet (x ± SD, n = 8, duration of experiment – 30 days)

Parameter	Control	*E. purpurea*	*E. purpurea* compared to the control, %	*S. marianum*	*S. marianum* compared to the control, %
Hemoglobin, g/L	126.8 ± 7.0	134.7 ± 6.8	106.2	119.7 ± 4.5	94.4
Hematocrit, %	40.5 ± 2.7	43.6 ± 1.8	107.6	37.9 ± 1.2	93.6
Erythrocytes, 10^{12}/L	6.93 ± 0.29	7.78 ± 0.14***	112.2	6.59 ± 0.23	95.0
Erythrocyte sedimentation rate (ESR), mm/h	1.17 ± 0.37	1.00 ± 0.00	85.7	1.14 ± 0.35	98.0
Thrombocytes, 10^9/L	339 ± 66	375 ± 74	110.6	241 ± 43*	71.2
Leukocytes, 10^9/L	8.6 ± 1.6	8.1 ± 2.4	94.2	7.8 ± 3.4	91.6
Leukocytic formula					
Basophils, %	0.0 ± 0.0	0.0 ± 0.0	–	0.0 ± 0.0	–
Eosinophils, %	1.50 ± 0.76	1.67 ± 0.94	111.1	1.00 ± 0.76	66.7
Mielocytes, %	0.0 ± 0.0	0.0 ± 0.0	–	0.0 ± 0.0	–
Neutrophils, %:					
– young	0.0 ± 0.0	0.0 ± 0.0	–	0.0 ± 0.0	–
– band	1.17 ± 0.69	0.00 ± 0.00***	0.0	0.57 ± 0.49	49.0
– with segmented nuclei	23.0 ± 8.2	15.0 ± 4.3	65.2	22.6 ± 7.5	98.1
Lymphocytes, %	68.8 ± 8.6	78.0 ± 2.9*	113.3	71.9 ± 7.0	104.4
Monocytes, %	5.5 ± 1.3	5.3 ± 1.2	97.0	4.0 ± 2.1	72.7

Note: see Table 3.

Table 5

Changes in the behavioral characteristics of the three rat groups over a 2-minute experiment, to whose diet *Echinacea purpurea* (L.) Moench and *Sylibum marianum* (L.) Gaertn. were added (x ± SD, n = 32, duration of the experiment was 30 days)

Characteristic	Control, 1–4th days	Control, 26–30th days	*E. purpurea*, 1–4th days	*E. purpurea*, 26–30th days	*S. marianum*, 1–4th days	*S. marianum*, 26–30th days
Number of visited peripheral squares	28.1 ± 18.0	24.3 ± 14.5	23.0 ± 10.6	22.8 ± 10.4	19.3 ± 10.4	13.6 ± 9.4
Number of visited central squares	1.00 ± 2.34	0.29 ± 1.04	1.88 ± 2.49	1.33 ± 2.19	0.00 ± 0.00	0.07 ± 0.38
Number of racks in peripheral squares	5.58 ± 4.53	3.79 ± 3.13	3.58 ± 1.47	3.17 ± 1.61	2.96 ± 2.47	2.57 ± 2.12
Number of racks in central squares	1.29 ± 1.43	0.71 ± 1.00	1.88 ± 2.54	2.21 ± 1.86	0.39 ± 0.83	0.61 ± 1.10
Number of grooming acts	0.58 ± 0.83	0.58 ± 0.93	2.67 ± 2.35	1.96 ± 1.85	0.43 ± 0.69	0.46 ± 0.88
Number of faecal boluses	2.25 ± 2.06	2.38 ± 1.56	1.50 ± 2.21	1.08 ± 1.61	0.32 ± 0.55	0.32 ± 0.55
Number of urination	0.333 ± 0.482	0.375 ± 0.495	0.125 ± 0.338	0.083 ± 0.282	0.107 ± 0.416	0.036 ± 0.189

Notes: there were no significant differences between the groups in any of the studied parameters.

Of particular interest is the fact of a decrease in the thymus relative mass in both experimental groups of animals. In the group with the addition of *E. purpurea* to the diet, this may indicate an active delymphotization of the organ due to the activation of cellular immunity, which is also confirmed by a significant increase in the number of blood lymphocytes. *Echinacea purpurea* is widely known as a traditional remedy which strengthens the body's resistance to disease. Preclinical studies have confirmed the hypothesis that *Echinacea* acts through immune mechanisms. A critical review by Barrett (2003) indicated that the ability of *Echinacea* extracts to activate macrophages, polymorphonuclear leukocytes and natural killers, as well as to increase the number of T- and B-lymphocytes. *Echinacea purpurea* extracts have also been clinically proven to be effective as anti-inflammatory agents only at the onset of upper respiratory symptoms; but long-term use of *Echinacea* preparations has not shown positive results (Block & Mead, 2003). Study by Abouelella et al. (2007) showed that the use of *Echinacea* increased the number of lymphocytes, leukocytes and monocytes in the blood in gamma-irradiated mice.

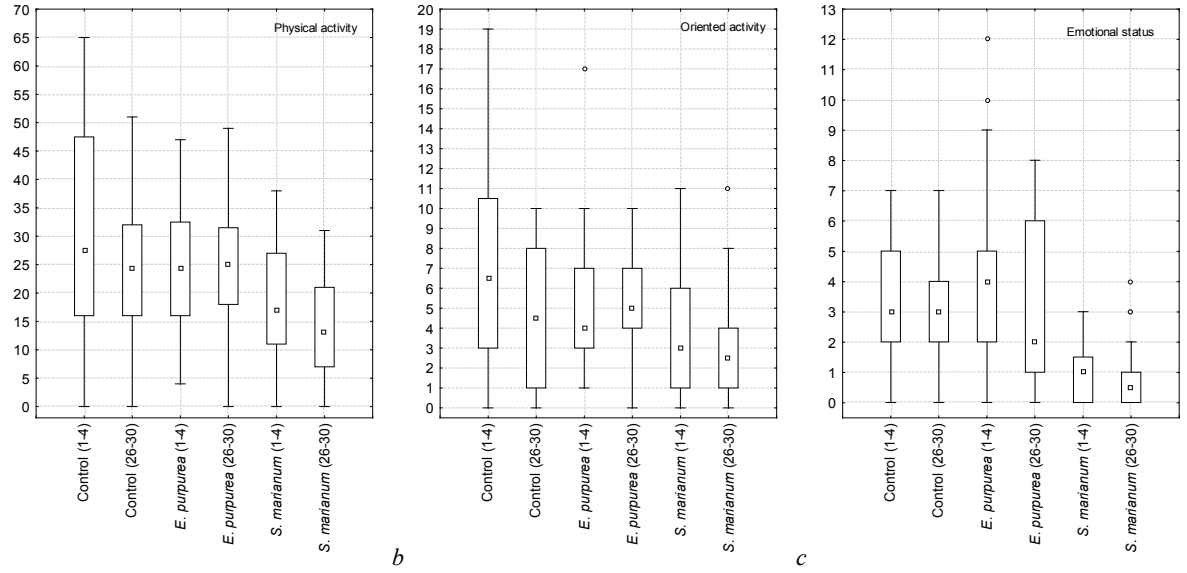

Fig. 2. Changes in the physical (*a*), orientation activity (*b*), and emotional status (*c*) of male rats when ground seeds of *Echinacea purpurea* (L.) Moench and *Sylibum marianum* (L.) Gaertn. were added to their diet: on the abscissa – animals' groups (n = 8) on a diet with an excess fat content and the addition of ground plant seeds (in parentheses are the days after the start of the experiment: 1–4th or 26–30th), the ordinate shows the absolute number of markers of this type of behaviour for 120 seconds of the experiment: for physical activity, the number of visited squares of the "open field", for orientation activity – the number of racks, for the emotional status – the number of grooming, defecation and urination acts; small square – median, upper and lower borders of the rectangle – 25% and 75% quartiles, vertical line – minimum and maximum values, circles – outliers

In the group with the addition of *S. marianum* to the diet, the low relative weight of the thymus is not accompanied by changes in the total number of leukocytes and, in particular, blood lymphocytes; therefore, one can assume an accelerated age-related involution of this organ. This issue requires further histological and cytological studies (Carrio & Lopez, 2013).

Under the influence of *E. purpurea*, the content of total protein in the blood serum increased (by 13.2%) due to the globulin fraction, which, together with a high level of C-reactive protein and an increased content of lymphocytes, may indicate the development of an inflammatory process in the body (Inyakina, 2011).

It is well known that abnormal levels of cholesterol, triglycerides, or free fatty acids are signs of a lipid disorder. Hyperlipidemia and hypercholesterolemia, and especially high concentrations of low-density lipoprotein cholesterol (LDL-C) in plasma, are the primary inducing factors in the development of atherosclerotic lesions. In our experiment, both the high-fat diet itself and the addition of *E. purpurea* and *S. marianum* seeds to it caused significant dyslipidemia in the blood of rats. In the control group of animals that consumed a diet with a high fat content (15%) for 30 days, an increase in triglycerides and low-density lipoprotein cholesterol (LDL-C) was found above the physiological norm. At the same time, the atherogenic index of plasma remained at a rather low level. The addition of *E. purpurea* seeds to the diet exacerbated dyslipidemia, causing an increase in cholesterol, low-density lipoprotein (LDL-C) cholesterol and a decrease in blood triglycerides and high-density lipoprotein (HDL-C) cholesterol. As a result, the atherogenic index of plasma increased to 1723.6%. *Sylibum marianum* seeds had the opposite effect: high-density lipoprotein (HDL-C) cholesterol increased, blood triglycerides decreased, and cholesterol and low-density lipoprotein (LDL-C) cholesterol did not change significantly, causing the atherogenic index of plasma to drop below that of the control group. This suggests that it is *S. marianum* that can be recommended for use in the treatment and prevention of diseases accompanied by lipid metabolism disorders. A study on the effect of long-term use of silymarin in patients with type 2 diabetes mellitus showed an antitriglyceride effect, but did not affect cholesterol levels and blood pressure (Khalili et al., 2017).

The concentration of total calcium, total bilirubin, urea and creatinine in the blood plasma of rats of the control and experimental groups did not change and did not exceed the reference values. Analyzing the enzymatic activity of blood plasma, we found that the seeds of *E. purpurea* and *S. marianum* in the rats' diet increased the activity of alkaline phosphatase (up to 310% and 350% of the values of the control group). At the same time, when *S. marianum* seeds were consumed, ALT activity decreased (to 80% of the control group level), but not to physiological values, and *E. purpurea* seeds reduced gamma-glutamyltransferase activity (to 53.8% of the control group level). At the same time, AST activity indicators did not differ significantly in the control and experimental groups, but were increased relatively to the reference norm values (Abrashova et al., 2017).

Of interest is the revealed moderate hypoglycemic effect of the studied plants seeds, found in rats of the experimental groups. Despite the fact that the glucose level in the control group rats was at the upper limit of the norm (7.39 mmol/L), additional consumption of *E. purpurea* and *S. marianum* seeds reduced the glucose level (to 79.4% and 75.2% of the control group value). The hypoglycemic effect of silymarin in metabolic syndrome, in particular in diabetes mellitus, is indicated in many scientific publications.

Despite the fact that some studies indicated that both *E. purpurea* and *S. marianum* themselves and their active substances in particular show effects aimed at stimulating the nervous system, reducing anxiety, increasing performance, relieving fatigue, neuroprotective activity, in our study no significant differences were found between groups of rats in the functional state of their nervous system. At the trend level, a decrease in the emotional status of animals was found when consuming *S. marianum* seeds.

Conclusions

The use of *E. purpurea* and *S. marianum* seeds as additions to a high-fat diet in rats caused significant changes in physical activity and blood biochemical parameters. Rats that consumed *E. purpurea* seeds increased their body weight during the 30 days of the experiment up to 199.2% of the weight gain in the control group, and *S. marianum* – only up to 44.2% of the values of the control group. The addition of *E. purpurea* to the feed did not affect the amount of food eaten, but increased the amount of water drunk (up to 121.4%), contributed to a significant increase in abdominal volume (up to 117.7% of the parameters of the control group rats). Consumption of *S. marianum* reduced the amount of food eaten, but did not affect water intake and abdominal volume.

Consumption of *E. purpurea* seeds for 30 days caused a decrease in the liver relative weight (to 89.1% of the values of the control group), spleen (to 81.8%), stomach (to 77.7%), and brain (to 73.9%). At the same time, both *E. purpurea* and *S. marianum* equally reduced the thymus relative weight (to 58.7% of the values of the control group).

The seeds of *E. purpurea* and *S. marianum* in the rats' diet caused significant changes in lipid metabolism. *Echinacea purpurea* in the blood of experimental animals increased the concentration of cholesterol (to 160.1% of the values of the control group), decreased the concentration of blood triglycerides (to 46.2%) and high-density lipoprotein (HDL-C) cholesterol (to 25.6%) against the background of an increase in low-density lipoprotein (LDL-C) cholesterol (up to 281.0%), which caused a sharp increase in the atherogenic index of plasma by more than 17 times. When *S. marianum* seeds were consumed, the level of blood triglycerides decreased (to 34.2% of the values in the control group of rats), but high-density lipoprotein (HDL-C) cholesterol increased (to 240.0%), while the content of low-density lipoprotein (LDL-C) cholesterol was within physiological limits and below the value of the control group, which caused a decrease in the atherogenic index of plasma (to 23.3% of the values in the control group).

Excess fat in the diet and the addition of *E. purpurea* and *S. marianum* seeds to it caused changes in the blood enzymatic activity parameters of animals. The addition of *E. purpurea* and *S. marianum* seeds to the diet caused a sharp increase in alkaline phosphatase activity to 240.6% and 271.0% of the control, respectively, but at the same time, *E. purpurea* reduced the activity of gamma-glutamyltransferase (to 53.8%), and *S. marianum* decreased the activity ALT (to 60.9% compared to the control group of rats). Under the influence of *E. purpurea*, the content of total protein in the blood serum increased (to 113.2%) due to the globulin fraction. *S. marianum* caused a decrease in glucose and non-organic phosphorus levels compared to the values of control rats, but these values did not go beyond the reference values of the norm.

Consumption of *E. purpurea* seeds caused an increase in the number of erythrocytes in the blood (up to 112.2% of the control group) and lymphocytes (up to 113.3%), and *S. marianum* – a decrease in the number of platelets (to 71.2%) compared with the control group of animals.

We did not observe significant changes in the open field test between groups of rats. An unreliable trend towards a decrease in the emotional status of rats by the end of the experiment was noted.

In general, the results of our studies indicate that the use of *E. purpurea* seeds for the correction of health problems associated with a high-fat diet and overweight is not recommended. The use of *S. marianum* seeds is promising in the mild correction of lipid metabolism disorders and the development of complex nutraceutical and pharmacological preparations. Further studies are needed to assess the effect of the dosage of these plantspreparations, as well as the effect of their chemical composition on the health status of model animals and humans.

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References

Abenavoli, L., Capasso, R., Milic, N., & Capasso, F. (2010). Milk thistle in liver diseases: Past, present, future. Phytotherapy Research, 24(10), 1423–1432.

Abenavoli, L., Izzo, A. A., Milić, N., Cicala, C., Santini, A., & Capasso, R. (2018). Milk thistle (*Silybum marianum*): A concise overview on its chemistry, pharmacological, and nutraceutical uses in liver diseases. Phytotherapy Research, 32(11), 2202–2213.

Abouelella, A. M., Shahein, Y. E., Tawfik, S. S., & Zahran, A. M. (2007). Phytotherapeutic effects of *Echinacea purpurea* in gamma-irradiated mice. Journal of Veterinary Science, 8(4), 341–351.

Abrashova, T. V., Gushhin, J. A., Kovaleva, M. A., Rybakova, A. V., Selezneva, A. I., Sokolova, A. P., & Hodko, S. V. (2013). Fiziologicheskie, biohimicheskie i biometricheskie pokazateli normy eksperimental'nyh zhivotnyh [Physiological, biochemical and biometric indicators of the norm of experimental animals]. Lema, Saint Petersburg (in Russian).

Ali, E. H. (2008). Protective effects of *Echinacea* on cyproterone acetate induced liver damage in male rats. Pakistan Journal of Biological Sciences, 11(21), 2464–2471.

Arafa, N. M. (2010). Efficacy of *Echinacea* on the action of cyproterone acetate in male rats. Pakistan Journal of Biological Sciences, 13(20), 966–976.

Aucoin, M., Cooley, K., Saunders, P. R., Carè, J., Anheyer, D., Medina, D. N., Cardozo, V., Remy, D., Hannan, N., & Garber, A. (2020). The effect of *Echinacea* spp. on the prevention or treatment of COVID-19 and other respiratory tract infections in humans: A rapid review. Advances in Integrative Medicine, 7(4), 203–217.

Awad, A., Fahim, H., El-Shhat, A. E., Mahrose, K., & Shazly, S. (2021). Dietary *Echinacea purpurea* administration enhanced egg laying performance, serum lipid profile, antioxidant status and semen quality in duck breeders during summer season. Journal of Animal Physiology and Animal Nutrition, 757–765.

Barnes, J., Anderson, L. A., Gibbons, S., & Phillipson, J. D. (2005). *Echinacea* species (*Echinacea angustifolia* (DC.) Hell., *Echinacea pallida* (Nutt.) Nutt., *Echinacea purpurea* (L.) Moench): A review of their chemistry, pharmacology and clinical properties. The Journal of Pharmacy and Pharmacology, 57(8), 929–954.

Barrett, B. (2003). Medicinal properties of *Echinacea*: A critical review. Phytomedicine, 10(1), 66–86.

Bashchenko, M. I., Boiko, O. V., Honchar, O. F., Gutyj, B. V., Lesyk, Y. V., Ostapyuk, A. Y., Kovalchuk, I. I., & Leskiv, K. Y. (2020). The effect of milk thistle, metiphen, and silimevit on the protein-synthesizing function of the liver of laying hens in experimental chronic cadmium toxicosis. Ukrainian Journal of Ecology, 10(6), 164–168.

Bauer, R. (1999). Chemistry, analysis and immunological investigations of *Echinacea* phytopharmaceuticals. In: Wagner, H. (Ed.). Immunomodulatory agents from plants. Springer Basel AG. Pp. 41–88.

Block, K. I., & Mead, M. N. (2003). Immune system effects of *Echinacea*, ginseng, and *Astragalus*: A review. Integrative Cancer Therapies, 2(3), 247–267.

Böhmer, B. M., Salisch, H., Paulicks, B. R., & Roth, F. X. (2009). *Echinacea purpurea* as a potential immunostimulatory feed additive in laying hens and fattening pigs by intermittent application. Livestock Science, 122(1), 81–85.

Bokelmann, J. M. (2021). *Echinacea* (*Echinacea purpurea*, *Echinacea angustifolia*). In: Bokelmann, J. M. (Ed.). Medicinal herbs in primary care. Elsevier. Pp. 317–322.

Bortlíková, V., Kolarič, L., & Šimko, P. (2019). Application of milk thistle (*Silybum marianum*) in functional biscuits formulation. Acta Chimica Slovaca, 12(2), 192–199.

Boyko, A. A., & Brygadyrenko, V. V. (2016). Influence of water infusion of medicinal plants on larvae of *Strongyloides papillosus* (Nematoda, Strongyloididae). Visnyk of Dnipropetrovsk University, Biology, Ecology, 24(2), 519–525.

Bozorgmehrifard, H., Feizi, A., & Peyman, B. (2010). Effect of *Echinacea purpurea* dried extract on producing antibody from Newcastle disease vaccine in broilers byhi test. Journal of Veterinary Research, 65, 119–122.

Brygadyrenko, V. V., Lieshchova, M. A., Bilan, M. V., Tishkina, N. M., & Horchanok, A. V. (2019). Effect of alcohol tincture of *Aralia elata* on the organism of rats and their gut microbiota against the background of excessive fat diet. Regulatory Mechanisms in Biosystems, 10(4), 497–506.

Cadiz, M. P., Schara, M. R., Kemp, B. H., & Gibbons Johnson, R. M. (2019). *Echinacea purpurea* root extract increases tumor necrosis factor production by concanavalin A-activated murine splenocytes. Journal of Medicinal Food, 22(11), 1146–1150.

Camini, F. C., & Costa, D. C. (2020). Silymarin: Not just another antioxidant. Journal of Basic and Clinical Physiology and Pharmacology, 31(4), 20190206.

Carrio, R., & Lopez, D. M. (2013). Insights into thymic involution in tumor-bearing mice. Immunologic Research, 57, 106–114.
Cheng, Z. Y., Sun, X., Liu, P., Lin, B., Li, L. Z., Yao, G. D., Huang, X. X., & Song, S. J. (2020). Sesquiterpenes from *Echinacea purpurea* and their anti-inflammatory activities. Phytochemistry, 179, 112503.
David, S., & Cunningham, R. (2019). *Echinacea* for the prevention and treatment of upper respiratory tract infections: A systematic review and meta-analysis. Complementary Therapies in Medicine, 44, 18–26.
De Avelar, C. R., Pereira, E. M., de Farias Costa, P. R., de Jesus, R. P., & de Oliveira, L. P. M. (2017). Effect of silymarin on biochemical indicators in patients with liver disease: Systematic review with meta-analysis. World Journal of Gastroenterology, 23(27), 5004–5017.
De Rosa, N., Giampaolino, P., Lavitola, G., Morra, I., Formisano, C., Nappi, C., & Bifulco, G. (2019). Effect of immunomodulatory supplements based on *Echinacea angustifolia* and *Echinacea purpurea* on the posttreatment relapse incidence of genital condylomatosis: A prospective randomized study. BioMed Research International, 2019, 3548396.
Derosa, G., Romano, D., D'Angelo, A., & Maffioli, P. (2015). *Berberis aristata* / *Silybum marianum* fixed combination (Berberol®) effects on lipid profile in dyslipidemic patients intolerant to statins at high dosages: A randomized, placebo-controlled, clinical trial. Phytomedicine, 22(2), 231–237.
Detaille, D., Sanchez, C., Sanz, N., Lopez-Novoa, J. M., Leverve, X., & El-Mir, M.-Y. (2008). Interrelation between the inhibition of glycolytic flux by silibinin and the lowering of mitochondrial ROS production in perifused rat hepatocytes. Life Sciences, 82(21–22), 1070–1076.
Di Pierro, F., Bellone, I., Rapacioli, G., & Putignano, P. (2015). Clinical role of a fixed combination of standardized *Berberis aristata* and *Silybum marianum* extracts in diabetic and hypercholesterolemic patients intolerant to statins. Diabetes, Metabolic Syndrome and Obesity: Targets and Therapy, 8, 89–96.
Di Pierro, F., Putignano, P., Montesi, L., Moscatiello, S., Marchesini Reggiani, G., & Villanova, N. (2013). Preliminary study about the possible glycemic clinical advantage in using a fixed combination of *Berberis aristata* and *Silybum marianum* standardized extracts versus only *Berberis aristata* in patients with type 2 diabetes. Clinical Pharmacology: Advances and Applications, 5, 167–174.
Dimitrov, I. V., Kamenov, V. I., Boyadjiev, N. P., Georgieva, K. N., Bivolarska, A. V., Draganova-Filipova, M. N., Angelova-Hristova, P. A., Delchev, S., Daskalova, E., Gerginska, F., Stankova, T. R., & Gramatikov, V. (2019). Impact of a high-fat diet on the development of chronic inflammation in heart of Wistar rats. Folia Medica, 61(3), 404–410.
Dobrange, E., Peshev, D., Loedolff, B., & Van den Ende, W. (2019). Fructans as immunomodulatory and antiviral agents: The case of *Echinacea*. Biomolecules, 9(10), 615.
Ebrahimpour Koujan, S., Gargari, B. P., Mobasseri, M., Valizadeh, H., & Asghari-Jafarabadi, M. (2015). Effects of *Silybum marianum* (L.) Gaertn. (silymarin) extract supplementation on antioxidant status and hs-CRP in patients with type 2 diabetes mellitus: A randomized, triple-blind, placebo-controlled clinical trial. Phytomedicine, 22(2), 290–296.
Fallahzadeh, M. K., Dormanesh, B., Sagheb, M. M., Roozbeh, J., Vessal, G., Pakfetrat, M., Daneshbod, Y., Kamali-Sarvestani, E., & Lankarani, K. B. (2012). Effect of addition of silymarin to renin-angiotensin system inhibitors on proteinuria in type 2 diabetic patients with overt nephropathy: A randomized, double-blind, placebo-controlled trial. American Journal of Kidney Diseases, 60(6), 896–903.
Flora, K., Hahn, M., Rosen, H., & Benner, K. (1998). Milk thistle (*Silybum marianum*) for the therapy of liver disease. American Journal of Gastroenterology, 93(2), 139–143.
Freier, D. O., Wright, K., Klein, K., Voll, D., Dabiri, K., Cosulich, K., & George, R. (2003). Enhancement of the humoral immune response by *Echinacea purpurea* in female swiss mice. Immunopharmacology and Immunotoxicology, 25(4), 551–560.
Goel, V., Chang, C., Slama, J. V., Barton, R., Bauer, R., Gahler, R., & Basu, T. K. (2002). Alkylamides of *Echinacea purpurea* stimulate alveolar macrophage function in normal rats. International Immunopharmacology, 2, 381–387.
Gurbuz, E., Balevi, T., Kurtoglu, V., Coskun, B., Oznurlu, Y., Kan, Y., & Kartal, M. (2010). Effects of *Echinacea* extract on the performance, antibody titres, and intestinal histology of layer chicks. British Poultry Science, 51(6), 805–810.
Gutyj, B. V., Martyshuk, T. V., Parchenko, V. V., Kaplaushenko, A. H., Bushueva, I. V., Hariv, I. I., Bilash, Y. P., Brygadyrenko, V. V., Turko, Y. I., & Radzykhovskyi, M. L. (2022). Effect of liposomal drug based on interferon and extract from *Silybum marianum* on antioxidative status of bulls against the background of contamination of fodders by cadmium and plumbum. Regulatory Mechanisms in Biosystems, 13(4), 419–425.
Hall, H., Fahlman, M. M., & Engels, H. J. (2007). *Echinacea purpurea* and mucosal immunity. International Journal of Sports Medicine, 28(9), 792–797.
Hashem, M. A., Neamat-Allah, A., Hammza, H., & Abou-Elnaga, H. M. (2020). Impact of dietary supplementation with *Echinacea purpurea* on growth performance, immunological, biochemical, and pathological findings in broiler chickens infected by pathogenic *E. coli*. Tropical Animal Health and Production, 52(4), 1599–1607.
Hou, R., Xu, T., Li, Q., Yang, F., Wang, C., Huang, T., & Hao, Z. (2020). Polysaccharide from *Echinacea purpurea* reduce the oxidant stress *in vitro* and *in vivo*. International Journal of Biological Macromolecules, 149, 41–50.
Hudson, J. B. (2012). Applications of the phytomedicine *Echinacea purpurea* (purple coneflower) in infectious diseases. Journal of Biomedicine and Biotechnology, 2012, 769896.
Inyakina, T. V. (2011). C-reaktivnyj belok Metodiki ocenki v biologicheskikh zhidkostiakh [C-reactive protein. Methods of valuation in biological fluids]. Medicine and Ecology, 1, 25–30 (in Russian).
Izzo, A. A., & Ernst, E. (2001). Interactions between herbal medicines and prescribed drugs. Drugs, 61(15), 2163–2175.
Jiang, L., Li, W., Wang, Y., Zhang, X., Yu, D., Yin, Y., Xie, Z., & Yuan, Y. (2014). Effects of cichoric acid extract from *Echinacea purpurea* on collagen-induced arthritis in rats. The American Journal of Chinese Medicine, 42(3), 679–692.
Karsch-Völk, M., Barrett, B., Kiefer, D., Bauer, R., Ardjomand-Woelkart, K., & Linde, K. (2014). *Echinacea* for preventing and treating the common cold. The Cochrane Database of Systematic Reviews, 2(2), CD000530.
Khalili, N., Fereydoonzadeh, R., Mohtashami, R., Mehrzadi, S., Heydari, M., & Huseini, H. F. (2017). Silymarin, olibanum, and nettle, a mixed herbal formulation in the treatment of type II diabetes: A randomized, double-blind, placebo-controlled, clinical trial. Journal of Evidence-Based Complementary and Alternative Medicine, 22(4), 603–608.
Kligler, B. (2003). *Echinacea*. American Family Physician, 67(1), 77–80.
Kostek, H., Szponar, J., Tchórz, M., Majewska, M., & Lewandowska-Stanek, H. (2012). Sylibinina i jej działanie hepatoprotekcyjne z punktu widzenia toksykologa [Silibinin and its hepatoprotective action from the perspective of a toxicologist]. Przeglad Lekarski, 69(8), 541–543.
Krystyjan, M., Gumul, D., & Adamczyk, G. (2022). The effect of milk thistle (*Silybum marianum* L.) fortification, rich in dietary fibre and antioxidants, on structure and physicochemical properties of biscuits. Applied Sciences, 12(23), 12501.
Levchuk, N. I., Lukashenia, O. S., & Kovzun, O. I. (2021). Eksperymentalne modeliuvannia metabolichnoho syndromu, indukovanoho dietoju, u laboratornykh tvaryn [Experimental modeling of diet-induced metabolic syndrome in laboratory animals]. Endokrynologia, 26(3), 298–310 (in Ukrainian).
Lieshchova, M. A., & Brygadyrenko, V. V. (2021). Influence of *Lavandula angustifolia*, *Melissa officinalis* and *Vitex angus-castus* on the organism of rats fed with excessive fat-containing diet. Regulatory Mechanisms in Biosystems, 12(1), 169–180.
Lieshchova, M. A., Bilan, M. V., Bohomaz, A. A., Tishkina, N. M., & Brygadyrenko, V. V. (2020). Effect of succinic acid on the organism of mice and their intestinal microbiota against the background of excessive fat consumption. Regulatory Mechanisms in Biosystems, 11(2), 153–161.
Lieshchova, M. A., Bilan, M. V., Evert, V. V., Kravtsova, M. V., & Mylostyvyi, R. V. (2022). Morphofunctional state of the rat's liver under the influence of *Aralia elata* alcohol tincture during the high-fat diet. Scientific Messenger of LNU of Veterinary Medicine and Biotechnologies, 24(108), 75–81.
Lieshchova, M. A., Bohomaz, A. A., & Brygadyrenko, V. V. (2021). Effect of *Salvia officinalis* and *S. sclarea* on rats with a high-fat hypercaloric diet. Regulatory Mechanisms in Biosystems, 12(3), 554–563.
Lieshchova, M. A., Tishkina, N. M., Bohomaz, A. A., Gavrilin, P. M., & Brygadyrenko, V. V. (2018). Combined effect of glyphosphate, saccharin and sodium benzoate on rats. Regulatory Mechanisms in Biosystems, 9(4), 591–597.
Lieshchova, M., & Brygadyrenko, V. (2022). Effects of *Origanum vulgare* and *Scutellaria baicalensis* on the physiological activity and biochemical parameters of the blood in rats on a high-fat diet. Scientia Pharmaceutica, 90(3), 49.
Maass, N., Bauer, J., Paulicks, B. R., Bohmer, B. M., & Roth-Maier, D. A. (2005). Efficiency of *Echinacea purpurea* on performance and immune status in pigs. Journal of Animal Physiology and Animal Nutrition, 89(7–8), 244–252.
Meddeb, W., Rezig, L., Zarrouk, A., Nury, T., Vejux, A., Prost, M., Bretillon, L., Mejri, M., & Lizard, G. (2018). Cytoprotective activities of milk thistle seed oil used in traditional tunisian medicine on 7-ketocholesterol and ^{24}S-hydroxycholesterol-induced toxicity on 158N murine oligodendrocytes. Antioxidants, 7(7), 95.
Neha, Kumar, A., Jaggi, A. S., Sodhi, R. K., & Singh, N. (2014). Silymarin ameliorates memory deficits and neuropathological changes in mouse model of high-fat-diet-induced experimental dementia. Naunyn-Schmiedeberg's Archives of Pharmacology, 387(8), 777–787.
Pinto Júnior, D. A. C., & Seraphim, P. M. (2012). Cafeteria diet intake for fourteen weeks can cause obesity and insulin resistance in Wistar rats. Revista de Nutrição, 25(3), 313–319.
Ramasahayam, S., Baraka, H. N., Abdel Bar, F. M., Abuasal, B. S., Widrlechner, M. P., Sayed, K. A., & Meyer, S. A. (2011). Effects of chemically characterized fractions from aerial parts of *Echinacea purpurea* and *E. angustifolia* on myelopoiesis in rats. Planta Medica, 77(17), 1883–1889.
Saad, B., Zaid, H., Shanak, S., & Kadan, S. (2017). Anti-diabetes and anti-obesity medicinal plants and phytochemicals. Springer, Cham.

Saeed, M., Babazadeh, D., Arain, M. A., Naveed, M., Shah, Q. A., Kamboh, A. A., Moshaveri, A., Modarresi-Ghazani, F., Hejazi, V., & Chao, S. (2018). The use of chicoric acid from *Echinacea purpurea* as a feed additive in poultry nutrition. World's Poultry Science Journal, 74(1), 69–78.

Saini, I., Chauhan, J., & Kaushik, P. (2020). Medicinal value of domiciliary ornamental plants of the Asteraceae family. Journal of Young Pharmacists, 12(1), 3–10.

Salamone, F., Galvano, F., Cappello, F., Mangiameli, A., Barbagallo, I., & Li Volti, G. (2012). Silibinin modulates lipid homeostasis and inhibits nuclear factor kappa B activation in experimental nonalcoholic steatohepatitis. Translational Research, 159(6), 477–486.

Salomone, F., Barbagallo, I., Godos, J., Lembo, V., Currenti, W., Cinà, D., Avola, R., D'Orazio, N., Morisco, F., Galvano, F., & Li Volti, G. (2017). Silibinin restores NAD+ levels and induces the SIRT1/AMPK pathway in non-alcoholic fatty liver. Nutrients, 9(10), 1086.

Salomone, F., Godos, J., Catania, M., Magrì, G., & Galvano, F. (2015). Silibinin exerts beneficial effects in experimental and human non-alcoholic steatohepatitis. Digestive and Liver Disease, 47, e234.

Schulten, B., Bulitta, M., Ballering-Brühl, B., Köster, U., & Schäfer, M. (2001). Efficacy of *Echinacea purpurea* in patients with a common cold. A placebo-controlled, randomised, double-blind clinical trial. Arzneimittel-Forschung, 51(7), 563–568.

Sharifi-Rad, M., Mnayer, D., Morais-Braga, M., Carneiro, J., Bezerra, C. F., Coutinho, H., Salehi, B., Martorell, M., Del Mar Contreras, M., Soltani-Nejad, A., Uribe, Y., Yousaf, Z., Iriti, M., & Sharifi-Rad, J. (2018). *Echinacea* plants as antioxidant and antibacterial agents: From traditional medicine to biotechnological applications. Phytotherapy Research, 32(9), 1653–1663.

Skaudickas, D., Kondrotas, A., & Baltrusaitis, K. (2004). The effect of *Echinacea purpurea* extract on sexual glands of male rats. Medicina, 40(12), 1211–1218.

Sperber, S. J., Shah, L. P., Gilbert, R. D., Ritchey, T. W., & Monto, A. S. (2004). *Echinacea purpurea* for prevention of experimental rhinovirus colds. Clinical Infectious Diseases, 38(10), 1367–1371.

Stevenson, J. L., Krishnan, S., Inigo, M. M., Stamatikos, A. D., Gonzales, J. U., & Cooper, J. A. (2016). *Echinacea*-based dietary supplement does not increase maximal aerobic capacity in endurance-trained men and women. Journal of Dietary Supplements, 13(3), 324–338.

Šutovská, M., Capek, P., Kazimierová, I., Pappová, L., Jošková, M., Matulová, M., Fraňová, S., Pawlaczyk, I., & Gancarz, R. (2015). *Echinacea* complex – chemical view and anti-asthmatic profile. Journal of Ethnopharmacology, 175, 163–171.

Tajmohammadi, A., Razavi, B. M., & Hosseinzadeh, H. (2018). *Silybum marianum* (milk thistle) and its main constituent, silymarin, as a potential therapeutic plant in metabolic syndrome: A review. Phytotherapy Research, 32(10), 1933–1949.

Thyagarajan, S. P., Jayaram, S., Gopalakrishnan, V., Hari, R., Jeyakumar, P., & Sripathi, M. S. (2002). Herbal medicines for liver diseases in India. Journal of Gastroenterology and Hepatology, 17, S370–S376.

Torres, M., Rodríguez-Serrano, F., Rosario, D. J., Rodríguez-Perez, F., & Toro, D. H. (2004). Does *Silybum marianum* play a role in the treatment of chronic hepatitis C? Puerto Rico Health Sciences Journal, 23(2 Suppl.), 69–74.

Voroshilin, R., Kurbanova, M., Rassolov, S., & Ulrih, E. (2020). Rabbit dietary supplementation with *Echinacea purpurea* L.: The quality profile of rabbit meat. Food Processing: Techniques and Technology, 50(2), 185–193.

Wesołowska, O., Łania-Pietrzak, B., Kuzdzał, M., Stanczak, K., Mosiadz, D., Dobryszycki, P., Ozyhar, A., Komorowska, M., Hendrich, A. B., & Michalak, K. (2007). Influence of silybin on biophysical properties of phospholipid bilayers. Acta Pharmacologica Sinica, 28(2), 296–306.

Wilson, K. M., Klein, J. D., Sesselberg, T. S., Yussman, S. M., Markow, D. B., Green, A. E., West, J. C., & Gray, N. J. (2006). Use of complementary medicine and dietary supplements among U.S. adolescents. The Journal of Adolescent Health, 38(4), 385–394.

Yamada, K., Hung, P., Park, T. K., Park, P. J., & Lim, B. O. (2011). A comparison of the immunostimulatory effects of the medicinal herbs *Echinacea*, Ashwagandha and Brahmi. Journal of Ethnopharmacology, 137(1), 231–235.

Yao, J., Zhi, M., Gao, X., Hu, P., Li, C., & Yang, X. (2013). Effect and the probable mechanisms of silibinin in regulating insulin resistance in the liver of rats with non-alcoholic fatty liver. Brazilian Journal of Medical and Biological Research, 46(3), 270–277.

Yao, L., Bai, L., Tan, Y., Sun, J., Qu, Q., Shi, D., Guo, S., & Liu, C. (2019). The immunoregulatory effect of sulfated *Echinacea purpurea* polysaccharide on chicken bone marrow-derived dendritic cells. International Journal of Biological Macromolecules, 139, 1123–1132.

Zazharskyi, V. V., Davydenko, P. O., Kulishenko, O. M., Borovik, I. V., & Brygadyrenko, V. V. (2019). Antimicrobial activity of 50 plant extracts. Biosystems Diversity, 27(2), 163–169.

Zazharskyi, V. V., Davydenko, P. O., Kulishenko, O. M., Borovik, I. V., Zazharska, N. M., & Brygadyrenko, V. V. (2020). Antibacterial and fungicidal activities of ethanol extracts of 38 species of plants. Biosystems Diversity, 28(3), 281–289.