Rocz Panstw Zakl Hig 2023;74(4):447-458
http://wydawnictwa.pzh.gov.pl/roczniki_pzh/ https://doi.org/10.32394/rpzh.2023.0281

ORIGINAL ARTICLE

INFLUENCE OF *INULA HELENIUM* RHIZOMES AND *MATRICARIA CHAMOMILLA* INFLORESCENCES ON THE BIOCHEMICAL AND PHYSIOLOGICAL PARAMETERS IN MALE RATS FED A HIGH-FAT DIET

Maryna Lieshchova[1], *Valentyn Yefimov*[1], *Victor Brygadyrenko*[1,2]

[1]Dnipro State Agrarian and Economic University, Dnipro, Ukraine
[2]Oles Honchar Dnipro National University, Dnipro, Ukraine

ABSTRACT

Background. Pharmacological correction of a high-fat diet is of great interest to prevent the development of obesity and hypertension. More and more research is being done on the preventive use of medicinal herbs for excess caloric intake.
Objective. The aim of this study was to determine the general physiological effect of *I. helenium* rhizomes and *M. chamomilla* inflorescences used in the diet of male rats consuming excess amounts of fat and calories in the daily diet.
Material and methods. In a 30-day experiment, we determined the effect of *I. helenium* rhizome and *M. chamomilla* on the physiological activity and metabolic processes of laboratory rats consuming a high-fat diet. The physical activity was evaluated according to the mass gain of animals and change in the relative mass of the internal organs, and also the functional conditions of the central nervous system. The influence on the metabolic processes was revealed by biochemical and clinical blood analyses.
Results. In a laboratory experiment on male rats, it was found that the addition of dry crushed rhizomes of *Inula helenium* L. and inflorescences of *Matricaria chamomilla* L. to the diet caused opposite changes in body weight. In the control group, the animals slightly increased their body weight (up to 111.5% of the initial weight by the end of the experiment); the rhizomes of *I. helenium* caused a decrease in body weight gain (up to 105.5% on the 30th day of the experiment compared to the initial weight); rats fed *M. chamomilla* inflorescences gained 123.2% of their initial body weight during the month of the experiment. The rhizomes of *I. helenium* caused an increase in the stomach relative mass. A decrease in the thymus relative weight was observed when animals were fed *M. chamomilla* inflorescences. The rhizomes of *I. helenium* stimulated an increase of blood protein concentration (mainly due to globulins), an increase in the alkaline phosphatase activity and cholesterol, and a decrease in the triglycerides concentration. *M. chamomilla* inflorescences reduced the blood urea concentration and increased the activity of alkaline phosphatase, causing strong changes in fat metabolism. Under the influence of the diet with the addition of *M. chamomilla* inflorescences, the atherogenic index increased in animals by 6.5 times relatively to the control group (due to a decrease in the concentration of high-density lipoprotein cholesterol and an increase in the concentration of low-density lipoprotein cholesterol, an increase in the total cholesterol concentration). When *M. chamomilla* was added to the diet, the blood triglycerides concentration in animals decreased sharply and the concentration of leukocytes increased. The concentration of monocytes exceeded the limits of the physiological norm both in the control group and in the group of animals fed on *M. chamomilla* inflorescences.
Conclusions. The results of the studies show the promise for further research of *I. helenium* rhizomes in the prevention of hypertension and also indicate strong risks when using *M. chamomilla* inflorescences for preventive purposes during high-fat and hypercaloric diet.

Key words: *relative organs' mass, increase in the bodyweight, high-fat diet, pharmaceutical chamomile, elecampane, phytotherapy, weight loss.*

INTRODUCTION

Excessive fat intake is one of the major health problems at the global level [27, 52, 65]. High-calorie food causes metabolic changes, disturbances in the digestive and endocrine systems, and most importantly, stimulates the vascular diseases development [7, 19, 46]. Problems of excess dietary energy are often addressed by adding spices, aromatics and medicinal herbs to human food [18, 50]. However, their use can not only cause positive health changes in the body but can also stimulate an increase in pathological changes caused by excess calorie intake [24, 33, 35, 37]. Some of the most widely used plants in traditional folk medicine and modern medical practice are elecampane and chamomile [70].

Corresponding author: Maryna Lieshchova, Dnipro State Agrarian and Economic University, Serhii Efremov st., 25, Dnipro, 49600, Ukraine. Tel.: +38-056-268-54-17, e-mail: lieshchova.m.o@dsau.dp.ua

Publisher: National Institute of Public Health NIH - National Research Institute

Elecampane (*Inula helenium L.*) has an expectorant and anti-inflammatory effect, reduces intestinal motility and secretion of gastric juice. *Tavares and Seca* [58] in their study with *Inula L.* identified the most promising secondary metabolites in the treatment of human diseases associated with oxidative stress. The most promising metabolite was β-caryophyllene, as it showed the highest antioxidant activity, even in comparison with ascorbic acid [62, 75].

The main biologically active substance of elecampane is believed to be alantolactone and associated terpenoids [32]. Studies have been conducted on alantolactone, obtained from the *I. helenium* rhizomes, which showed the effectiveness of this substance against tumor cells [3, 12, 67, 74]. *Koc* et al. [31] revealed antioxidant and antitumor activity of *I. helenium* extract. Alantolactone has also been shown to have anti-inflammatory activity by enhancing the phagocytic activity of macrophages [22, 49]. The essential oil of *I. helenium* rhizomes has bactericidal and fungicidal properties [26, 29]. In folk medicine, tinctures and rhizome's extracts are used for malaria, oedema, urolithiasis, hypoacid gastritis, migraine, whooping cough, bronchial asthma, as a haemostatic, diuretic, and anti-inflammatory agent for skin diseases [10, 19, 64].

Since ancient times, chamomile (*Matricaria chamomilla* L.) has been known as a valuable medicinal plant, which is used both in traditional medicine as well as in the official pharmacopoeia of 26 countries [57, 58]. Chamomile contains a number of phytochemicals: flavonoids and other phenolic compounds, essential oil, polysaccharides, amino acids, fatty acids and mineral elements [39, 44, 53].

Due to the diverse composition of active substances, chamomile preparations have numerous pharmacological effects. Chamazulene has anti-inflammatory, sedative, antiallergic and local anaesthetic properties [26], it activates the function of immune organs, and enhances regenerative processes. There have been shown antidiabetic and hypocholesterolemic effects of chamomile extract in animal studies [47, 71, 72]. *Zand* et al. [73] showed weak estrogenic and progestogenic effects of chamomile extract at high concentrations, and *Kabiri* et al. [28] compared the effect of chamomile syrup and cabergoline in reducing the blood prolactin concentration in patients with idiopathic prolactinemia.

Chamomile preparations have a pronounced effect on the nervous system [54]. It has been experimentally shown that chamomile essential oil enhances reflex activity, stimulates the medulla oblongata, strengthens and quickens breathing, increases heart rate, dilates the blood vessels of animals' brain, but in large doses can cause central nervous system depression and muscle tone decrease [16, 51].

Preparations made from chamomile are often used externally primarily as an anti-inflammatory agent, but the infusion of chamomile inflorescence inflorescence has a haemostatic, antiseptic, mild astringent, analgesic, sedative, anticonvulsant, diaphoretic, and choleretic effect [25]. Research with animals suggests antispasmodic, anxiolytic, anti-inflammatory and some antimutagenic and cholesterol-lowering effects for chamomile [45].

Thus, both medicinal plants – elecampane and chamomile – are recommended for the treatment of certain pathological changes amid hypertension, are widely used in folk and official medicine, and are used by people in everyday life. However, comprehensive studies of changes in the body against the background of excessive fat consumption have not yet been carried out for the herbal preparations *I. helenium* and *M. chamomilla*.

The objective of this study was to determine the general physiological effect of *I. helenium* rhizomes and *M. chamomilla* inflorescences used in the diet of male rats consuming excess amounts of fat and calories in the daily diet.

MATERIAL AND METHODS

Ethics. 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 (Decision No. 3/20-21 of 20.09.2020). 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, 18th March, 1986, ETS No. 123) and in Law of Ukraine "On protection of animals from cruel treatment" (Kyiv, 21st February, 2006, No. 3447-IV).

Animals. The experiment used 24 adult white outbred male laboratory rats, weighing 200 ± 10 g. The rats were divided into a control group and two experimental groups (n = 8). The rats were kept in groups of 4 in polycarbonate cages with steel mesh lids and a food recess. The temperature in the room where the experimental animals were located was 20–22 °C, relative air humidity was 50–65%, and the light regime was 12 hours light/12 hours dark.

Diet. Animals received water and food ad libitum. The diet of all animals had an excess fat content (3600 kcal/kg). The high-fat diet was based on a standard diet (75% grain mixture (corn, sunflower grain, wheat, barley, soybeans), 8% root vegetables (potatoes, carrots), 2% meat-and-bone meal, 2% mineral-vitamin complex) with the introduction of 15 % sunflower oil.

The control group of animals received a high-fat diet, and the experimental group received a semi-synthetic diet, to which a dry medicinal plant of 5% of the feed weight was added. In the first experimental group, 5% of dry *I. helenium* crushed rhizomes of the second year of life were additionally added to the high-fat diet. The raw materials were collected in mid-August. The rhizomes were washed in water, cut into pieces 5–7 mm thick, and dried at room temperature without direct sunlight. The second experimental group had 5% of crushed dry chamomile inflorescences to the high-fat diet, which were collected manually as the flowers bloomed on the plants. They were dried without direct sunlight at room temperature.

The main ingredients of the diet were ground in a mill (grain, meat-and-bone meal, mineral and vitamin complex, dry rhizomes and inflorescences of medicinal plants) and mixed, then sunflower oil was added and granules were made at the rate of 4,200 g for each group for the entire period of the experiment (30 days). Root vegetables in appropriate quantities were additionally given fresh every day. During the experiment, we took into account the amount of food consumed by each group per day and the total amount of food consumed during the entire period of the experiment.

Morphometric parameters (live weight, abdominal volume) were determined on days 1–4 and on days 26–30 of the experiment. The total increase in animal weight and daily increase in live weight were calculated. Orientation-physical activity and the emotional state of experimental animals were studied in the "open field" test [55]. We used an installation consisting of 1 m^2 square area, divided into 16 squares and limited by an opaque wall 20 cm high. The experiment was carried out in complete silence with intense lighting of the field itself. The animal, taken from a cage in a previously darkened room, was placed in the centre of the field. The exposure time was 2 minutes. Animals were tested for four days (days 1–4) at the beginning of the experiment and four days at its end (days 26–30). The number of crossed squares was counted: peripheral and central – physical activity was assessed; peripheral (with support on the wall) and central (without support on the wall) racks – orientation activity; the number of grooming acts, defecation and urination – emotional state [34, 36].

Animals were euthanized on the 30th day of the experiment under anaesthesia (80 mg/kg ketamine and 12 mg/kg xylazine, intraperitoneally) by total bleeding from the heart. After the autopsy, the condition of the internal organs was visually assessed for the presence of pathological changes, organs were selected and weighed (heart, liver, lungs, thymus, spleen, stomach, small and large intestines, kidneys). The mass of internal organs was determined with an accuracy of 10 mg [8].

Biochemical studies of blood serum were carried out on an automatic analyser Miura 200 (Italy) using reagent kits High Technology (USA), PZ Cormay S.A. (Poland) and Spinreact S.A. (Spain). Total protein was determined using the biuret method; globulins and protein coefficient - by calculation; albumin concentration – by reaction with bromocresol green; activity of aspartate aminotransferase (AST) and alanine aminotransferase (ALT) – by a kinetic method based on the *Warburg* optical test; alkaline phosphatase – enzymatically by reaction with p-nitrophenyl phosphate; glucose – using the glucose oxidase method [11].

Characteristics of lipid metabolism. The concentration of total cholesterol was determined enzymatically using cholesterol oxidase; triglycerides – after cleavage by lipoprotein lipase with detection by the Trinder reaction; HDL and LDL – using selective detergents with subsequent staining of the enzymatic reaction products, the atherogenic index was calculated.

On the biochemical markers of inflammation, C-reactive protein was determined by immunoturbidimetry.

The number of red and white blood cells, haematocrit and haemoglobin content were determined in the rats' blood after the addition of K3EDTA using a PCV-80 Vet automatic haematology analyser. For leukogram, blood smears were prepared according to *Pappenheim*.

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

RESULTS

The addition of dry crushed rhizomes of *Inula helenium* L. and inflorescences of *Matricaria chamomilla* L. to the diet of rats caused opposite changes in the dynamics of the animals' body weight. If in the control group the weight of male rats increased up to 110.0% of the weight on the first day of the experiment, slightly increasing to 111.5% by the end of the experiment, then the rhizomes of *I. helenium* caused a decrease in the body weight of animals to 95.0% of the initial weight on the 13th day of the experiment (Figure 1a). On the 26th day, the weight of the rats reached a maximum (107.9% from the beginning of the experiment), decreasing to 105.5% by the 30th day. Rats fed *M. chamomilla* inflorescences increased their body weight relatively evenly, reaching 123.2% of their body weight at the beginning of the experiment (Fig. 1b). Thus, the addition of *I. helenium* rhizomes to the diet reduced the weight of animals by 6.0% by the end of the experiment compared to the control, and the addition of *M. chamomilla* inflorescences increased it by 11.7% compared to the control group (Figure 1).

Probably, the bitter taste of the rhizomes of *I. helenium* caused a decrease in food consumption in male rats to the level of 81.9% of the control group, but we did not observe any changes in fluid consumption by the animals (Table 1). It is interesting that with a significant increase in body weight in animals fed on *M. chamomilla* inflorescences (1505 ± 198 compared to 700 ± 271 mg/day in the control group), their food consumption did not increase (96.0% of the control group), there was only slightly increased water consumption (105.1% of the control group, Table 1). Abdominal volume also increased when rats ate *M. chamomilla* inflorescences and amounted to 111.2% of the control group, remaining unchanged on the diet

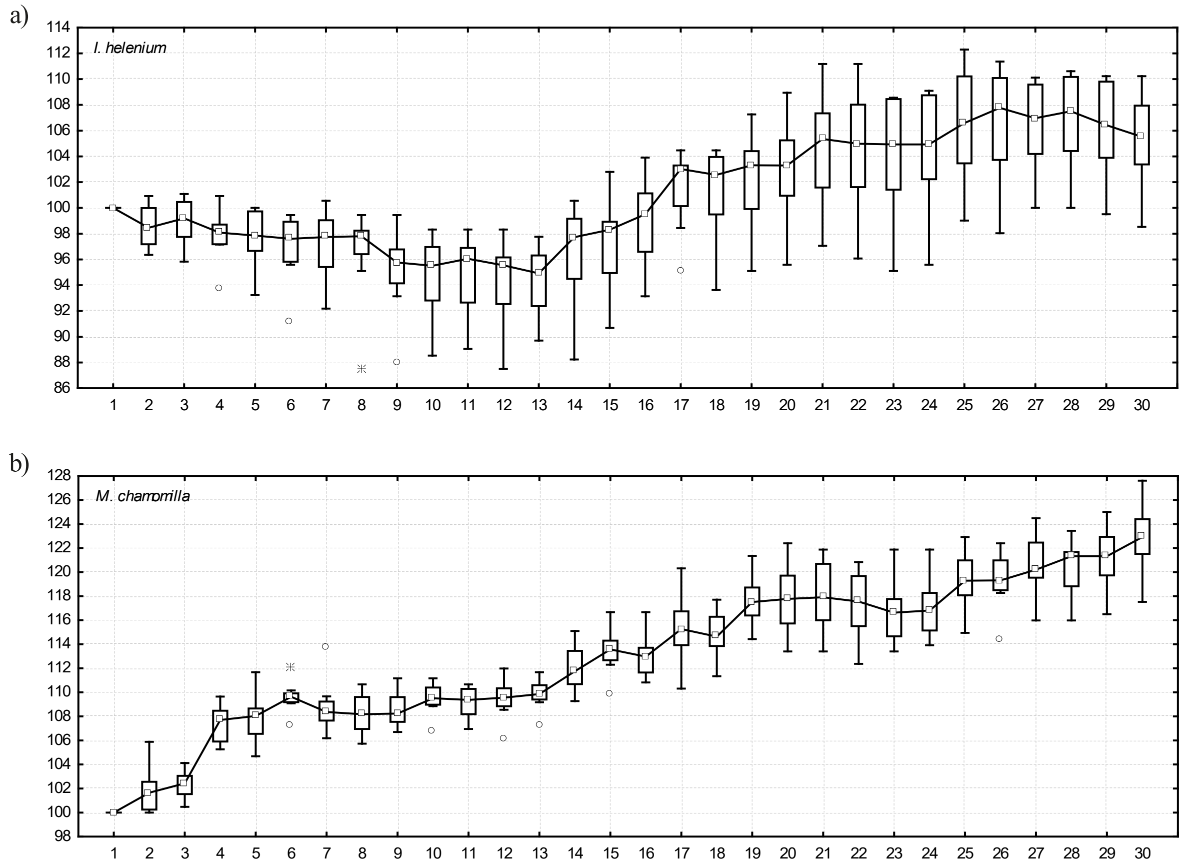

Figure 1. Changes in the rats' body weight when adding crushed rhizomes of *Inula helenium* L. (a) and *Matricaria chamomilla* L. inflorescences (b) to the diet of animals: the x-axis is the day of the experiment, the y-axis is the animals' body weight (% relative to the initial body weight of each animal, taken as 100% at the beginning of the experiment); small square – median, upper and lower borders of the rectangle – 25% and 75% quartiles, vertical line – minimum and maximum values, circles – outliers; n=8

Table 1. Change in the body weight and fodder consumption of young male rats under the impact of *Inula helenium* L. and *Matricaria chamomilla* L supplementation to their diet (x ± SD, n = 8, duration of experiment – 30 days)

Parameter	Control	*I. helenium*	*I. helenium* compared to control, %	*M. chamomilla*	*M. chamomilla* compared to control, %
Consumption of food by animals, g/day	20.09	16.46	81.9	19.29	96.0
Consumption of water by animals, g/day	18.42	17.27	93.8	19.36	105.1
Change in body weight, µg/day	700 ± 271	325 ± 208*	46.4	1505 ± 198***	215.0
Change in body weight, %/30 day	13.6 ± 5.9	5.3 ± 3.5*	38.9	22.9 ± 2.9**	167.6
Abdominal volume, cm	14.00 ± 0.46	14.50 ± 0.56	103.6	15.57 ± 0.68**	111.2

Note: * – P < 0.05, ** – P < 0.01, *** – P < 0.001 probability of differences compared to control within one line of the table according to the results of comparison using the ANOVA with Bonferroni correction.

supplemented with *I. helenium* rhizomes (103.6% of the control group, Table 1).

The rhizomes of *I. helenium* caused an increase of up to 138.3% of the control group in the relative weight of the stomach (Table 2). A significant decrease in the relative weight of the thymus to 42.7% of the control group was observed when animals were fed *M. chamomilla* inflorescences (Table 2). The relative weight of other organs did not change statistically significantly compared to the control group.

The rhizomes of *I. helenium* in animals' diet increased the blood total protein concentration to 117.0% (mainly due to globulins, which reached a level of 123.6% of the control group, Table 3). *I. helenium*

Table 2. Change in relative organ mass (%) of male rats under the influence of *Inula helenium* L. and *Matricaria chamomilla* L. addition to their diet (x ± SD, n = 8, duration of experiment – 30 days)

Organ	Control	*I. helenium*	*I. helenium* compared to control, %	*M. chamomilla*	*M. chamomilla* compared to control, %
Heart	0.352 ± 0.023	0.345 ± 0.031	98.0	0.355 ± 0.012	101.0
Liver	4.08 ± 0.17	4.23 ± 0.24	103.7	4.15 ± 0.47	101.7
Lungs	0.979 ± 0.169	0.846 ± 0.153	86.4	0.952 ± 0.327	97.3
Thymus	0.285 ± 0.046	0.210 ± 0.076	73.7	0.122 ± 0.065***	42.7
Spleen	0.370 ± 0.036	0.353 ± 0.050	95.6	0.334 ± 0.035	90.4
Stomach	0.699 ± 0.060	0.966 ± 0.126***	138.3	0.599 ± 0.069	85.6
Small intestine	2.58 ± 0.52	2.60 ± 0.31	100.7	2.17 ± 0.24	83.9
Cecum	0.509 ± 0.176	0.458 ± 0.124	90.0	0.392 ± 0.084	77.0
Colon	0.374 ± 0.085	0.388 ± 0.065	103.8	0.315 ± 0.050	84.3
Rectum	0.398 ± 0.073	0.381 ± 0.109	95.6	0.368 ± 0.089	92.4
Right kidney	0.358 ± 0.031	0.313 ± 0.030	87.4	0.341 ± 0.035	95.2
Left kidney	0.372 ± 0.040	0.303 ± 0.032	81.4	0.333 ± 0.026	89.5
Brain	0.867 ± 0.052	0.829 ± 0.065	95.7	0.613 ± 0.253	70.7

Note: see Table 1

Table 3. Changes in blood biochemical parameters of male rats under effect of *Inula helenium* L. and *Matricaria chamomilla* L supplementation (x ± SD, n = 8, duration of experiment – 30 days)

Parameters	Control	*I. helenium*	*I. helenium* compared to control, %	*M. chamomilla*	*M. chamomilla* compared to control, %
Total protein, g/L	77.0 ± 4.9	90.1 ± 6.2**	117.0	67.6 ± 27.7	87.8
Albumins, g/L	39.6 ± 2.6	43.9 ± 2.6	110.9	41.2 ± 2.0	104.0
Globulins, g/L	37.4 ± 3.9	46.3 ± 4.5*	123.6	37.7 ± 2.1	100.6
Albumin/Globulin ratio, U	1.10 ± 0.15	0.83 ± 0.32	75.0	1.10 ± 0.10	100.0
Urea, mmol/L	6.84 ± 1.02	6.06 ± 1.24	88.6	3.97 ± 0.40***	58.0
Creatinine, µmol/L	63.0 ± 4.4	65.5 ± 7.3	104.0	69.5 ± 5.3	110.3
AST, U/L,	186 ± 61	183 ± 54	98.4	171 ± 31	91.6
ALT, U/L	131 ± 41	186 ± 77	142.0	111 ± 16	84.7
Alkaline phosphatase, U/L	129 ± 64	330 ± 90***	255.6	525 ± 101***	407.0
Total bilirubin, µmol/L	6.07 ± 1.67	6.96 ± 1.91	114.7	4.00 ± 1.42	65.9
Glucose, mmol/L	7.39 ± 1.04	6.88 ± 0.85	93.1	7.43 ± 0.88	100.6
Total calcium (Ca), mmol/L	2.53 ± 0.09	2.38 ± 0.19	93.9	2.43 ± 0.11	96.2
Inorganic phosphorus (P), mmol/L	3.07 ± 0.58	2.99 ± 0.84	97.3	2.78 ± 0.35	90.6
Gamma-glutamyl-transferase, U/L	9.29 ± 2.60	9.38 ± 2.60	101.0	7.33 ± 0.94	79.0
Cholesterol, mmol/L	1.27 ± 0.14	1.55 ± 0.17*	121.9	1.57 ± 0.21*	123.2
Blood triglycerides, mmol/L	2.13 ± 0.55	0.98 ± 0.37***	45.8	0.65 ± 0.11***	30.5
HDL cholesterol, mmol/L	0.650 ± 0.128	0.575 ± 0.348	88.5	0.212 ± 0.047***	32.6
LDL cholesterol, mmol/L	0.517 ± 0.290	0.733 ± 0.305	141.6	1.460 ± 0.158***	282.3
C-reactive protein, mg/L	12.5 ± 5.4	19.0 ± 10.6	151.6	8.8 ± 1.9*	70.2
Atherogenic index of plasma	1.04 ± 0.45	2.69 ± 1.88	258.0	6.76 ± 1.92***	648.9

Note: see Table 1.

rhizomes also greatly increased alkaline phosphatase activity (up to 255.6% of the control group), cholesterol concentration (up to 121.9% of the control group) and decreased triglyceride concentration (up to 45.8% of the control group). Despite the increase in the average atherogenic index value by 2.58 times (Table 3), but this change was statistically insignificant, since it was not observed in all animals in this group.

M. chamomilla inflorescences (Table 3) had little effect on blood albumins and globulins and their ratio, but reduced the blood urea and urea nitrogen concentrations in animals by up to 58.0% of the control group. Under the influence of *M. chamomilla*, alkaline phosphatase activity increased to 407.0% of the control group. However, the most dramatic changes occurred in fat metabolism: under the influence of a diet with *M. chamomilla* inflorescences supplementation (Table 5), the atherogenic index increased in male rats up to 648.9% relatively to the control group (both due to a decrease in the concentration of high-density lipoprotein cholesterol (HDL) to 32.6%, and due to an increase up to 282.3% in low-density lipoprotein cholesterol concentration (LDL)). The cholesterol concentration also significantly increased (up to 123.2% of the control group level). When *M. chamomilla* was added to the diet, the blood triglycerides concentration in animals sharply decreased – to 30.5% of the level of the control group (Table 3).

When supplementing with *I. helenium* rhizomes, we did not observe significant changes in the rats' blood cytological composition (Table 4). The addition of *M. chamomilla* inflorescences to the diet (Table 4) also generally did not cause significant changes in complete blood count and leukogram, except for a sharp increase in the leukocytes concentration up to 162.5% of the control group level (however, the concentration of leukocytes was still within the physiological norm). It should be noted that being on a hypercaloric diet with the addition of *I. helenium* rhizomes caused a decrease in haematocrit. The number of monocytes under the *I. helenium* rhizomes influence tended to decrease, however, it was not considerable due to the significant individual indicator variability (Table 4).

The physical activity (Figure 2a) of the animals did not change significantly by the end of the experiment when fed crushed rhizomes of *I. helenium* and inflorescences of *M. chamomilla*. Under the influence of the same plants, the orientation activity of male rats did not change significantly (Figure 2b). Important changes in emotional state were also not observed during the experiment, however, in the animals group fed the diet supplemented with *I. helenium* crushed rhizomes, a tendency towards a decrease in emotional state was recorded, especially noticeable towards the end of the experiment (Figure 2c). No significant changes in the open field test were observed between the groups of rats with *I. helenium* and *M. chamomilla* in the diet and the control group at the beginning and end of the experiment (Table 5).

DISCUSSION

The use of elecampane rhizome ethanol extract (*Inula helenium* L.) in doses from 0 to 1000 mg/kg of

Table 4. Change in complete blood count and leukogram of male rats under effect supplementing with *Inula helenium* L. and *Matricaria chamomilla* L. ($x \pm SD$, $n = 8$, duration of experiment – 30 days)

Parameter	Control	*I. helenium*	*I. helenium* compared to control, %	*M. chamomilla*	*M. chamomilla* compared to control, %
Hemoglobin, g/L	127 ± 7	117 ± 13	92.4	116 ± 14	91.8
Hematocrit, %	40.5 ± 2.7	36.5 ± 4.3	90.2	40.6 ± 6.8	100.3
Erythrocytes, 10^{12}/L	6.93 ± 0.29	6.48 ± 0.73	93.5	6.88 ± 0.79	99.3
Erythrocyte sedimentation rate (ESR), mm/h	1.17 ± 0.37	1.00 ± 0.00	85.7	1.00 ± 0.00	85.7
Thrombocytes, 10^9/L	339 ± 66	272 ± 93	80.3	298 ± 67	88.1
Leukocytes, 10^9/L	8.6 ± 1.6	8.6 ± 4.3	100.1	13.9 ± 3.3***	162.5
Leukocytic formula	–	–	–	–	–
Basophils, %	0.0 ± 0.0	0.0 ± 0.0	–	0.0 ± 0.0	–
Eosinophils, %	1.50 ± 0.76	0.50 ± 0.50	33.3	0.60 ± 0.49	40.0
Neutrophils, %:	–	–	–	–	–
– band	1.17 ± 0.69	1.13 ± 0.78	96.4	0.60 ± 0.80	51.4
– with segmented nuclei	23.0 ± 8.2	22.8 ± 7.3	98.9	18.6 ± 3.2	80.9
Lymphocytes, %	68.8 ± 8.6	72.9 ± 7.7	105.9	71.0 ± 8.1	103.1
Monocytes, %	5.5 ± 1.3	2.8 ± 2.2	50.0	5.6 ± 2.1	101.8

Note: see Table 1.

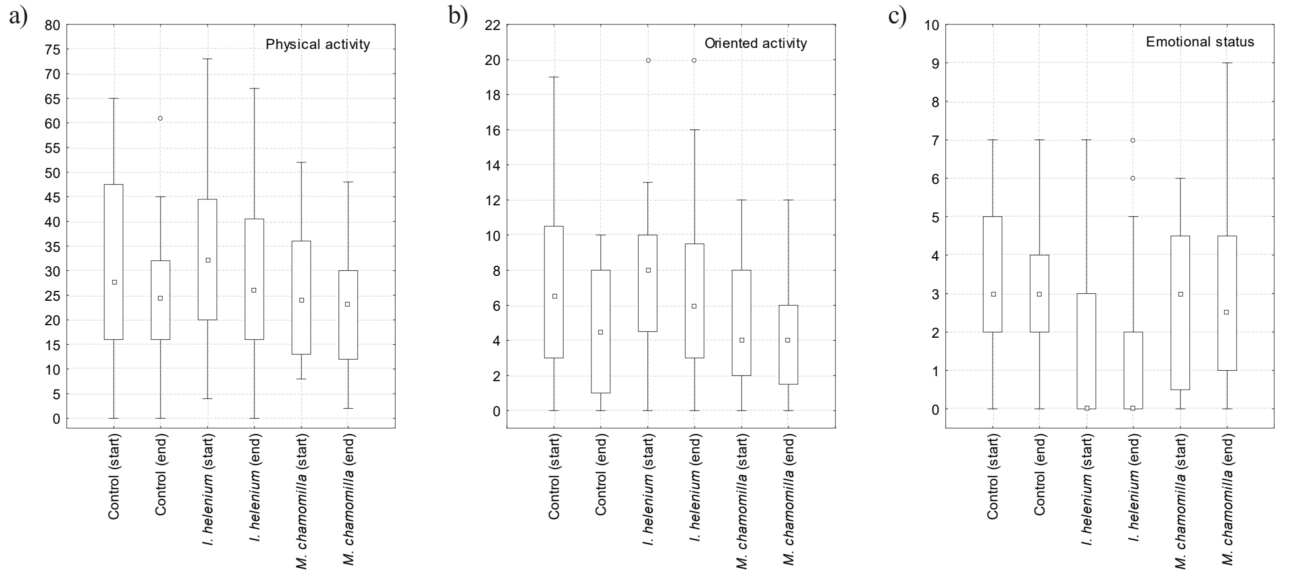

Figure 2. Changes in physical (a) and orientation activity (b), as well as emotional state (c) of male rats when crushed rhizomes of *Inula helenium* L. and inflorescences of *Matricaria chamomilla* L. were added to their diet: along the x-axis – animals' group (n = n1 * n2 = 32: n1 = 8 animals, n2 = 4 experiments with each animal), on a diet with excess fat and the addition of plants (the days after the start of the experiment are indicated in parentheses: 1–4th – start or 26–30 - end), along the ordinate - the absolute number of markers of this behavior type during 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 emotional state - the number of grooming, defecation and urination acts; small square – median, upper and lower edges of the rectangle – 25% and 75% quartiles, vertical line – minimum and maximum values, circles – outliers; Tukey's test results showed no significant ($P<0.05$) differences between animal's groups.

Table 5. Changes in behavioral characteristics of three rats groups over a 2-minute experiment, to whose diet *Inula helenium* L. rhizomes and *Matricaria chamomilla* L inflorescences were added (x ± SD, n = 32, duration of the experiment was 30 days)

Характеристика	Control, 1–4th days	Control, 26–30th days	*I. helenium*, 1–4th days	*I. helenium* 26–30th days	*M. chamomilla*, 1–4th days	*M. chamomilla*, 26–30th days
Number of visited peripheral squares	28.1 ± 18.0	24.3 ± 14.5	31.9 ± 15.0	27.3 ± 15.9	24.8 ± 12.8	22.8 ± 11.9
Number of visited central squares	1.00 ± 2.34	0.29 ± 1.04	1.00 ± 1.50	1.34 ± 2.50	0.50 ± 1.17	0.25 ± 0.70
Number of racks in peripheral squares	5.58 ± 4.53	3.79 ± 3.13	5.56 ± 2.90	4.44 ± 3.22	3.61 ± 2.67	2.86 ± 2.19
Number of racks in central squares	1.29 ± 1.43	0.71 ± 1.00	2.22 ± 1.72	2.22 ± 2.11	1.50 ± 1.86	1.29 ± 1.63
Number of grooming acts	0.583 ± 0.830	0.583 ± 0.929	0.438 ± 0.801	0.375 ± 0.907	0.714 ± 1.150	0.679 ± 1.020
Number of faecal boluses	2.25 ± 2.03	2.38 ± 1.56	1.16 ± 1.73	1.00 ± 1.74	1.93 ± 1.72	2.25 ± 1.96
Number of urination	0.333 ± 0.482	0.375 ± 0.495	0.063 ± 0.246	0.031 ± 0.177	0.071 ± 0.262	0.000 ± 0.000

Notes: No significant differences ($P<0.05$) were found between the groups for all studied parameters according to the results of the Tukey test.

diet causes a linear increase in broiler chickens' weight gain due to an increase in the digestibility of feed dry matter, but the level of its consumption remains unchanged [1, 2]. Our studies noted a decrease in food consumption and, accordingly, a decrease in body weight in rats. This is likely due to the bitter and pungent aftertaste characteristic of the *I. helenium* rhizomes [38], which could cause a decrease in animals' appetite. Inula helenium contains sesquiterpene lactones, which, depending on the dose, can cause both an anti-inflammatory and a local irritant effect [14]. This may probably explain the increase in stomach weight in rats fed the roots and rhizomes of *I. helenium* (considering that C-reactive protein level both on average in the group and in individual animals was higher than in the control group, although changes were reliable). At the same time, the WBC number under the influence of *I. helenium* did not change, which also indicates the absence of a pronounced inflammatory process.

The RBC number, as well as hemoglobin concentration and hematocrit in the animals' blood of all groups were within the reference values [20]. At the same time, the use of *M. chamomilla* inflorescences led to a significant increase in the WBC number, which was also noted by *Alsaadi* et al. [4] in rabbits when they used an aqueous solution of chamomile inflorescences, as well as when they injected a methanolic extract of this plant into mice [21]. The noted changes were accompanied by a trend towards an increase in the concentration of lymphocytes, which was also noted by *Shwaikh* et al. [56]. It is possible that stimulation of the humoral immune system by the chamomile inflorescences active substances also caused a decrease in thymus mass in these animals, although this effect requires further study.

The number of monocytes in the rats' blood of the control group and animals that received *M. chamomilla* inflorescences did not differ significantly. Although the number of these blood cells was within the reference level [15], it was close to its upper limit. At the same time, the noted tendency towards a decrease in the concentration of monocytes under the influence of *I. helenium* rhizomes can be regarded as a positive phenomenon for health due to a possible reduction in the risk of developing atherosclerosis. The high monocytes concentration in the pigs' blood [30] correlates with their adhesion level to the endothelium of large arterial vessels, which subsequently leads to the appearance of atherosclerotic changes in the circulatory system.

Changes in the blood cellular composition and organs morphological features of rats on a high-fat diet should be assessed taking into account biochemical parameters, which are largely the first to undergo changes.

Total protein concentration was higher in rats fed *I. helenium*. Significant changes were due to a significant increase in the content of globulin protein fractions. According to *Petterino and Argentino-Storino* [43], the total protein and albumin blood concentration in rats is 58–81 and 24–41 g/L, respectively. The noted increase in globulin concentration under the *I. helenium* influence may be associated with the immunostimulating effect of phenolic compounds and alantolactone, which are part of elecampane rhizomes [23].

Transamination enzymes, AST and ALT, are considered to be biomarkers of liver damage. An increase in their blood serum concentration is characteristic of the hepatocyte cytolysis process [66]. Our studies showed high activity of both enzymes compared to reference values [66], with no significant differences between animals' groups. Obviously, the reason for this is the development of non-alcoholic fatty liver disease (NAFLD), which is characteristic of animals and humans on a high-fat diet [5, 41, 63]. NAFLD is either a cause or a consequence of the metabolic syndrome development, which is characterized by a combination of risk factors for cardiovascular disease and type 2 diabetes [48].

Cholesterol is an essential component of cell membranes, ensuring their permeability and fluidity. In addition, cholesterol is a precursor to steroid hormones, bile acids, and vitamin D [69]. Our work noted an increase in the concentration of cholesterol in the blood serum both under the *I. helenium* rhizomes and *M. chamomilla* inflorescences influence. More importantly, there was an increase in the cholesterol level that is part of low-density lipoproteins amid a decrease in the content of high-density lipoprotein cholesterol. A relationship has been established between an increase in LDL-C levels, a decrease in HDL-C and the risk development of cardiovascular diseases due to the development of vascular atherosclerosis [13, 40], which is confirmed by a significant increase in the atherogenic index in experimental animals. Reducing serum LDL cholesterol concentrations is not always prophylactically effective due to high triglyceride concentrations [9]. Therefore, the decrease we noted in the concentration of triglycerides under the influence of the elecampane and chamomile active ingredients can be regarded as a positive factor, since triglycerides and their metabolites can contribute to the development of atherosclerosis by modulating inflammation, oxidative stress and foam cells formation [68].

Some chamomile components like flavonoids have effects on the central nervous system through neurotransmitter (GABAergic, serotonergic, noradrenergic and dopaminergic) and have sedative, anxiolytic and antidepressant effects [6, 42, 61]. In our experiment, the consumption of the studied plants amid a high-fat diet did not affect the physical and orientation activity of the animals. In the group of animals that received *I. helenium* in addition to a high-fat diet, by the end of the experiment a tendency towards a decrease in emotional state was revealed, which indicates the need to continue further research about the elecampane and its active substances effects on behavioural reactions and the nervous system as a whole.

CONCLUSIONS

When rats were kept on a hypercaloric diet, a number of characteristic features were noted indicating the development of metabolic syndrome in them, accompanied by an increase in body weight, as well as an increase in the transamination enzymes activity, indicating the manifestation of non-alcoholic fatty liver disease syndrome. The introduction of elecampane rhizomes into their menu leads to a decrease in the body weight gain intensity with a simultaneous increase in the stomach size, apparently due to the specific taste qualities and the presence of irritating substances in the plant composition itself. The active substances of elecampane rhizomes stimulate an increase in the concentration of total protein globulin fractions in the blood serum, which may be a consequence of the plant immunomodulatory effect.

The active ingredients of chamomile inflorescences have the most pronounced effect on the rats' body receiving a high-fat diet, which is manifested by an increase in rats' body weight against the background of an increase in abdominal volume, nitrogen retention in the body, and an increase in the activity of bone tissue cellular components. In addition, we have established an increase in the WBC concentration due to stimulation, presumably, of the immune system's humoral component.

A number of morphological and physiological-biochemical changes that we noted, together with a positive effect on the body of rats, indicate the possibility of increasing the risk of cardiovascular diseases amid the long-term oral use of chamomile inflorescences and elecampane rhizomes due to the development of dyslipidaemia. However, this requires additional research, given the decrease in triglyceride concentrations under the influence of herbal remedies from both plant species.

Funding

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

Conflict of interest

The authors declare no conflict of interest.

REFERENCES

1. *Abolfathi M. E., Tabeidian S. A., Foroozandeh Shahraki A. D., Tabatabaei S. N., Habibian M.*: Effects of ethanol extract of elecampane (*Inula helenium* L.) rhizome on growth performance, diet digestibility, gut health, and antioxidant status in broiler chickens. Livestock Science 2019;223:68–75. http://doi.org/10.1016/j.livsci.2019.03.006.
2. *Abolfathi M.-E., Tabeidian S. A., Foroozandeh Shahraki A. D., Tabatabaei S. N., Habibian, M.*: Comparative effects of n-hexane and methanol extracts of elecampane (*Inula helenium* L.) rhizome on growth performance, carcass traits, feed digestibility, intestinal antioxidant status and ileal microbiota in broiler chickens. Archives of Animal Nutrition 2019;73(2):88–110. http://doi.org/10.1080/1745039x.2019.1581027.
3. *Ahmad B., Gamallat Y., Su P., Husain A., Rehman A. U., Zaky M. Y., Bakheet A. M. H., Tahir N., Xin Y., Liang W.*: Alantolactone induces apoptosis in THP-1 cells through STAT3, survivin inhibition, and intrinsic apoptosis pathway. Chemical Biology and Drug Design 2020;97(2):266–272. http://doi.org/10.1111/cbdd.13778.
4. *Alsaadi S.A.R.A., Al-Perkhdri A.S.A., Al-Hadeedy I.Y.H.*: Effects of *Matricaria chamomilla* flower aqueous extract on some hematological, biochemical parameters and carcass traits in Iraqi local rabbits. Plant Archives 2020;20:1044–1049.
5. *Alanazi S. M., Alsaqer R. A., Alsaeed F. I., Almakhaytah R. M., Buwashl N. T., Mohamed M. E., Younis N. S.*: Studying the actions of sage and thymoquinone combination on metabolic syndrome induced by high-fat diet in rats. European Review for Medical and Pharmacological Sciences 2023;27(6):2404–2418.
6. *Avallone R., Zanoli P., Puia G., Kleinschnitz M., Schreier P., Baraldi M.*: Pharmacological profile of apigenin, a flavonoid isolated from *Matricaria chamomilla*. Biochemical Pharmacology 2000;59(11):1387–1394. https://doi.org/10.1016/s0006-2952(00)00264-1.
7. *Bravo-Ruiz I., Medina M. Á., Martínez-Poveda B.*: From food to genes: Transcriptional regulation of metabolism by lipids and carbohydrates. Nutrients 2021;13(5):1513.
8. *Brygadyrenko V. V., Lieshchova M. A., Bilan M. V., Tishkina N. M., Horchanok A. V.*: 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 2019;10(4):497–506. http://doi.org/10.15421/021973.
9. *Bubb K. J., Nelson A. J., Nicholls S. J.*: Targeting triglycerides to lower residual cardiovascular risk. Expert Review of Cardiovascular Therapy 2022;20(3):185–191. https://doi.org/10.1080/14779072.2022.2058489.
10. *Buza V., Cătană L., Andrei S. M., Ștefănuț L. C., Răileanu Ș., Matei M. C., Vlasiuc I., Cernea M.*: *In vitro* anthelmintic activity assessment of six medicinal plant aqueous extracts against donkey strongyles. Journal of Helminthology 2020;94:e147. http://doi.org/10.1017/s0022149x20000310.
11. *Chawla R.*: Practical clinical biochemistry: Methods and interpretations, 4th ed.; JP Medical Ltd.: London, UK, 2014.
12. *Chun J., Song K., Kim Y. S.*: Sesquiterpene lactones-enriched fraction of Inula helenium L. induces apoptosis through inhibition of signal transducers and activators of transcription 3 signaling pathway in MDA-MB-231 breast cancer cells. Phytotherapy Research 2018;32(12): 2501–2509. http://doi.org/10.1002/ptr.6189.
13. *Crismaru I., Pantea Stoian A., Bratu O. G., Gaman M.-A., Stanescu A. M. A., Bacalbasa N., Diaconu C. C.*: Low-density lipoprotein cholesterol lowering treatment: the current approach. Lipids in Health and Disease 2020;19(1). https://doi.org/10.1186/s12944-020-01275-x.

14. *da Silva L. P., Borges B. A., Veloso M. P., Chagas-Paula D. A., Gonçalves R. V., Novaes R. D.*: Impact of sesquiterpene lactones on the skin and skin-related cells? A systematic review of *in vitro* and *in vivo* evidence. Life Sciences 2021;265:118815. https://doi.org/10.1016/j.lfs.2020.118815.
15. *de Kort M., Weber K., Wimmer B., Wilutzky K., Neuenhahn P., Allingham P., Leoni A.-L.*: Historical control data for hematology parameters obtained from toxicity studies performed on different Wistar rat strains: Acceptable value ranges, definition of severity degrees, and vehicle effects. Toxicology Research and Application 2020;4:239784732093148. https://doi.org/10.1177/2397847320931484.
16. *Ebrahimi F., Farzaei M. H., Bahramsoltani R., Heydari M., Naderinia K., Rahimi R.*: Plant-derived medicines for neuropathies: A comprehensive review of clinical evidence. Reviews in the Neurosciences 2019;30(6):671–684. http://doi.org/10.1515/revneuro-2018-0097.
17. *Ekbatan M. R., Khoramjouy M., Gholamine B., Faizi M., Sahranavard S.*: Evaluation of anticonvulsant effect of aqueous and methanolic extracts of seven *Inula* species. Iranian Journal of Pharmaceutical Research 2019;18:208–220. http://doi.org/10.22037/ijpr.2019.15509.13151.
18. *Ejelonu O. C., Oluba S. O., Awolokun B. O., Elekofehinti O. O., Adanlawo I. G.*: Saponin-rich extracts reverse obesity and offer protection against obesity-induced inflammation in high-fat diet mice. Journal of Medicinal Plants for Economic Development 2021;5:101. doi:10.4102/jomped.v5i1.101.
19. *Fan W.*: Epidemiology in diabetes mellitus and cardiovascular disease. Cardiovascular Endocrinology 2017);6(1):8–16.
20. *Filho J.W., Lima C. C., Paunksnis M. R. R., Silva A. A., Perilhão M. S., Caldeira M., de Souza R. R.:* Reference database of hematological parameters for growing and aging rats. The Aging Male 2018;21(2):145-148.
21. *Ghonime M., Eldomany R., Abdelaziz A., Soliman H.*: Evaluation of immunomodulatory effect of three herbal plants growing in Egypt. Immunopharmacology and Immunotoxicology 2010;33(1):141–145. https://doi.org/10.3109/08923973.2010.487490.
22. *Gierlikowska B., Gierlikowski W., Bekier K., Skalicka-Woźniak K., Czerwińska M. E., Kiss A. K.: Inula helenium* and *Grindelia squarrosa* as a source of compounds with anti-inflammatory activity in human neutrophils and cultured human respiratory epithelium. Journal of Ethnopharmacology 2020;249:112311. http://doi.org/10.1016/j.jep.2019.112311.
23. *Grigore A., Neagu G., Nita S., Ionita C., Ionita L., Goanta A.-M., Fernoaga C., Danacu V., Albulescu R.*: Biomedical application of a herbal product based on two Asteraceae species. Applied Sciences 2020;10(18): article 6444. http://doi.org/10.3390/app10186444.
24. *He S., Zhang C., Zhou P., Zhang X., Ye T., Wang R., Sun G., Sun X.*: Herb-induced liver injury: Phylogenetic relationship, structure-toxicity relationship, and herb-ingredient network analysis. International Journal of Molecular Sciences 2019;20(15):3633. http://doi.org/10.3390/ijms20153633.
25. *He X., Zhao W.-Y., Shao B., Zhang B.-J., Liu T.-T., Sun C.-P., Huang H. L., Wu J. R., Liang J. H., Ma X.-C.*: Natural soluble epoxide hydrolase inhibitors from *Inula helenium* and their interactions with soluble epoxide hydrolase. International Journal of Biological Macromolecules 2020;158:1362–1368. http://doi.org/10.1016/j.ijbiomac.2020.04.227.
26. *Heghes S. C., Vostinaru O., Rus L. M., Mogosan C., Iuga C. A., Filip L.*: Antispasmodic effect of essential oils and their constituents: A review. Molecules 2019;24(9):1675. http://doi.org/10.3390/molecules24091675.
27. *Hernández Bautista R. J., Mahmoud A. M., Königsberg M., López Díaz Guerrero N. E.*: Obesity: Pathophysiology, monosodium glutamate-induced model and anti-obesity medicinal plants. Biomedicine and Pharmacotherapy 2019;111:503–516. doi:10.1016/j.biopha.2018.12.108.
28. *Kabiri M., Kamalinejad M., Bioos S., Shariat M., Sohrabvand F.*: Comparative study of the effects of chamomile (*Matricaria chamomilla* L.) and cabergoline on idiopathic hyperprolactinemia: A pilot randomized controlled trial. Iranian Journal of Pharmaceutical Research 2019;18(3):1612–1621. http://doi.org/10.22037/ijpr.2019.1100758.
29. *Kamatou G. P. P., Viljoen A. M.*: A review of the application and pharmacological properties of α-bisabolol and α-bisabolol-rich oils. Journal of the American Oil Chemists' Society 2009;87(1):1–7. http://doi.org/10.1007/s11746-009-1483-3.
30. *Kim D. N., Schmee J., Thomas W. A.*: Dietary fish oil added to a hyperlipidemic diet for swine results in reduction in the excessive number of monocytes attached to arterial endothelium. Atherosclerosis 1990;81(3):209–216. https://doi.org/10.1016/0021-9150(90)90068-t.
31. *Koc K., Ozdemir O., Ozdemir A., Dogru U., Turkez H.*: Antioxidant and anticancer activities of extract of *Inula helenium* (L.) in human U-87 MG glioblastoma cell line. Journal of Cancer Research and Therapeutics 2018;14(3):article 658. http://doi.org/10.4103/0973-1482.187289.
32. *Kumar C., Kumar A., Nalli Y., Lone W. I., Satti N. K., Verma M. K., Ahmed Z., Ali A.*: Design, synthesis and biological evaluation of alantolactone derivatives as potential anti-inflammatory agents. Medicinal Chemistry Research 2019;28(6):849–856. http://doi.org/10.1007/s00044-019-02337-1.
33. *Lieshchova M. A., Bohomaz A. A., Brygadyrenko V. V.*: Effect of *Salvia officinalis* and *S. sclarea* on rats with a high-fat hypercaloric diet. Regulatory Mechanisms in Biosystems 2021; 12(3):554–563. https://doi.org/10.15421/022176.
34. *Lieshchova M. A., Brygadyrenko V. V.*: 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 2021;12(1):169–180. https://doi.org/10.15421/022125.

35. *Lieshchova M., Brygadyrenko V.*: 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 2022;90:49.
36. *Lieshchova M., Brygadyrenko V.*: Effect of *Rhodiola rosea* Rhizomes and *Punica granatum* Fruit Peel on the metabolic processes and physiological activity of rats fed with excessive fat diet. Food Technology and Biotechnology 2023;61(2):202–211. https://doi.org/10.17113/ftb.61.02.23.7913.
37. *Lieshchova M. A., Brygadyrenko V. V.* Effect of *Echinacea purpurea* and *Silybum marianum* seeds on the body of rats with an excessive fat diet. Biosystems Diversity 2023;31(1):90–99. https://doi.org/10.15421/012310.
38. *Lunz K., Stappen I.*: Back to the roots – an overview of the chemical composition and bioactivity of selected root-essential oils. Molecules 2021;26(11):3155. https://doi.org/10.3390/molecules26113155.
39. *McKay D. L., Blumberg J. B.*: A Review of the bioactivity and potential health benefits of chamomile tea (*Matricaria recutita* L.). Phytotherapy Research 2006;20(7):519–530. https://doi.org/10.1002/ptr.1900.
40. *Mangalesh S., Yadav P., Dudani S., Mahesh N. K.*: Atherogenic index of plasma predicts coronary artery disease severity and major adverse cardiac events in absence of conventional risk factors. Coronary Artery Disease 2022;33(7):523–530. https://doi.org/10.1097/mca.0000000000001166.
41. *Monserrat-Mesquida M., Bouzas C., Mascaró C. M., Tejada S., Sureda A.*: Probiotics as potential therapy in the management of non-alcoholic fatty liver disease (NAFLD). Fermentation 2023;9(4):395. https://doi.org/10.3390/fermentation9040395.
42. *Nakazawa T., Yasuda T., Ueda J., Ohsawa K.*: Antidepressant-Like Effects of Apigenin and 2,4,5-Trimethoxycinnamic Acid from Perilla frutescens in the Forced Swimming Test. Biological and Pharmaceutical Bulletin 2003;26(4):474–480. https://doi.org/10.1248/bpb.26.474.
43. *Petterino C., Argentino-Storino A.*: Clinical chemistry and haematology historical data in control Sprague-Dawley rats from pre-clinical toxicity studies. Experimental and Toxicologic Pathology 2006;57(3):213–219. https://doi.org/10.1016/j.etp.2005.10.002.
44. *Petronilho S., Maraschin M., Coimbra M. A., Rocha S. M.*: *In vitro* and *in vivo* studies of natural products: A challenge for their valuation. The case study of chamomile (*Matricaria recutita* L.). Industrial Crops and Products 2012;40:1–12. https://doi.org/10.1016/j.indcrop.2012.02.041.
45. *Pisoschi A. M., Pop A., Cimpeanu,C., Predoi G.*: Antioxidant capacity determination in plants and plant-derived products: A review. Oxidative Medicine and Cellular Longevity 2016;9130976. http://doi.org/10.1155/2016/9130976.
46. *Portnychenko A. G., Vasylenko M. I., Aliiev R. B., Kozlovska M. G., Zavhorodnii M. O., Tsapenko P. K., Rozova K. V., Portnichenko V. I.*: The prerequisites for the development of type 2 diabetes or prediabetes in rats fed a high-fat diet. Regulatory Mechanisms in Biosystems 2023;14(1);16–22. https://doi.org/10.15421/022303.
47. *Rafraf M., Zemestani M., Asghari-Jafarabadi M.*: Effectiveness of chamomile tea on glycemic control and serum lipid profile in patients with type 2 diabetes. Journal of Endocrinological Investigation 2014);38(2):163–170. https://doi.org/10.1007/s40618-014-0170-x.
48. *Radu F., Potcovaru C.-G., Salmen T., Filip P. V., Pop C., Fierbințeanu-Braticievici C.*: The link between NAFLD and metabolic syndrome. Diagnostics 2023;13(4):614. https://doi.org/10.3390/diagnostics13040614.
49. *Rasul A., Khan M., Ali M., Li J., Li X.*: Targeting apoptosis pathways in cancer with alantolactone and isoalantolactone. The Scientific World Journal 2013;248532. http://doi.org/10.1155/2013/248532.
50. *Saad B., Zaid H., Shanak S., Kadan S.*: Anti-obesity Medicinal Plants. Anti-Diabetes and Anti-Obesity Medicinal Plants and Phytochemicals 2017;59–93. https://doi.org/10.1007/978-3-319-54102-0_3.
51. *Saghahazrati S., Ayatollahi S., Kobarfard F., Minaii Zang B.*: The synergistic effect of glucagon-like peptide-1 and chamomile oil on differentiation of mesenchymal stem cells into insulin-producing cells. Cell Journal 2020;21(4):371–378. http://doi.org/10.22074/cellj.2020.6325.
52. *Salazar-Gómez A., Ontiveros-Rodríguez J. C., Pablo-Pérez S. S., Vargas-Díaz M. E., Garduño-Siciliano L.*: The potential role of sesquiterpene lactones isolated from medicinal plants in the treatment of the metabolic syndrome – A review. S. Afr. J Bot. 2020;135:240–251. http://doi.org/10.1016/j.sajb.2020.08.020.
53. *Sanchez M., Gonzalez-Burgos E., Gomez-Serranillos M. P.*: The pharmacology and clinical efficacy of *Matricaria recutita* L.: A systematic review of *in vitro*, *in vivo* studies and clinical trials. Food Reviews International 2020;1–35. http://doi.org/10.1080/87559129.2020.1834577.
54. *Sayyar Z., Yazdinezhad A., Hassan M., Jafari Anarkooli I.*: Protective effect of *Matricaria chamomilla* ethanolic extract on hippocampal neuron damage in rats exposed to formaldehyde. Oxidative Medicine and Cellular Longevity 2018;6414317. https://doi.org/10.1155/2018/6414317.
55. *Seibenhener M. L., Wooten M. C.*: Use of the open field maze to measure locomotor and anxiety-like behavior in mice. Journal of Visualized Experiments 2015;96. https://doi.org/10.3791/52434.
56. *Shwaikh A. K., Hassan A. J., Rashid K. H.:* The effects of Methotrexate and *Matricaria Chamomilla* extract on some immunological and hematological parameters in male albino rats. Annals of the Romanian Society for Cell Biology 2021;25(6):15319–15330.
57. *Singh O., Khanam Z., Misra N., Srivastava M. K.*: Chamomile (*Matricaria chamomilla* L.): An overview. Pharmacognosy reviews 2011;5(9):82–95. https://doi.org/10.4103/0973-7847.79103.
58. *Srivastava J. K., Shankar E., Gupta S.*: Chamomile: A herbal medicine of the past with a bright future

(review). Molecular Medicine Reports 2010;3(6):895–901. http://doi.org/10.3892/mmr.2010.377.
59. *Tavares W. R., Seca A.*: *Inula* L. secondary metabolites against oxidative stress-related human diseases. Antioxidants 2019;8(5):122. http://doi.org/10.3390/antiox8050122.
60. *Vigneshwar R., Arivuchelvan A., Mekala P., Imayarasi K.*: Sex-specific reference intervals for Wistar albino rats: hematology and clinical biochemistry. Indian Journal of Animal Health 2021;60(1):58–65. https://doi.org/10.36062/ijah.60.1.2021.58-65.
61. *Viola H., Wasowski C., Levi de Stein M., Wolfman C., Silveira R., Dajas F., Medina J., Paladini A.*: Apigenin, a Component of Matricaria recutita Flowers, is a Central Benzodiazepine Receptors-Ligand with Anxiolytic Effects. Planta Medica 1995;61(03):213–216. https://doi.org/10.1055/s-2006-958058.
62. *Wang J., Zhao Y. M., Tian Y. T., Yan C. L., Guo C. Y.*: Ultrasound-assisted extraction of total phenolic compounds from *Inula helenium*. The Scientific World Journal 2013;157527. http://doi.org/10.1155/2013/157527.
63. *Wang M., Luo W., Ye L., Jin L., Yang B., Zhang Q., Qian J., Wang Y., Zhang Y., Liang G.*: Dectin-1 plays a deleterious role in high fat diet-induced NAFLD of mice through enhancing macrophage activation. Acta Pharmacologica Sinica 2022;44(1):120–132. https://doi.org/10.1038/s41401-022-00926-2.
64. *Wang Q., Gao S., Wu G., Yang N., Zu X., Li W., Xie N., Zhang R. R., Li C. W., Hu Z. L. Zhang W*: Total sesquiterpene lactones isolated from *Inula helenium* L. attenuates 2,4-dinitrochlorobenzene-induced atopic dermatitis-like skin lesions in mice. Phytomedicine 2018;46:78–84. http://doi.org/10.1016/j.phymed.2018.04.036.
65. *Wei X., Gao Y., Cheng F., Yun S., Chang M., Cao J., Cheng Y., Feng C.*: The Effects of a High-Fat/Cholesterol Diet on the Intestine of Rats Were Attenuated by Sparassis latifolia Polysaccharides. Food Technology and Biotechnology 2022;60(4):469–487. https://doi.org/10.17113/ftb.60.04.22.7561.
66. *Yip T. C.-F., Lyu F., Lin H., Li G., Yuen P.-C., Wong V. W.-S., Wong G. L.-H.*: Non-invasive biomarkers for liver inflammation in non-alcoholic fatty liver disease: present and future. Clinical and Molecular Hepatology 2023;29:171–183. https://doi.org/10.3350/cmh.2022.0426.
67. *Yan Y. Y., Zhang Q., Zhang B., Yang B., Lin N. M.*: Active ingredients of *Inula helenium* L. exhibits similar anti-cancer effects as isoalantolactone in pancreatic cancer cells. Natural Product Research 2019;34(17):2539–2544. http://doi.org/10.1080/14786419.2018.1543676.
68. *Zand R. R. S., Jenkins D. J. A., Diamandis E. P.*: Effects of natural products and nutraceuticals on steroid hormone-regulated gene expression. Clinica Chimica Acta 2001;312(1–2):213–219. https://doi.org/10.1016/s0009-8981(01)00626-x.
69. *Zampelas A., Magriplis E.*: New insights into cholesterol functions: a friend or an enemy? Nutrients 2019;11(7):1645. https://doi.org/10.3390/nu11071645.
70. *Zazharskyi V. V., Davydenko P. O., Kulishenko O. M., Borovik I. V., Brygadyrenko V. V.*: Antimicrobial activity of 50 plant extracts. Biosystems Diversity 2019;27(2):163–169. http://doi.org/10.15421/011922.
71. *Zemestani M., Rafraf M., Asghari-Jafarabadi M.*: Chamomile tea improves glycemic indices and antioxidants status in patients with type 2 diabetes mellitus. Nutrition 2016;32(1):66–72. https://doi.org/10.1016/j.nut.2015.07.011.
72. *Zemestani M., Rafraf M., Asghari-Jafarabadi M.*: Effects of chamomile tea on inflammatory markers and insulin resistance in patients with type 2 diabetes mellitus. Trends in General Practice 2018;1(3):1–6. https://doi.org/10.15761/tgp.1000117.
73. *Zhang B.-H., Yin F., Qiao Y.-N., Guo S.-D.*: Triglyceride and Triglyceride-Rich Lipoproteins in Atherosclerosis. Frontiers in Molecular Biosciences 2022;9:909151 https://doi.org/10.3389/fmolb.2022.909151.
74. *Zhao P., Pan Z., Luo Y., Zhang L., Li X., Zhang G., Zhang Y., Cui R., Sun M., Zhang X.*: Alantolactone Induces Apoptosis and Cell Cycle Arrest on Lung Squamous Cancer SK-MES-1 Cells. Journal of Biochemical and Molecular Toxicology 2015;29(5):199–206. https://doi.org/10.1002/jbt.21685.
75. *Zlatić N., Jakovljević D., Stanković M.*: Temporal, plant part, and interpopulation variability of secondary metabolites and antioxidant activity of *Inula helenium* L. Plants 2019;8(6):179. http://doi.org/10.3390/plants8060179

Received: 05.10.23
Accepted: 08.11.23
Published online first: 21.11.2023