Regulatory Mechanisms in Biosystems

ISSN 2519-8521 (Print)
ISSN 2520-2588 (Online)
Regul. Mech. Biosyst.

Effect of *Tribulus terrestris* supplementation on the organism of rats fed a high-fat diet

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

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

Article info

Received 25.05.2025
Received in revised form 21.06.2025
Accepted 18.07.2025

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

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

Lieshchova, M. A., & Brygadyrenko, V. V. (2025). Effect of Tribulus terrestris supplementation on the organism of rats fed a high-fat diet. Regulatory Mechanisms in Biosystems, 16(3), e25118. doi:10.15421/0225118

Recently, the global community has turned its attention to the use of antioxidant-rich herbs. Researchers have also looked into using them to develop natural antioxidant formulas in the fields of medicine and nutrition. In particular, they have been studied for their potential to treat or correct metabolic disorders caused by an unbalanced diet. *Tribulus terrestris* is rich in secondary metabolites and is known for its antitumor, antilithic, antidiabetic, anti-inflammatory, and antioxidant properties. It has also been used for a long time to treat sexual dysfunction. In a laboratory study, 20 adult male rats were fed a high-fat diet supplemented with 0.5% or 2.0% dry *T. terrestris* herb for 32 days. The rats' weight gain, internal organ mass, and indicators of protein, lipid, carbohydrate, and mineral metabolism and blood enzyme activity were observed. The body weight of the animals that received a high-fat diet increased to 105.7% of the initial weight by the end of the experiment. The addition of *T. terrestris* herb to the diet at a dose of 0.5% did not affect the rate of body weight gain, while a dose of 2.0% contributed to an increase in body weight gain (up to 107.4%). *Tribulus terrestris* did not significantly alter feed intake or water consumption. A high-fat diet supplemented with *T. terrestris* caused a dose-dependent increase in liver weight; the 2.0% dose significantly increased the relative weight of the testicles and stomach. Both doses of the *T. terrestris* herb significantly reduced blood glucose levels. The 2.0% dose increased high-density lipoprotein (HDL) cholesterol and inorganic phosphorus levels while decreasing the Ca/P ratio. Depending on the dose, *T. terrestris* herb had a mixed effect on creatinine levels, causing either a decrease at 0.5% or an increase at 2.0%. AST and ALT activity was sharply increased by a 2.0% dose of *T. terrestris* herb. These results suggest the potential use of *T. terrestris* as a dietary supplement for correcting metabolic disorders in an unbalanced diet.

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

Introduction

The prevalence of obesity and the burden of obesity-related diseases are increasing worldwide (Dai et al., 2020). In 2021, the number of deaths and disability-adjusted life years (DALYs) associated with high body mass index (BMI) increased 2.54- and 2.68-fold, respectively, compared to 1990 (Wu et al., 2024). Herbal remedies based on biologically active compounds from plant cells are a promising approach to the prevention and treatment of obesity because they have a systemic effect on the body (Chandrasekaran et al., 2012; Preeti, 2018; Wang et al., 2020; Singh & Bhatti, 2021). Compared to their synthetic counterparts, natural plant compounds usually demonstrate a broader spectrum of action and fewer negative side effects (Khalili et al., 2017; Liu et al., 2017).

The genus *Tribulus*, which belongs to the family Zygophyllaceae, has about 20 species in the world. Among these, *Tribulus terrestris* L. is the most common and popular as a medicinal and food herb of this genus (Chhatre et al., 2014; Parham et al., 2020). *Tribulus terrestris* is an annual plant which grows mainly in subtropical regions and countries around the Mediterranean Sea. This plant is considered small, growing as a shrub that spreads along the ground. It is 10 to 60 cm tall. Its leaves are pinnate and contain five to eight pairs of opposite, uneven, oblong or elliptical-lanceolate leaflets. *Tribulus terrestris* fruits are 3–6 mm long, axe-shaped, 7–12 mm in diameter, radially arranged, and firm. The root is thin, cylindrical, fibrous, and often branched (Chhatre et al., 2014). The fruits and roots, either alone or combined with other medicinal plants, have been used in folk medicine for over five thousand years in various Asian countries. In traditional Chinese medicine, the leaves of the *T. terrestris* are used to treat stomach problems, bladder stones, male reproductive disorders, and ophthalmic pathology (Singh & Kumari, 2015; Yuan et al., 2020). The herb has also been used to treat sexual dysfunction and for other pharmaceutical purposes, such as protecting the heart. *Tribulus terrestris* is known for its antitumor, antilithic, antidiabetic, anti-inflammatory, and antioxidant effects.

A wide range of biologically active compounds have been identified in the herb, including vitamins, alkaloids, saponins, flavonoids, steroids, tannins, flavanol glycosides, and unsaturated fatty acids (Adewoyin et al., 2017; Khaleghi et al., 2017). Saponins include the following types: furostanol, spirostanol, tigogenin, neotigogenin, hitogenin, neohitogenin, hectogenin, neohectogenin, diosgenin, chlorogenin, ruscogenin, and sarsasapogenin. In addition, four sulfated saponins of the tigogenin and diosgenin type were also isolated. Protodioscin, a steroidal glycoside, is the main saponin found in extracts made from air-dried aerial parts of the *T. terrestris* herb (Adaikan et al., 2001). Saponins vary in type, structure, and composition of their aglycone fragment and oligosaccharide chains, but many saponins have surface-active properties. *Tribulus terrestris* is characterized by a significant concentration and variety of flavonoids. The main flavonoids in the leaves and fruits of *T. terrestris* contain kaempferol, kaempferol-3-glucoside, kaempferol-3-rutinoside, and tribuloside (kaempferol-3-beta-D-(6-O-cis-p-coumaroyl) glucoside), as well as caffeoyl derivatives, quercetin glycosides (e.g., rutin and kaempferol glycosides [quercetin 3-O-rutinoside, quercetin 3-O-glycoside, and kaempferol 3-O-glycoside]). The flavonoids isolated from the plant exhibit pronounced antioxidant and phytoestrogenic properties. The plant material also contains a mixture of beta-carboline alkaloids, including harmane, norharmane, tetrahydroharmane, harmine, harmaline, harmol and harmalol (Al-Bayati & Al-Mola, 2008; Lal & Sutradhar, 2024). *Tribulus terrestris* contains a number of phytochemicals with potential biological activity.

The following effects have been reported and described in the current scientific literature: diuretic, antiurolithic, immunomodulatory, antidiabetic, hypolipidemic, cardiotonic, hepatoprotective, anti-inflammatory, analgesic, antispasmodic, antibacterial, anthelmintic effects, and larvicidal, anti-mite, and antitumor activity (Sirotkin & Kolesárová, 2021).

The increasing popularity of herbal remedies derived from *T. terrestris* is due to its well-documented aphrodisiac properties and its effect on sex hormone levels in both men and women (Neychev & Mitev, 2016). The ability of *T. terrestris* to treat loss of libido and infertility is one of its most well-known effects in folk and official medicine (Sahin et al., 2016; Gamal El Din, 2017). Studies have shown that *T. terrestris* can enhance sperm quality, including the number, mobility, and shape of sperm in both male animals (Haghmorad et al., 2019; Shiva et al., 2020) and humans (Asadmobini et al., 2017; Khaleghi et al., 2017; Sanagoo et al., 2019). Methanol extracts of *T. terrestris* counteract the degradation of sperm quality caused by nicotine hydrogen tartrate and lead in male rats due to the presence of several biologically active compounds (Aldaddou et al., 2022). The presence of phytoestrogenic saponins in the plant is associated with a significant effect on reproductive function. Dioscin, diosgenin, and protodioscin, in particular, may positively affect libido, and phytosterols, especially beta-sitosterols, may benefit prostate function and male fertility (Sellami et al., 2018). Saponins can promote libido and influence physical fitness due to their antioxidant and anti-inflammatory effects (Parama et al., 2020; Dutta et al., 2025). In a study on rats that underwent physical exercise, *T. terrestris* extract increased muscle mass and physical performance. The authors attributed this effect to an increase in plasma insulin-like growth factor 1 (IGF-1) levels (Wu et al., 2017). The ability of *T. terrestris* to increase bone mineral density explains its bone-protective effect in rats. This phenomenon may be partially due to an increase in serum levels of dehydroepiandrosterone (DHEA), but not testosterone or oestradiol, a Ca^{2+}-sparing effect (Marques et al., 2019).

The use of *T. terrestris* fruits as a rejuvenating herb has demonstrated antidepressant and anxiolytic activity, which can be attributed to the presence of harmin and β-carboline alkaloids in the fruit's composition. Harmin is a monoamine oxidase inhibitor that increases dopamine levels in the brain (Deole et al., 2011).

The diuretic properties of *T. terrestris* are associated with its high nitrate and essential oil content, as well as its high potassium salt concentration (Chhatre et al., 2014). An aqueous extract of *T. terrestris*, when taken orally at a dose of 5 g/kg, caused positive diuresis and increased the concentration of sodium and chlorides in the urine. It also increased the tone of smooth muscles, making it a promising treatment for urolithiasis (Al-Ali et al., 2003). A lyophilized mixture of saponins isolated from *T. terrestris* has been proven to have a spasmolytic effect due to a dose-dependent decrease in small intestine peristalsis in rabbits (Arcasoy et al., 1998).

Ethanol extract of *T. terrestris* has been shown to inhibit the expression of mediators associated with inflammation and inflammatory cytokines. This has a beneficial effect on various inflammatory conditions. Methanol extract has demonstrated dose-dependent inhibition of rat paw volume in carrageenan-induced inflammation (Oh et al., 2012; Garapati, 2022). In carrageenan- and zymosan-induced oedema models in rats, thick *T. terrestris* extract at doses ranging from 50 to 200 mg/kg exhibited anti-inflammatory activity (Yunusova et al., 2023).

Tribulus terrestris seed extract has demonstrated concentration-dependent cytotoxicity against oral cancer cells. A concentration of 100 μg/mL showed significant growth inhibition and proapoptotic effects, resulting in noticeable morphological changes in cancer cells treated with the extract (Malik et al., 2024). An aqueous extract of *T. terrestris* roots and fruits at 800 mg/kg showed chemopreventive potential against 7,12-dimethylbenz[a]anthracene (DMBA) and croton oil-induced papillomagenesis (Kumar et al., 2006).

Saponins isolated from *T. terrestris* fruits have been shown to enhance phagocytosis in a dose-dependent manner, indicating stimulation of a non-specific immune response. An ethanol extract of *T. terrestris* exhibited a dose-dependent increase in humoral antibody titers and delayed-type hypersensitivity reactions, suggesting an enhancement of the specific immune response (Chhatre et al., 2014). Phytochemicals from *T. terrestris* could replace conventional antibiotics and antiviral drugs, including those used to treat HIV (Parham et al., 2020). Hussein (2018) established the antibacterial activity of the aqueous extract of *T. terrestris* against pathogenic microorganisms of two Gram-positive bacteria (*Bacillus subtilis* and *Staphylococcus aureus*) and two Gram-negative bacteria (*Escherichia coli* and *Pseudomonas aeruginosa*) using the agar plate diffusion method. Extracts of the Iraqi herb obtained using polar and nonpolar solvents showed significant antibacterial activity in vitro against Gram-positive (*Staphylococcus aureus*) and Gram-negative (*E. coli*, *Proteus vulgaris*, *Pseudomonas aerugiuosa*, and *Klebsiella pneumonia*) bacteria. Additionally, alcohol and acetonitrile extracts significantly increased free testosterone levels in the blood serum of male mice (Mohammed et al., 2010). The methanol extract of *T. terrestris* fruits exhibited the greatest activity against Gram-positive and Gram-negative bacteria, while moderate activity was observed in its petroleum ether and chloroform extracts (Mohammed, 2008).

Tribusponin, a mixture of steroid glycosides, has a pronounced anti-sclerotic effect. It is used in the treatment of atherosclerosis, hypertension, and angina pectoris. *Tribulus terrestris* has shown significant efficacy in treating heart diseases, including myocardial infarction, ischemic heart disease, cerebral atherosclerosis, and the consequences of cerebral thrombosis. Tribulosin plays a significant role in protecting the myocardium from ischemia/reperfusion injury by activating protein kinase (Zhang et al., 2010). The aqueous extract of *T. terrestris* fruit has been shown to significantly inhibit acetylcholinesterase (ACE) *in vitro*. Methanol and water extracts of the plant have also been shown to have antihypertensive activity, causing direct relaxation of smooth muscle in arteries in experiments on spontaneously hypertensive rats (Phillips et al., 2006; Eljabri et al., 2015).

Tribulus terrestris extract protects against the harmful effects of methotrexate, particularly with regard to blood and liver health. Prior administration of the extract increases glutathione levels, reduces oxidative damage, and stabilizes liver enzyme activity. The extract's active components, particularly steroidal saponins and flavonoids, play a significant role in its antioxidant and anti-inflammatory properties. *Tribulus terrestris* may be a useful addition to traditional treatments for methotrexate toxicity (Al-Hamadani et al., 2025). Research on male albino mice susceptible to sulfasalazine (SSZ)-induced hepatotoxicity revealed the antihepatotoxic properties of aqueous and alcoholic extracts of *T. terrestris* fruits. This was evident from a notable enhancement in liver enzyme levels and histological structure. The aqueous extract demonstrated the strongest hepatoprotective effect (Al-Hathoot et al., 2025). An extract of *T. terrestris* (250 mg/kg) exhibited significant hepatoprotective activity against acetaminophen-induced hepatotoxicity in freshwater fish (*Oreochromis mossambicus*), normalizing elevated biochemical parameters and reducing enzyme levels (Kavitha et al., 2011).

While most clinical trials have not reported any serious side effects from the long-term use of *T. terrestris*, there are isolated reports of negative effects from excessive use. These effects include exhaustion, fatigue, tachycardia, hypertension, sleep disorders, and the possible development of photosensitivity (Sellami et al., 2018; Chen et al., 2019; Sirotkin & Kolesárová, 2021). The Australian Institute of Sport does not recommend that athletes use *T. terrestris*. Currently, published data on *T. terrestris* does not provide substantial evidence of its usefulness or safety in sports (Pokrywka et al., 2014).

Tribulus terrestris may effectively treat hypercholesterolemia by lowering cholesterol, homocysteine, leptin, and resistin levels, as well as increasing adipokine expression. It has also demonstrated hypolipidemic and antihyperglycemic activity (Samani et al., 2016; Abdel-Mottaleb et al., 2022). In a double-blind randomized clinical trial evaluating the efficacy of *T. terrestris* hydroalcoholic extract, Samani et al. (2016) found that it significantly reduced blood sugar levels. Due to the abundance of reports on this plant's impact on lipid and carbohydrate metabolism (Altug Tuncer et al., 2009; Povydysh et al., 2023; Kaliaperumal et al., 2024), our study aimed to assess the herb's impact on body weight, internal organ weight, and blood biochemical parameters in white laboratory rats on a high-fat diet.

Materials and methods

Animals and experimental design. Twenty adult male rats (aged 5–6 weeks and weighing 200–220 g) participated in the study. Prior to the intervention, the animals underwent a seven-day acclimatization period under standardized environmental conditions: an ambient temperature of 22 ± 2 °C and a 12-hour light/dark cycle (lights on at 7:00 a.m. and off at 7:00 p.m.). The animals had free access to water and feed throughout the study. After the acclimatization period, the rats were randomly assigned to one of four groups of five rats each (n = 5): group 1 (control) – received a high-fat diet; group 2 – received a high-fat diet + 0.5% *T. terrestris* herb; group 3 – received a high-fat diet + 2.0% *T. terrestris* herb; group 4 – received a high-fat diet + Rosuvastatin (20 mg/kg) The groups were assigned using a randomization procedure, and each rat was assigned an identification number. After the intervention, the animals were euthanized, and blood and organ samples were collected for further biochemical analysis. All procedures were performed in accordance with national and institutional guidelines for the care and use of laboratory animals. The study protocol was approved by the institutional ethics committee (protocol number No. 2/23-24, September 18, 2023). Every effort was made to minimize the number of animals used and their suffering.

Diet preparation and drug administration. The experimental diet was based on rodent feed made from a blend of grains (corn, sunflower seeds, wheat, barley, and soybeans), essential nutrients (meat and bone meal, minerals, and vitamins), and root vegetables (carrots). The excess fat in the diet came from the addition of 15% sunflower oil to a mixture of crushed dry feed components, followed by granulation (Levchuk et al., 2021). In addition to the high-fat diet, crushed *Tribulus terrestris* herb was added in doses of 0.5% and 2.0%. As a positive control, Rosuvastatin (Rosuvastatin-Darnitsa tablets, film-coated, 20 mg, No. 30) was added, calculated per kilogram of body weight according to the instructions for use. For the experiment, the official form of the medicinal herb *Tribulus terrestris*, purchased from a commercial pharmacy, was used.

Body weight and food consumption monitoring. Body weight was measured daily in the morning using electronic scales (Metrinco AB224, China). The amount of food consumed was measured each day by weighing the food given and then subtracting the uneaten portion left the following morning. The food intake of each cage was assessed and then normalized to obtain the approximate intake per animal. Fresh feed and water were provided daily to ensure continuous consumption.

Sample collection and biochemical blood tests. On day 32, animals from four groups were removed from the experiment by overdose of anesthetic (80 mg/kg of ketamine and 12 mg/kg of xylazine, administered intraperitoneally). Blood samples were taken from the animals for biochemical analysis. Possible changes in the mass of internal organs were determined during autopsy. The heart, liver, lungs, thymus, spleen, stomach, intestines, kidneys and testicles were examined for pathological changes and weighed to an accuracy of 10 mg. The relative weight of the organs was calculated in relation to body weight. Blood serum was obtained by standing the blood for a while and then centrifuging it in a CM-3M.01 MICROmed centrifuge (200×g for 5 minutes; MICROmed, Shenzhen, China). Biochemical parameters were determined using a Miura 200 automatic analyzer (Italy) and reagent kits from High Technology (USA), PZ Cormay S.A. (Poland) and Spinreact S.A. (Spain). Protein metabolism indicators were assessed based on the following biochemical parameters: total protein was determined using the biuret method, albumin concentration using the bromocresol green reaction, and globulins and the protein coefficient were calculated. The total bilirubin concentration was measured using the enzymatic method. Assessment of urea was carried out using an enzyme-based method. Blood urea nitrogen was also identified at the same time. Creatinine was determined kinetically. This was based on the Jaffe reaction with picric acid. A yellow-red complex is formed when creatinine in blood serum reacts with picric acid in an acidic solution. Carbohydrate metabolism was assessed by measuring glucose levels using the glucose oxidase method (Chawla, 2014). Lipid metabolism was characterized by indicators of total cholesterol concentration, which was determined enzymatically using cholesterol oxidase. Triglyceride levels were determined after cleavage by lipoprotein lipase and detection by the Trinder reaction. HDL and LDL were determined using selective detergents, after which the products of the enzymatic reaction were stained. The atherogenic index was also calculated. Total calcium and inorganic phosphorus, which are indicators of mineral metabolism, were determined using the spectrophotometric method. A colored complex was formed when calcium in the sample reacted with arsenazo III (Cormay Diagnostics, Warsaw, Poland), and a second reaction involved phosphorus reacting with ammonium molybdate. The Ca/P ratio indicator is the ratio of total calcium to inorganic phosphorus. Changes in the activity of enzymes in the blood plasma were monitored in relation to the activities of aspartate aminotransferase (AST) and alanine aminotransferase (ALT). To do this, we used a method based on the Warburg test. We determined the activities of AST and ALT according to the rates of NADH (nicotinamide adenine dinucleotide), and we measured the absorbances spectrophotometrically at λ = 340 nm. The De Ritis ratio indicator was identified by the ratio of aspartate aminotransferase activity to alanine aminotransferase activity. The activity of alkaline phosphatase (U/L) was determined using an enzyme-based method that involved the formation of 4-nitrophenol, which was then measured by taking the absorption readings at a λ = 405 nm wavelength. The activity of alkaline phosphatase directly affects the rates of formation of 4-nitrophenol. The activity of γ-glutamyltransferase was assessed kinetically. This assessment was based on the breakdown of L-γ-glutamyl-3-carboxy-4-nitroanilide, which led to the formation of 5-amino-2-nitrobenzoate. The formation rates were measured using a spectrophotometer; the activity of γ-glutamyltransferase was directly proportional to the absorption at λ = 365–405 nm.

Statistical analysis. The data were analyzed using the Statistica 12.0 programme (StatSoft Inc., USA). The results are demonstrated in the tables as x ± SE (mean ± standard error). The differences between the values of the control and experimental groups were determined using the Tukey test (with consideration of Bonferroni's correction). The differences were considered to be significant at $P < 0.05$.

Results

A post-experimental increase in body weight was shown by rats fed a high-fat diet, starting from the first days of the experiment and reaching a maximum on day 28, followed by a slight slowdown in weight gain until day 32. There was significant variability in the body weight of individual animals in this group (see Fig. 1). The same trend was observed in the group of animals that received 0.5% *T. terrestris* herb in addition to a high-fat diet. However, weight gain was observed up to and including day 32 of the experiment in the group of animals that received 2.0% *T. terrestris* herb. A different picture emerged in the group of animals whose diet included Rosuvastatin: no change in body weight occurred during the first four days; a slight increase in body weight was observed by day 12, followed by a sharp decrease by day 20 of the experiment. By the end of the study, the animals had gradually gained weight, but on day 32, this only slightly exceeded their initial body weight (see Fig. 1). Animals that were fed a high-fat diet put on 5.7% of their initial weight over 32 days. Adding 0.5% *T. terrestris* herb to the diet did not affect the rate of weight gain; however, at a dose of 2.0%, the rate of weight gain increased to 107.4% of the initial weight.

The average body weight of rats at the end of the experiment in the high-fat diet group was 319 ± 20 g, whereas adding the medicinal plant *T. terrestris* to the diet had the opposite effect on final body weight. In the group of animals that consumed an additional 0.5% *T. terrestris*, body weight was 23.8% higher, while in the group that consumed 2.0%, body weight was 20.1% lower compared to the control group (receiving only a high-fat diet). Meanwhile, the average body weight of rats receiving Rosuvastatin was only 9.1% higher than that of the control group (Table 3).

Adding *T. terrestris* or Rosuvastatin to a high-fat diet did not significantly affect the amount of feed or water consumed (Table 1). Adding the *T. terrestris* herb to a high-fat diet significantly affected the morphometric parameters of some of the rats' internal organs. Consumption of 0.5% *T. terrestris* led to a 12.5% increase in liver weight, while consumption of 2.0% led to a 26.7% increase compared to rats that received only a high-fat diet. Consumption of the herb also affected the mass indicators of the digestive system.

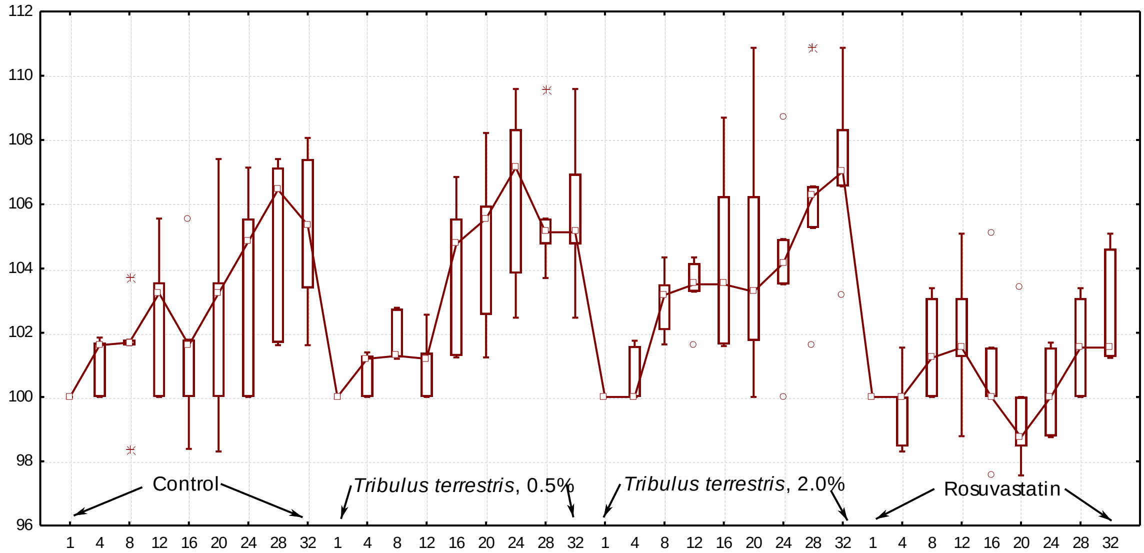

Fig. 1. Change in body weight of rats when crushed herb of *Tribulus terrestris* and Rosuvastatin were added to the diet: the x-axis shows the days of the experiment, the y-axis shows the body weight of the animals (% relative to the initial body weight of each animal, taken as 100% at the beginning of the experiment); small square – median, upper and lower boundaries of the rectangle – 25% and 75% quartiles, vertical line – minimum and maximum values, circles and stars – outliers; n = 5

Table 1
Change in food consumption by young male rats when *Tribulus terrestris* and Rosuvastatin were added to their diet (x ± SD, n = 5, experiment duration – 32 days)

Parameter	Control	*T. terrestris*	*T. terrestris* compared to the control, %	*T. terrestris*	*T. terrestris* compared to the control, %	Rosuvastatin	Rosuvastatin compared to the control, %
Consumption of food by animals, g/day	29.14	30.00	102.9	29.25	100.4	28.50	97.8
Consumption of water by animals, g/day	40.57	31.79	78.3	33.70	83.1	34.60	85.3

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

In both groups, *T. terrestris* caused a significant increase in caecum mass: by 112.8% at a dose of 0.5%, and by 95.8% at a dose of 2.0%. A dose of 2.0% caused a significant 17.3% increase in stomach mass. However, this medicinal plant did not affect the length of the small intestine, colon or rectum. Animals that received a 0.5% dose of the herb had a body length that was 6.7% higher than the control indicator. Adding 2.0% *T. terrestris* to a high-fat diet increased the relative weight of the testicles by 49.2%. Meanwhile, Rosuvastatin in a high-fat diet did not significantly affect the weight of internal organs (Table 2).

Table 2
Change in the relative mass of male rats' organs (%) when their diet was supplemented with *Tribulus terrestris* and Rosuvastatin (x ± SD, n = 5, experiment duration – 32 days)

Organ	Control	*T. terrestris*, 0.5%	*T. terrestris* compared to the control, %	*T. terrestris*, 2.0%	*T. terrestris* compared to the control, %	Rosuvastatin	Rosuvastatin compared to the control, %
Heart	0.342 ± 0.050	0.355 ± 0.021	103.8	0.363 ± 0.063	106.3	0.318 ± 0.029	93.2
Liver	2.57 ± 0.15	2.89 ± 0.10*	112.5	3.26 ± 0.34**	126.7	2.75 ± 0.37	107.0
Lungs	0.71 ± 0.06	0.71 ± 0.12	99.3	0.86 ± 0.14	120.7	0.69 ± 0.12	97.2
Thymus	0.094 ± 0.034	0.126 ± 0.037	134.6	0.113 ± 0.027	120.3	0.105 ± 0.027	112.1
Spleen	0.208 ± 0.026	0.258 ± 0.103	123.9	0.297 ± 0.125	142.8	0.179 ± 0.033	86.1
Stomach	0.532 ± 0.020	0.482 ± 0.049	90.7	0.624 ± 0.060**	117.3	0.508 ± 0.040	95.4
Small intestine	1.55 ± 0.33	1.53 ± 0.29	98.8	1.97 ± 0.32	127.3	1.65 ± 0.43	106.6
Caecum	0.24 ± 0.04	0.51 ± 0.14**	212.8	0.47 ± 0.17*	195.8	0.37 ± 0.12	157.1
Colon	0.471 ± 0.021	0.402 ± 0.101	85.4	0.445 ± 0.081	94.6	0.402 ± 0.169	85.4
Right kidney	0.309 ± 0.017	0.312 ± 0.019	100.8	0.339 ± 0.037	109.5	0.306 ± 0.019	98.8
Left kidney	0.316 ± 0.029	0.326 ± 0.029	103.1	0.350 ± 0.049	110.5	0.302 ± 0.009	95.6
Testicle	0.421 ± 0.035	0.410 ± 0.088	97.5	0.627 ± 0.126***	149.2	0.416 ± 0.035	99.0
Brain	0.585 ± 0.030	0.514 ± 0.045	87.9	0.708 ± 0.078	121.1	0.610 ± 0.177	104.3
Body weight, g	319 ± 20	395 ± 43***	123.8	255 ± 40	79.9	348 ± 31	109.1
Length of small intestine, cm	122.6 ± 14.3	130.6 ± 3.6	106.5	135.2 ± 7.3	110.3	137.6 ± 3.3	112.2
Length of colon and rectum, cm	20.2 ± 0.8	19.2 ± 3.4	95.0	15.8 ± 2.2	78.2	18.8 ± 4.8	93.1
Body length, cm	20.8 ± 0.8	22.2 ± 0.8*	106.7	20.2 ± 0.8	97.1	21.2 ± 0.8	101.9

Note: see Table 1.

No changes in protein metabolism, with the exception of creatinine levels, were caused by the addition of *T. terrestris* herb to a high-fat diet. Thus, a 16.2% decrease in this indicator in the blood of rats was caused by 0.5% *T. terrestris* herb, while, on the contrary, creatinine levels were increased by 27.8% with a 2.0% dose of the medicinal herb, whereas in the Rosuvastatin group, this indicator increased by 12.8% (Table 3). In rats fed a high-fat diet, the blood glucose level was 5.02 mmol/L, which is within the physiological range. *Tribulus* *terrestris* caused a significant decrease in this indicator: by 17.1% at a dose of 0.5%, and by 25.5% at a dose of 2.0%. Adding Rosuvastatin to the high-fat diet reduced the animals' blood glucose levels to the lower limit of the reference value, which was a 48.6% decrease compared to the group receiving only a high-fat diet.

Tribulus terrestris herb also caused changes in mineral metabolism. Inorganic phosphorus increased by 57.4% with the addition of 2.0% of the herb, which caused a 35.6% decrease in the Ca/P ratio. More significant changes in mineral metabolism indicators were found in the positive control group (Rosuvastatin). The level of inorganic phosphorus in the blood doubled compared to the control group, while total calcium decreased by 6.3%. This led to a 52.5% decrease in the Ca/P ratio.

Rats on a high-fat diet showed elevated levels of AST, ALT, and alkaline phosphatase activity in their blood, which went beyond the reference values when these values were evaluated. Adding 0.5% *T. terrestris* herb to a high-fat diet did not cause any significant changes. However, at a dose of 2.0%, AST activity increased by 44.9% and ALT activity by 36.7%. The addition of the medicinal herb to the high-fat diet had no effect on the activity of alkaline phosphatase, gamma-glutamyltransferase, and alpha-amylase. Rosuvastatin in the diet caused a 61.0% increase in ALT activity, compared to the increase observed in the high-fat diet group (Table 3).

Table 3
Change in blood biochemical parameters in male rats under the influence of *Tribulus terrestris* and Rosuvastatin (x ± SD, n = 5, experiment duration – 32 days)

Parameters	Control	*T. terrestris*, 0.5%	*T. terrestris* compared to the control, %	*T. terrestris*, 2.0%	*T. terrestris* compared to the control, %	Rosuvastatin	Rosuvastatin compared to the control, %
Total protein, g/L	74.4 ± 2.6^{a}	74.2 ± 2.2^{a}	99.7	72.8 ± 3.1^{a}	97.8	74.2 ± 7.3^{a}	99.7
Albumins, g/L	30.0 ± 2.4^{a}	31.4 ± 2.2^{a}	104.7	31.6 ± 2.3^{a}	105.3	32.0 ± 1.9^{a}	106.7
Globulins, g/L	44.6 ± 2.6^{a}	42.8 ± 2.9^{a}	96.0	41.2 ± 1.1^{a}	92.4	42.2 ± 6.6^{a}	94.6
Albumin / globulin ratio	0.68 ± 0.08^{a}	0.74 ± 0.11^{a}	108.8	0.78 ± 0.04^{a}	114.7	0.76 ± 0.11^{a}	111.8
Urea, mmol/L	5.2 ± 1.1^{a}	4.5 ± 1.9^{a}	87.2	6.3 ± 1.1^{a}	122.9	6.3 ± 1.2^{a}	121.3
Blood urea nitrogen, mg/100 g	98 ± 21^{a}	86 ± 37^{a}	88.3	122 ± 21^{a}	124.3	119 ± 22^{a}	122.1
Creatinine, µmol/L	46.8 ± 5.2^{a}	39.2 ± 3.8^{b}	83.8	59.8 ± 2.9^{c}	127.8	52.8 ± 9.7^{ac}	112.8
AST, U/L	154 ± 16^{a}	150 ± 27^{a}	97.0	224 ± 21^{b}	144.9	206 ± 103^{ab}	133.7
ALT, U/L	61.6 ± 11.9^{a}	56.8 ± 8.8^{a}	92.2	84.2 ± 6.7^{b}	136.7	99.2 ± 24.2^{b}	161.0
De Ritis ratio (AST/ALT), U	2.60 ± 0.52^{a}	2.66 ± 0.46^{a}	102.3	2.66 ± 0.30^{a}	102.3	2.00 ± 0.66^{a}	76.9
Alkaline phosphatase, U/L	386 ± 183^{a}	438 ± 141^{a}	113.5	453 ± 288^{a}	117.6	458 ± 84^{a}	118.8
Alpha-amylase, U/L	1458 ± 220^{a}	1291 ± 148^{a}	88.6	1668 ± 764^{a}	114.4	1487 ± 67^{a}	102.0
Total bilirubin, µmol/L	2.98 ± 0.34^{a}	3.02 ± 0.08^{a}	101.3	2.92 ± 0.64^{a}	98.0	3.20 ± 0.57^{a}	107.4
Direct bilirubin, µmol/L	0.72 ± 0.13^{a}	0.70 ± 0.39^{a}	97.2	0.84 ± 0.15^{a}	116.7	1.00 ± 0.32^{a}	138.9
Indirect bilirubin, µmol/L	2.40 ± 0.12^{a}	2.32 ± 0.32^{a}	96.7	2.06 ± 0.48^{a}	85.8	2.20 ± 0.57^{a}	91.7
Glucose, mmol/L	5.02 ± 0.63^{a}	4.16 ± 0.40^{b}	82.9	3.74 ± 1.05^{b}	74.5	2.58 ± 0.64^{b}	51.4
Total calcium, mmol/L	2.52 ± 0.08^{ab}	2.58 ± 0.11^{a}	102.4	2.48 ± 0.08^{ab}	98.4	2.36 ± 0.09^{b}	93.7
Inorganic phosphorus, mmol/L	2.16 ± 0.22^{a}	2.06 ± 0.15^{a}	95.4	3.40 ± 0.50^{b}	157.4	4.32 ± 0.64^{c}	200.0
Ca/P	1.18 ± 0.15^{a}	1.28 ± 0.08^{a}	108.5	0.76 ± 0.11^{b}	64.4	0.56 ± 0.09^{c}	47.5
Gamma-glutamyltransferase, U/L	3.0 ± 1.2^{a}	1.8 ± 0.8^{a}	60.0	2.2 ± 1.6^{a}	73.3	1.8 ± 0.4^{a}	60.0
Cholesterol, mmol/L	1.96 ± 0.17^{a}	1.86 ± 0.46^{a}	94.9	2.00 ± 0.59^{a}	102.0	1.68 ± 0.13^{b}	85.7
High-density lipoprotein (HDL) cholesterol, mmol/L	0.65 ± 0.14^{a}	0.77 ± 0.13^{a}	118.5	0.94 ± 0.16^{b}	144.6	0.95 ± 0.07^{c}	146.1
Low-density lipoprotein (LDL) cholesterol, mmol/L	0.91 ± 0.56^{a}	1.46 ± 0.30^{a}	160.4	0.63 ± 0.09^{a}	69.2	0.64 ± 0.16^{a}	70.3
Atherogenic index of plasma	1.98 ± 1.08^{a}	0.80 ± 0.39^{a}	40.4	1.73 ± 0.97^{a}	87.4	1.19 ± 0.35^{a}	60.1

Note: see Table 1.

Analysis of lipid metabolism indicators revealed that the supplementation of a high-fat diet with *T. terrestris* herb did not result in changes in blood cholesterol levels. However, a dose of 2.0% of the herb significantly increased high-density lipoprotein (HDL) cholesterol levels by 44.6%, compared to rats on a high-fat diet. The effect of Rosuvastatin on lipid metabolism is also noteworthy. In animals receiving Rosuvastatin alongside a high-fat diet, cholesterol levels decreased significantly by 14.3%, while HDL cholesterol levels increased by 46.1%.

Discussion

Obesity, which is defined as having a high body mass index (BMI), is a significant public health problem that has worsened worldwide due to rapid economic growth, changes in eating habits and decreased physical activity (Coulthard et al., 2021). It is a biological risk factor associated with an increased risk of morbidity and mortality from cardiovascular disease, diabetes, cancer and musculoskeletal disorders. Those who are obese typically report a lower quality of life than people of normal weight (Sarma et al., 2021).

Herbal medicines are a potential alternative to traditional synthetic treatments due to their minimal side effects, and they are considered safe and effective in treating many diseases, including metabolic disorders. Various methods of treating obesity exist, but cheaper methods with fewer negative side effects are more popular. Herbal preparations meet these requirements as they have fewer side effects and are effective at reducing weight in obese individuals by regulating the plasma lipid profile, inhibiting lipase and amylase, increasing ghrelin levels and suppressing appetite (Singh & Bhatti, 2021). In a series of experiments on rats, the influence of *Sambucus nigra* inflorescences, *Viola tricolor* herbs, *Silybum marianum* seeds, *Origanum vulgare* herb, *Inula helenium* root, and herbs such as *Salvia sclarea*, *Helichrysum arenarium* and *Bidens tripartita* was investigated (Lieshchova et al., 2021; Lieshchova, & Brygadyrenko, 2022, 2023b, 2023c; Lieshchova et al., 2023a; Bilan et al., 2024; Lieshchova, & Brygadyrenko, 2024a, 2024b).

Tribulus terrestris is a natural source of antioxidants, which are found in abundance in its fruits, leaves and flowers. These antioxidants are renowned for their ability to scavenge free radicals, and are considered to be less hazardous than their synthetic counterparts. *Tribulus terrestris* is a key ingredient in many over-the-counter supplements widely recommended as enhancers of human vitality. In an experiment in which rats were fed a high-fat diet and given *T. terrestris* herb for 32 days, only a high dose (2.0% of the diet) affected weight gain. By the end of the experiment, the weight of the rats in the group that received an additional 2.0% of the herb was 20.1% lower than the weight of the rats that received only a high-fat diet. A study conducted on 30 healthy men who took a 770 mg *T. terrestris* supplement and underwent CrossFit® training for six weeks showed that the herbal supplement did not improve performance or body composition (body weight, fat mass or composition), but it did increase blood testosterone levels. Consequently, *T. terrestris* can be considered a potential supplement for maintaining optimal testosterone levels and reducing fatigue and catabolism associated with physical exercise (Fernández-Lázaro et al., 2021). Several studies have demonstrated the hypoglycemic activity of *T. terrestris*, both in its natural form and in the form of isolated biologically active substances (Mohammed et al., 2010; Sailaja et al., 2013; Samani et al., 2016; Abdel-Mottaleb et al., 2022; Povydysh et al., 2023; Kaliaperumal et al., 2024). In our study, the herb showed a dose-dependent hypoglycemic effect. Adding 0.5% of the herb to a high-fat diet reduced blood glucose levels by 17.1%, while 2.0% reduced them by 25.5% (Table 3). A single dose of *T. terrestris* methanol extract was found to significantly reduce fasting blood glucose levels, producing effects similar to those observed with glibenclamide treatment (El-Shaibany et al., 2016).

Saponins isolated from *T. terrestris* sharply reduced blood glucose levels after feeding (Su et al., 2009). *Tribulus terrestris* significantly reduced serum glucose, triglyceride and cholesterol levels while increasing serum superoxide dismutase activity in mice with alloxan-induced diabetes. In mice, the *T. terrestris* decoction was found to be effective in the inhibition of gluconeogenesis. An alcohol extract of *T. terrestris* at a concentration of 2 g/kg body weight exhibited a protective effect in rats with streptozotocin-induced diabetes by suppressing oxidative stress (Amin et al., 2009; Chhatre et al., 2014). A comparative study of two species of this herb revealed that extracts of *T. alatus* and *T. terrestris* both significantly reduced fasting glucose levels in diabetic rats. However, *T. alatus* extract had a more pronounced hypoglycaemic effect as it significantly reduced glucose levels after four and six hours compared to *T. terrestris* extract. Both extracts caused a significant reduction in glycated hemoglobin, total cholesterol, triglycerides and LDL cholesterol (El-Tantawy & Hassanin, 2007). Although there is promising evidence of the antidiabetic properties of *T. terrestris*, including confirmation by our experiment, further research is needed to fully understand its hypoglycemic potential and the underlying mechanisms involved in treating diabetes.

In our study, a 2.0% dose of *T. terrestris* in a high-fat diet for 32 days caused a decrease in high-density lipoprotein (HDL) cholesterol levels only, while total cholesterol, low-density lipoprotein (LDL) cholesterol and the atherogenic index remained similar to those of rats consuming a high-fat diet. The hypolipidemic activity of *T. terrestris* is associated with the presence of phenolic compounds, which increase lipoprotein lipase activity in muscle tissue and decrease it in adipose tissue (Khan et al., 2011; Chhatre et al., 2014). An aqueous extract of *T. terrestris* fruit, administered at a dose of 580 mg/kg, reduced cholesterol-induced hyperlipidemia and lowered cholesterol, triglycerides, low-density lipoproteins (LDL), very low-density lipoproteins (VLDL) and the atherogenic index (AI). It also increased high-density lipoproteins (HDL) in the blood of Wistar rats (Khan et al., 2011). The addition of *T. terrestris* to a high-cholesterol diet significantly improved the blood lipid profile of rabbits and reduced endothelial damage and the frequency of vessel rupture (Altug Tuncer et al., 2009). In rats with induced type 2 diabetes, *T. terrestris* saponin supplements combined with inulin significantly reduced liver steatosis while restoring the blood plasma lipid profile (Misiakiewicz-Has et al., 2021).

Many medicinal plants have been studied as potential mild correctors of metabolic processes, particularly in cases of imbalanced diets (Lieshchova & Brygadyrenko, 2021; 2023a; Lieshchova et al., 2023b; Lieshchova, 2024). *Tribulus terrestris* has been reported to have hepatoprotective activity and to protect against liver damage (Saeed et al., 2024). A study found that an aqueous extract of *T. terrestris* combined with silymarin significantly protected against carbon tetrachloride-induced liver damage in rats. *Tribulus terrestris* extract exhibits cytoprotective properties, restoring the histostructure of the liver and reducing levels of liver enzymes (ALT, AST and ALP), as well as lipid peroxidation. Meanwhile, it increases the levels of antioxidants (such as glutathione and superoxide dismutase) in liver tissue (Kilany et al., 2020). The hepatoprotective effect of *T. terrestris* in experimental liver fibrosis caused by carbon tetrachloride was demonstrated by Altay et al. (2019).

However, in this study, we did not observe a significant hepatoprotective effect of the *T. terrestris* herb. In fact, consuming *T. terrestris* as part of a high-fat diet caused a dose-dependent increase in relative liver weight, which may indicate the development of pathological processes. Additionally, analyzing blood enzyme activity indicators revealed that rats on a high-fat diet exhibited significantly elevated AST, ALT, alkaline phosphatase, and alpha-amylase activity. Introducing *T. terrestris* into the diet, especially at a dose of 2.0%, contributed to an even greater increase in AST and ALT activity, indicating possible liver cell damage. In other words, the herb in a high-fat diet causes pronounced morphofunctional changes in the liver. The increase in mass indicators for individual sections of the digestive system can be explained by the higher fiber content of the diet (since dry *T. terrestris* herb was used), compared to the group that received only a high-fat diet.

It is believed that *T. terrestris* can increase testosterone levels by stimulating the release of gonadotropin-releasing hormone (GnRH), which triggers the production of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) (Akhtari et al., 2014; Salahshoor et al., 2020; Sirotkin & Kolesárová, 2021). While we did not determine the level of sex hormones in our study, *T. terrestris* was found to have a significant effect on the reproductive system, increasing the relative mass of the testicle (Table 2).

We used Rosuvastatin as a positive control in our study. Rosuvastatin is a selective competitive inhibitor of HMG-CoA reductase, an enzyme that converts 3-hydroxy-3-methylglutaryl-CoA to mevalonate, a cholesterol precursor. Rosuvastatin is a well-known statin medication used to prevent cardiovascular disease in high-risk individuals and to treat abnormal lipids (Lee, 2022). In our study, Rosuvastatin had a stronger effect on metabolism than the *T. terrestris* herb when administered alongside a high-fat diet. Rosuvastatin significantly reduced blood glucose and cholesterol levels, as well as increasing high-density lipoprotein (HDL) cholesterol levels, more effectively than the *T. terrestris* herb. Similar to 2.0% *T. terrestris*, Rosuvastatin increased blood creatinine levels and significantly impacted mineral metabolism, increasing phosphorus levels while decreasing calcium levels and the Ca/P ratio.

Conclusions

Rats fed a high-fat diet containing *T. terrestris* herb for 32 days gained more weight, had larger relative internal organs and showed greater metabolic changes than rats given Rosuvastatin. At the end of the study, there was no significant change in body weight in rats given a dose of 0.5% *T. terrestris*. However, a dose of 2.0% led to a slight increase in this indicator. *Tribulus terrestris* caused a dose-dependent increase in liver and caecum weight; 2.0% increased the relative weight of the testicles by 49.2% and that of the stomach by 17.3%. Adding *T. terrestris* to a high-fat diet caused changes in blood biochemical parameters. The concentration of glucose in the blood of the animals decreased significantly under the influence of the *T. terrestris* herb: by 17.1% at a dose of 0.5%, and by 25.5% at a dose of 2.0%. A dose of 0.5% of *T. terrestris* caused a 16.2% decrease in creatinine levels, while a dose of 2.0% caused a 27.8% increase. Regarding lipid metabolism, adding 2.0% dry *T. terrestris* herb increased high-density lipoprotein (HDL) cholesterol levels by 44.6%. A dose of 2.0% of *T. terrestris* herb caused a significant increase in inorganic phosphorus (by 57.4%) and a decrease in the Ca/P ratio (by 35.6%). *Tribulus terrestris* herb at a dose of 2.0% increased AST activity by 44.9% and ALT activity by 36.7%. Rosuvastatin in a high-fat diet did not significantly affect the weight of internal organs. It caused an increase in creatinine levels (12.8%), HDL cholesterol (46.1%), inorganic phosphorus (100%), and a decrease in glucose levels (48.6%), cholesterol (14.3%), total calcium (6.3%), and the Ca/P ratio (52.5%).

These results complement existing data on the significant impact of *T. terrestris* active substances on metabolic processes and modelling, which could inform the development of bioproducts for the treatment and prevention of obesity.

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

References

Abdel-Mottaleb, Y., Ali, H. S., El-Kherbetawy, M. K., Elkazzaz, A. Y., ElSayed, M. H., Elshormilisy, A., Eltrawy, A. H., Abed, S. Y., Alshahrani, A. M., Hashish, A. A., Alamri, E. S., & Zaitone, S. A. (2022). Saponin-rich extract of *Tribulus terrestris* alleviates systemic inflammation and insulin resistance in dietary obese female rats: Impact on adipokine / hormonal disturbances. Biomedicine and Pharmacotherapy, 147, 112639.

Adaikan, P. G., Gauthaman, K., & Prasad, R. N. V. (2001). History of herbal medicines with an insight on the pharmacological properties of *Tribulus terrestris*. The Aging Male, 4(3), 163–169.

Akhtari, E., Raisi, F., Keshavarz, M., Hosseini, H., Sohrabvand, F., Bioos, S., Kamalinejad, M., & Ghobadi, A. (2014). *Tribulus terrestris* for treatment of sexual dysfunction in women: Randomized double-blind placebo-controlled study. Daru, 22(1), 40.

Al-Ali, M., Wahbi, S., Twaij, H., & Al-Badr, A. (2003). *Tribulus terrestris*: preliminary study of its diuretic and contractile effects and comparison with *Zea mays*. Journal of Ethnopharmacology, 85(2–3), 257–260.

Al-Bayati, F. A., & Al-Mola, H. F. (2008). Antibacterial and antifungal activities of different parts of *Tribulus terrestris* L. growing in Iraq. Journal of Zhejiang University, Science B, 9(2), 154–159.

Aldaddou, W. A., Aljohani, A. S. M., Ahmed, I. A., Al-Wabel, N. A., & El-Ashmawy, I. M. (2022). Ameliorative effect of methanolic extract of *Tribulus terrestris* L. on nicotine and lead-induced degeneration of sperm quality in male rats. Journal of Ethnopharmacology, 295, 115337.

Al-Hamadani, R., Diab, H., & Al-Hamdani, M. (2025). Protective effects of *Tribulus terrestris* extract on methotrexate-induced liver damage in male rabbits: An experimental study. Babcock University Medical Journal, 8(S1), 37–43.

Al-Hathoot, S. J. M., & Sulaiman Musa, M. I. (2025). Anti-hepatotoxic effects of aqueous and alcoholic extracts of *Tribulus terrestris* fruits and Ferti-Plus® on Albino rats exposed to sulfasalazine. Tikrit Journal of Pharmaceutical Sciences, 19(1), 1–17.

Altay, D., Dogan, Y., Orhan, C., Tuzcu, M., Sahin, N., Ozercan, I., & Sahin, K. (2019). Hepatoprotective effects of *Tribulus terrestris*, ashwagandha and N-acetylcysteine on liver fibrosis in carbon tetrachloride-induced rats. Acta Poloniae Pharmaceutica – Drug Research, 76(5), 805–813.

Altug Tuncer, M., Yaymaci, B., Sati, L., Cayli, S., Acar, G., Altug, T., & Demir, R. (2009). Influence of *Tribulus terrestris* extract on lipid profile and endothelial structure in developing atherosclerotic lesions in the aorta of rabbits on a high-cholesterol diet. Acta Histochemica, 111(6), 488–500.

Amin, A., Lotfy, M., Shafiullah, M., & Adeghate, E. (2006). The protective effect of *Tribulus terrestris* in diabetes. Annals of the New York Academy of Sciences, 1084(1), 391–401.

Arcasoy, H. B., Erenmemisoglu, A., Tekol, Y., Kurucu, S., & Kartal, M. (1998). Effect of *Tribulus terrestris* L. saponin mixture on some smooth muscle preparations: a preliminary study. Bollettino Chimico Farmaceutico, 137(11), 473–475.

Asadmobini, A., Bakhtiari, M., Khaleghi, S., Esmaeili, F., & Mostafaei, A. (2017). The effect of *Tribulus terrestris* extract on motility and viability of human sperms after cryopreservation. Cryobiology, 75, 154–159.

Bilan, M. V., Lieshchova, M. A., & Brygadyrenko, V. V. (2023). Impacts on gut microbiota of rats with high-fat diet supplemented by herbs of *Melissa officinalis*, *Lavandula angustifolia* and *Salvia officinalis*. Regulatory Mechanisms in Biosystems, 14(2), 155–160.

Bilan, M. V., Lieshchova, M. A., Bohomaz, A. A., & Brygadyrenko, V. V. (2024). Effect of *Viola tricolor* flower supplementation on body and intestinal microbiota in rats fed a high-fat diet. Regulatory Mechanisms in Biosystems, 15(3), 626–634.

Chandrasekaran, C. V., Vijayalakshmi, M. A., Prakash, K., Bansal, V. S., Meenakshi, J., & Amit, A. (2012). Review article: herbal approach for obesity management. American Journal of Plant Sciences, 3(7), 1003–1014.

Chen, Y., Quinn, J. C., Weston, L. A., & Loukopoulos, P. (2019). The aetiology, prevalence and morbidity of outbreaks of photosensitisation in livestock: A review. PLoS One, 14(2), e0211625.

Chhatre, S., Nesari, T., Kanchan, D., Somani, G., & Sathaye, S. (2014). Phytopharmacological overview of *Tribulus terrestris*. Pharmacognosy Reviews, 8(15), 45.

Coulthard, H., Sharps, M., Cunliffe, L., & van den Tol, A. (2021). Eating in the lockdown during the Covid 19 pandemic: self-reported changes in eating behaviour, and associations with BMI, eating style, coping and health anxiety. Appetite, 161, 105082.

Dai, H., Alsalhe, T. A., Chalghaf, N., Riccò, M., Bragazzi, N. L., & Wu, J. (2020). The global burden of disease attributable to high body mass index in 195 countries and territories, 1990–2017: An analysis of the global burden of disease study. PLoS Medicine, 17(7), e1003198.

Deole, Y., Chavan, S., Ashok, B., Ravishankar, B., Thakar, A., & Chandola, H. (2011). Evaluation of anti-depressant and anxiolytic activity of Rasayana Ghana tablet (a compound Ayurvedic formulation) in albino mice. AYU, 32(3), 375–379.

Dutta, U., Dey, S., Boruah, M., Sailo, B., Muchahary, B., & Kunnumakkara, A. (2025). Anti-cancer potential of diosgenin, a steroidal saponin, against human oral cancer cells. Asian Pacific Journal of Cancer Prevention, 26(6), 2049–2059.

Eljabri, N. A. E.-A., Ahmed, A. K., & Ahmed, A. (2015). Antihypertensive and hematological effects of *Tribulus terrestris* aqueous extract. International Journal for Innovation Education and Research, 3(8), 67–73.

El-Shaibany, A., Al-Habori, M., Al-Tahami, B., & Al-Massarani, S. (2016). Anti-hyperglycaemic activity of *Tribulus terrestris* L. aerial part extract in glucose-loaded normal rabbits. Tropical Journal of Pharmaceutical Research, 14(12), 2263–2268.

El-Tantawy, W. H., & Hassanin, L. A. (2007). Hypoglycemic and hypolipidemic effects of alcoholic extract of *Tribulus alatus* in streptozotocin-induced diabetic rats: A comparative study with *T. terrestris* (caltrop). Indian Journal of Experimental Biology, 45(9), 785–790.

Fernández-Lázaro, D., Mielgo-Ayuso, J., del Valle Soto, M., Adams, D. P., González-Bernal, J. J., & Seco-Calvo, J. (2021). The effects of 6 weeks of *Tribulus terrestris* L. supplementation on body composition, hormonal response, perceived exertion, and CrossFit® performance: A randomized, single-blind, placebo-controlled study. Nutrients, 13(11), 3969.

Gamal El Din, S. F. (2017). Role of *Tribulus terrestris* in male infertility: Is it real or fiction? Journal of Dietary Supplements, 15(6), 1010–1013.

Garapati, P. (2022). *In-vitro* & *in-vivo* evaluation of anti-inflammatory and antioxidant activity on *Hyptis suoveolens* (seeds) & *Tribulus terrestris* (fruit). International Journal of Science and Research, 11(7), 1211–1218.

Haghmorad, D., Mahmoudi, M. B., Haghighi, P., Alidadiani, P., Shahvazian, E., Tavasolian, P., Hosseini, M., & Mahmoudi, M. (2019). Improvement of fertility parameters with *Tribulus terrestris* and *Anacyclus pyrethrum* treatment in male rats. International Brazilian Journal of Urology, 45(5), 1043–1054.

Hussein, M. (2018). Study of antibacterial effect of *Tribulus terrestris* extract. International Journal of Advanced Research, 6(1), 469–471.

Kaliaperumal, K., Bhat, B. A., Subramanian, K., Ramakrishnan, T., Chakravarthy, E., Al-Keridis, L. A., Ahmad, I., Alabdallah, N. M., Saeed, M., & Karunakaran, R. (2024). *In-vivo* anti-hyperglycemic effect of herbal extracts *Tribulus terrestris* (L) and *Curcuma amada* (R) on streptozotocin-induced diabetic rats and its associated histopathological studies. Heliyon, 10(1), e24009.

Kavitha, P., Ramesh, R., Bupesh, G., Stalin, A., & Subramanian, P. (2011). Hepatoprotective activity of *Tribulus terrestris* extract against acetaminophen-induced toxicity in a freshwater fish (*Oreochromis mossambicus*). In Vitro Cellular and Developmental Biology – Animal, 47(10), 698–706.

Khaleghi, S., Bakhtiari, M., Asadmobini, A., & Esmaeili, F. (2016). *Tribulus terrestris* extract improves human sperm parameters *in vitro*. Journal of Evidence-Based Complementary and Alternative Medicine, 22(3), 407–412.

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.

Khan, S., Kabir, H., Jalees, F., Asif, M., & Naquvi, K. J. (2011). Antihyperlipidemic potential of fruits of *Tribulus terrestris* Linn. International Journal of Biomedical Research, 2(1), 98–101.

Kilany, O. E., El-Beltagy, M. A., & El-Sherbeeny, N. A. (2020). *Tribulus terrestris* ameliorates carbon tetrachloride-induced hepatotoxicity in male rats through suppression of oxidative stress and inflammation. Environmental Science and Pollution Research, 27(20), 24967–24981.

Kumar, M., Soni, A. K., Shukla, S., & Kumar, A. (2006). Chemopreventive potential of *Tribulus terrestris* against 7,12-dimethylbenz(a)anthracene induced skin papillomagenesis in mice. Asian Pacific Journal of Cancer Prevention, 7(2), 289–294.

Kumari, M., & Singh, P. (2018). *Tribulus terrestris* improves metronidazole-induced impaired fertility in the male mice. African Health Sciences, 18(3), 645.

Lal, M., & Sutradhar, D. (2024). A comprehensive analysis of phytochemicals, antioxidant, anti-inflammatory, antibacterial, antifungal and phytoestrogenic properties of different parts of *Tribulus terrestris*. Natural Product Research, 2024, 2424390.

Lee, S.-H. (2022). Meta-analysis on comparison of efficacy and tolerability of Rosuvastatin 5 mg/ezetimibe 10 mg versus Rosuvastatin 20 mg monotherapy. Atherosclerosis, 355, 217–218.

Lieshchova, M. A. (2024). The role of medicinal plants in correcting metabolism during an unhealthy diet. In: Scientific aspects of conserving and restoring natural resources under the modern development of society. Baltija Publishing, Riga. P. 295–379.

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., & Brygadyrenko, V. V. (2023b). Effect of *Echinacea purpurea* and *Silybum marianum* seeds on the body of rats with an excessive fat diet. Biosystems Diversity, 31(1), 90–99.

Lieshchova, M. A., & Brygadyrenko, V. V. (2023c). The effect on the organism of rats of adding *Helichrysum arenarium* inflorescences to a hypercaloric diet, high in sugar and fat. Biosystems Diversity, 31(3), 350–357.

Lieshchova, M. A., & Brygadyrenko, V. V. (2024a). Effect of *Bidens tripartita* leaf supplementation on the organism of rats fed a hypercaloric diet high in fat and fructose. Regulatory Mechanisms in Biosystems, 15(3), 648–655.

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

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., Oliyar, A. V., & Bohomaz, A. A. (2023b). Morphofunctional characteristics of liver in rat exposed to high-fat diet and lemon balm. Theoretical and Applied Veterinary Medicine, 11(3), 10–15.

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.

Lieshchova, M., & Brygadyrenko, V. (2023a). 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, 61(2), 202–211.

Lieshchova, M., Yefimov, V., & Brygadyrenko, V. (2023a). Influence of *Inula helenium* rhizomes and *Matricaria chamomilla* inflorescences on the biochemical and physiological parameters in male rats fed a high-fat diet. Roczniki Państwowego Zakładu Higieny, 74(4), 447–458.

Liu, Y., Sun, M., Yao, H., Liu, Y., & Gao, R. (2017). Herbal medicine for the treatment of obesity: An overview of scientific evidence from 2007 to 2017. Evidence-Based Complementary and Alternative Medicine, 2017(1), 8943059.

Malik, M. Y., Alex, A., Sivalingam, A. M., Neha, B., & Vimal, S. (2024). Evaluation of the phytochemical screening of methanolic seed extracts of *Tribulus terrestris*: an *in vitro* application of anti-cancer, anti-oxidant, and anti-microbial activities. Cureus, 16(8), e66674.

Marques, M. A. A., Lourenço, B. H. L. B., Reis, M. de P., Pauli, K. B., Soares, A. L., Belettini, S. T., Donadel, G., Palozi, R. A. C., Froehlich, D. L., Lívero, F. A. dos R., Gasparotto Jr., A., & Lourenço, E. L. B. (2019). Osteoprotective effects of *Tribulus terrestris* L.: Relationship between dehydroepiandrosterone levels and Ca^{2+}-sparing effect. Journal of Medicinal Food, 22(3), 241–247.

Misiakiewicz-Has, K., Maciejewska-Markiewicz, D., Rzeszotek, S., Pilutin, A., Kolasa, A., Szumilas, P., Stachowska, E., & Wiszniewska, B. (2021). The obscure effect of *Tribulus terrestris* saponins plus inulin on liver morphology, liver fatty acids, plasma glucose, and lipid profile in SD rats with and without induced type 2 diabetes mellitus. International Journal of Molecular Sciences, 22(16), 8680.

Mohammed, A. A., Hussain Al-Amiery, A. A., Al-Mosowy, I., Heba, H. I. A.-M., & Abbas, A. H. (2010). Study the biological activities of *Tribulus terrestris* extracts. Journal of Biotechnology Research Center, 4(1), 55–60.

Mohammed, M. J. (2008). Biological activity of saponins isolated from *Tribulus terrestris* (fruit) on growth of some bacteria. Tikrit Journal of Pure Science, 13(3), 1–4.

Neychev, V., & Mitev, V. (2016). Pro-sexual and androgen enhancing effects of *Tribulus terrestris* L.: Fact or fiction. Journal of Ethnopharmacology, 179, 345–355.

Oh, J. S., Baik, S. H., Ahn, E.-K., Jeong, W., & Hong, S. S. (2012). Anti-inflammatory activity of *Tribulus terrestris* in RAW264.7 Cells (54.2). The Journal of Immunology, 188(1S), 54.2.

Parama, D., Boruah, M., Yachna, K., Rana, V., Banik, K., Harsha, C., Thakur, K. K., Dutta, U., Arya, A., Mao, X., Ahn, K. S., & Kunnumakkara, A. B. (2020). Diosgenin, a steroidal saponin, and its analogs: Effective therapies against different chronic diseases. Life Sciences, 260, 118182.

Parham, S., Kharazi, A. Z., Bakhsheshi-Rad, H. R., Nur, H., Ismail, A. F., Sharif, S., RamaKrishna, S., & Berto, F. (2020). Antioxidant, antimicrobial and antiviral properties of herbal materials. Antioxidants, 9(12), 1309.

Phillips, O. A., Mathew, K. T., & Oriowo, M. A. (2006). Antihypertensive and vasodilator effects of methanolic and aqueous extracts of *Tribulus terrestris* in rats. Journal of Ethnopharmacology, 104(3), 351–355.

Pokrywka, A., Obmiński, Z., Malczewska-Lenczowska, J., Fijatek, Z., Turek-Lepa, E., & Grucza, R. (2014). Insights into supplements with *Tribulus terrestris* used by athletes. Journal of Human Kinetics, 41(1), 99–105.

Povydysh, M. N., Titova, M. V., Ivkin, D. Y., Krasnova, M. V., Vasilevskaya, E. R., Fedulova, L. V., Ivanov, I. M., Klushin, A. G., Popova, E. V., & Nosov, A. M. (2023). The hypoglycemic and hypocholesterolemic activity of *Dioscorea deltoidea*, *Tribulus terrestris* and *Panax japonicus* cell culture biomass in rats with high-fat diet-induced obesity. Nutrients, 15(3), 656.

Preeti, S. (2018). Herbal approach for obesity management. New Insights in Obesity: Genetics and Beyond, 2(1), 5–16.

Saeed, M., Munawar, M., Bi, J. B., Ahmed, S., Ahmad, M. Z., Kamboh, A. A., Arain, M. A., Naveed, M., & Chen, H. (2024). Promising phytopharmacology, nutritional potential, health benefits, and traditional usage of *Tribulus terrestris* L. herb. Heliyon, 10(4), e25549.

Sahin, K., Orhan, C., Akdemir, F., Tuzcu, M., Gencoglu, H., Sahin, N., Turk, G., Yilmaz, I., Ozercan, I. H., & Juturu, V. (2016). Comparative evaluation of the sexual functions and NF-κB and Nrf2 pathways of some aphrodisiac herbal extracts in male rats. BMC Complementary and Alternative Medicine, 16, 318.

Sailaja, K. V., Shivaranjani, V. L., Poornima, H., Rahamathulla, S. B., & Devi, K. L. (2013). Protective effect of *Tribulus terrestris* L. fruit aqueous extracton lipid profile and oxidative stress in isoproterenol induced myocardial necrosis in male albino Wistar rats. EXCLI Journal, 12, 373–383.

Salahshoor, M. R., Abdolmaleki, A., Faramarzi, A., Jalili, C., & Shiva, R. (2020). Does *Tribulus terrestris* improve toxic effect of malathion on male reproductive parameters? Journal of Pharmacy and Bioallied Sciences, 12(2), 183–191.

Samani, N. B., Jokar, A., Soveid, M., Heydari, M., & Mosavat, S. H. (2016). Efficacy of the hydroalcoholic extract of *Tribulus terrestris* on the serum glucose and lipid profile of women with diabetes mellitus. Journal of Evidence-Based Complementary and Alternative Medicine, 21(4), NP91–NP97.

Sarma, S., Sockalingam, S., & Dash, S. (2021). Obesity as a multisystem disease: Trends in obesity rates and obesity-related complications. Diabetes, Obesity and Metabolism, 23(S1), 3–16.

Sellami, M., Slimeni, O., Pokrywka, A., Kuvačić, G., D Hayes, L., Milic, M., & Padulo, J. (2018). Herbal medicine for sports: A review. Journal of the International Society of Sports Nutrition, 15, 14.

Shayakhmetova, G. M., Kovalenko, V. M., Basovska, O. G., & Vozna, A. V. (2020). Clinical biochemical parameters of healthy adult white male rats blood serum (retrospective assessment). Pharmacology and Drug Toxicology, 13(6), 428–433.

Shiva, R., Salahshoor, M., Abdolmaleki, A., Faramarzi, A., & Jalili, C. (2020). Does *Tribulus terrestris* improve toxic effect of malathion on male reproductive parameters? Journal of Pharmacy and Bioallied Sciences, 12(2), 183–191.

Singh, L., & Bhatti, M. (2021). Obesity management via herbal approach: A review. Journal of Pharmaceutical Research International, 33(63B), 204–217.

Singh, P., & Kumari, M. (2015). *Tribulus terrestris* ameliorates metronidazole-induced spermatogenic inhibition and testicular oxidative stress in the laboratory mouse. Indian Journal of Pharmacology, 47(3), 304–310.

Sirotkin, A., & Kolesárová, A. (2021). Puncture vine (*Tribulus terrestris* L.) in control of health and reproduction. Physiological Research, 70(S4), S657–S667.

Su, L., Chen, G., Feng, S.-G., Wang, W., Li, Z.-F., Chen, H., Liu, Y.-X., & Pei, Y.-H. (2009). Steroidal saponins from *Tribulus terrestris*. Steroids, 74(4–5), 399–403.

Wang, H.-N., Xiang, J.-Z., Qi, Z., & Du, M. (2020). Plant extracts in prevention of obesity. Critical Reviews in Food Science and Nutrition, 62(8), 2221–2234.

Wu, Y., Yang, H., & Wang, X. (2017). The function of androgen/androgen receptor and insulin growth factor-1/insulin growth factor-1 receptor on the effects of *Tribulus terrestris* extracts in rats undergoing high intensity exercise. Molecular Medicine Reports, 16(3), 2931–2938.

Wu, Z., Xia, F., Wang, W., Zhang, K., Fan, M., & Lin, R. (2024). The global burden of disease attributable to high body mass index in 204 countries and territories from 1990 to 2021 with projections to 2050: An analysis of the Global Burden of Disease Study 2021. European Journal of Heart Failure, 27(2), 354–365.

Yuan, Z., Du, W., He, X., Zhang, D., & He, W. (2020). *Tribulus terrestris* ameliorates oxidative stress-induced ARPE-19 cell injury through the PI3K/Akt-Nrf2 signaling pathway. Oxidative Medicine and Cellular Longevity, 2020, 7962393.

Yunusova, S., Rozhkovskyi, Y., Prystupa, B., & Bohatu, S. (2023). Study of the anti-inflammatory properties of a thick extract of *Tribulus terrestris* L. Česká a Slovenská Farmacie, 72(4), 184–189.