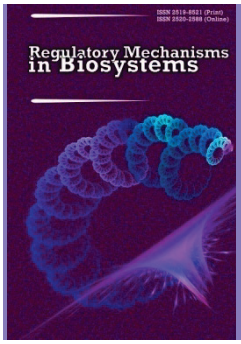

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The state of the liver, reproductive and musculoskeletal systems in female rats with prolonged exposure to α-cypermethrin

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Pyrethroid insecticides are currently a widely used class of pesticides. It is therefore important to determine the mechanism of disorders in some mammalian systems due to prolonged exposure to these pesticides and to justify means to prevent poisoning complications. The study was conducted on 30 female rats aged 3 months for 120 days. Intoxication was modeled using α-cypermethrin at a dose of 10 mg/kg. The prevention of intoxication complications was carried out with an adaptogenic complex consisting of the flavonoid quercetin, vitamins C and D_3, and minerals (Ca, Mn, Zn, Cu, Se, Mg) at a dose of 500 mg/kg. Assessment of intoxication and preventive efficacy of the adaptogen was performed by determining the duration of the estrous cycle, ovarian organ index, degree of atrophy of the alveolar process in the jaws, as well as density, content of mineral and organic components in the bones. Hepatotoxicity markers were determined in the blood serum, osteoresorption markers in the alveolar bone homogenate, inflammation indicators in the liver, and antioxidant system status in all tissues. Prolonged exposure to α-cypermethrin was associated with a prolongation of the estrous cycle due to a reduction in the diestrus stage and a decrease in the ovarian organ index along with a significant increase in blood estradiol levels. In intoxicated animals, the degree of atrophy of the alveolar process increased and the density of femurs and vertebrae decreased due to a decrease in the weight fraction of the mineral component in the bones. In the bone tissue of the alveolar process, an increase in the activity of elastase and markers of oxidative stress (an increase in the content of malondialdehyde and a decrease in catalase activity), as well as a compensatory increase in the alkaline phosphatase activity were found. In the rat liver, an increase in the acid phosphatase activity and the inhibition of antioxidant defense were observed. An increase in the elastase activity and a decrease in the catalase activity with an increase in the malondialdehyde content were found in the serum of the animals. The use of an adaptogen under conditions of α-cypermethrin intoxication contributed to the normalization of the estrous cycle, ovarian organ index and blood estradiol levels. In the tissue of the femur and vertebrae of rats, the treatment by the prophylactic complex led to an increase in bone density due to an increase in the content of the mineral component, and a decrease in the degree of atrophy of the jaws, in the tissue of which the activation of the enzymatic link of antioxidant defense and a decrease in the activity of destructive elastase too were found. The developed adaptogen prevented the development of oxidative stress and inflammation in the blood serum and liver in the animals. The results of the research indicate a negative effect of α-cypermethrin on the sexual function in female rats, on the state of bone tissue along with the hepatotoxic effect of the pesticide. The proposed agent for the prevention of α-cypermethrin intoxication effectively prevented endocrine disruption in the ovaries, bone destruction and inflammation in the liver.

Keywords: α-cypermethrin; estrous cycle; ovaries; estradiol; hepatotoxicity; osteodystrophy; quercetin; calcium citrate.

Introduction

Pyrethroid insecticides (pyrethroids) are a type of synthetic pesticides that are complex esters of chrysanthemic acid in chemical composition. By their mechanism of action, these compounds are neurotoxins that bind to sodium channels and change the membrane potential of insect nerve cells, leading to their death due to excitation paralysis. Pyrethroids have been widely used in agriculture since the late twentieth century due to their high efficiency and low toxicity to non-target species compared to organophosphate, carbamate and organochlorine insecticides. Currently, sales of pyrethroid insecticides in the world account for about 19% of the total number of pesticides on the global market, and they are becoming more and more popular every year. The pyrethroids market reached a value of $3.4 billion in 2021, with a forecast to grow to $4.5 billion by 2027 (Ahamad & Kumar, 2023; Yue et al., 2023). In the environment, pyrethroid residues may be present in water, soil, dust and air. These compounds can enter the human body through the respiratory system, skin contacts and ingestions. They can be transmitted through the food chain, which also poses a risk of ingestion into the human body (Kozak et al., 2020; Shepelska et al., 2021; Faly et al., 2023). Due to their lipophilic properties, pyrethroids accumulate in the body in adipose and nervous tissues, liver and breast milk (Yue et al., 2023).

Despite the fact that acute intoxication with pyrethroids has a very low level of danger to humans and other vertebrates, there is increasing evidence in the scientific literature that under chronic exposure these compounds can cause neurological disorders, damage to genetic material, the formation of malignant tumors, initiation of apoptosis, cardiovascular disease and hepatotoxic effects. Pyrethroids are metabolised in the liver through hydrolytic cleavage of esters and oxidative pathways involving cytochrome P_{450} enzymes. As a result, an excess of reactive oxygen species is formed, which exceeds the ability of the cell's antioxidant system to neutralize the latter. This leads to an increase in pro-oxidant processes; excessive amounts of free radicals cause damage to proteins, lipids, cell organelles and DNA. One of the main dangers of using these substances is

that they and their metabolites are potential disruptors of the sexual endocrine system due to their ability to bind to sex hormone receptors and act as full or partial receptor agonists and antagonists, as well as damage the gonadal tissue through increased oxidative stress in both sexes (Shepelska et al., 2021b; Ahamad & Kumar, 2023).

It is known that in male rats, when intoxicated with pyrethroids such as deltamethrin, fenvalerate, cypermethrin, the quality of sperm deteriorates, sperm DNA is damaged and sperm motility decreases; testosterone levels in blood plasma, testicular weight and the number of Leydig and Sertoli cells decrease. In men, increased concentrations of the nonspecific metabolite of more than 18 pyrethroid pesticides, 3-phenoxybenzoic acid (3-PBA), may be associated with impaired testosterone production, increased serum estradiol levels, due to the induction of aromatase activity by some pesticides (Ravula & Yenugu, 2021; Xu & Bo, 2022).

In female rats exposed to fenvalerate, deltamethrin, bifenthrin, permethrin, cypermethrin, follicular atresia, granulose cell apoptosis, decreased ovarian weight, degenerative changes in the structure of mitochondria and endoplasmic reticulum in follicular cells and corpus luteum cells and increased duration of the estrous cycle were observed. There is evidence that the concentration of 3-PBA is directly proportional to the level of follicle-stimulating and luteinizing hormones and inversely proportional to the level of estradiol in the blood serum; increased concentrations of this metabolite also correlate with delayed puberty in girls (Ye et al., 2017; Li et al., 2018; Song et al., 2022). These changes lead to exhaustion of ovarian function and an imbalance in the activity of sex hormones, creating conditions for the development of primary ovarian insufficiency – a reproductive-endocrine disease characterized by the cessation of menstrual function before the age of 40 (Song et al., 2022). Primary ovarian insufficiency is accompanied by typical symptoms of menopause: dry skin, urogenital syndrome, emotional disorders, hot flashes, lipid metabolism disorders, atherosclerosis, osteoporosis, but these symptoms are more pronounced because the woman's body does not gradually adapt to the decrease in the production of sex hormones (Ventskivska et al., 2018).

Pyrethroid insecticides are also characterised by hepatotoxicity, as their chronic exposure can be accompanied by damage to liver cells due to inhibition of the activity of antioxidant defence enzymes such as superoxide dismutase, catalase, glutathione peroxidase and, as a result, initiation of lipid peroxidation of hepatocyte cell membranes (Dar et al., 2019).

Cypermethrin (R,S)-a-cyano-3-phenoxybenzyl (1RS)-cis-trans-3-(2,2-dichlorovinyl)-2,2-dimethylcyclopropanecarboxylate) and its stereoisomers are among the most widely used pyrethroid insecticides in the world. There are 8 possible isomers for the cypermethrin molecule, each of which has a predominant insecticidal effect against certain taxonomic groups of insect pests; among all isomers, α-cypermethrin – a racemate of cypermethrin is the most commonly used that includes two cis-enantiomers, 1R-cis-αS and 1S-cis-αS. Cypermethrin has toxic effects, the clinical manifestations of which are typical for the other pyrethroids were listed above including those of the gonads, but there is little evidence of hepatotoxicity and sexual toxicity of α-cypermethrin in women and no information about methods to the prevent the development of chronic intoxication complications (Shepelska et al., 2021a; Yue et al., 2023).

Given the increased risk of excessive production of reactive oxygen species and osteoresorptive phenomena due to premature ovarian failure caused by pyrethroids, we had assumed the preventive effectiveness of the calcium complex together with other macro- and microelements, vitamins and the flavonoid quercetin in conditions of prolonged pesticide exposure. The rationale for the complex composition is listed below.

Calcium is an integral part of the mineral component of bone tissue, which is the main depot of this element in the body, where it is found in the form of a crystalline compound – hydroxyapatite, in combination with phosphorus. Calcium deficiency is one of the most common risk factors for osteoporosis. It is worth noting that low estrogen levels reduce intestinal calcium absorption and accelerate its excretion in the urine (Vannucci et al., 2018; Knosla & Pacifici, 2021). Vitamin D_3 is considered to be an essential agent of bone metabolism, which prevents the bone resorption and increases the calcium absorption in the digestive tract. It also participates in the processes of cell differentiation, immune response, and controls the formation of cytokines in various tissue types. Vitamin D deficiency indirectly affects the transcriptional activation of the RANK receptor, which leads to an increase in the number of osteoclasts (Povoroznyuk & Grigoryeva, 2001; Shymanskyi et al., 2017). Vitamin D_3 is a precursor of its active metabolite calcitriol – $1,25(OH)_2D$, which is formed in the kidneys and bones by the enzyme 1α-hydroxylase, whose activity depends on estrogen levels. The reduced intestinal calcium absorption and tubular reabsorption due to a lack of $1,25(OH)_2D$, which occurs among other things due to ovarian dysfunction, leads to a deterioration in calcium availability, increased parathyroid hormone secretion, phosphaturia, decreased bone mineralization and osteomalacia (Goltzman, 2018).

Manganese activates osteogenic marker genes and improves the formation of extracellular bone matrix. It is involved in the metabolism of carbohydrates and lipids, acts as an activator or a cofactor in some enzymes, in particular in manganese-containing superoxide dismutase, an antioxidant enzyme whose deficiency can lead to non-alcoholic fatty liver disease (Westhauser et al., 2020; Zhang et al., 2021). Selenium is an important microelement for maintaining human health. Most selenoproteins, including glutathione peroxidase and thioredoxin reductase, exhibit the functions of oxidoreductases, preventing the development of oxidative stress (Huang et al., 2022). It is also known that low levels of selenium in the body are associated with a decrease in the rate of bone renewal and a decrease in bone density (Gilbert et al., 2022).

Magnesium is able to act as an "intracellular buffer" that interacts with negatively charged molecules such as RNA and DNA, reactive oxygen species, ATP, participating in the stabilisation of nucleic acids, modulation of enzyme activity, and protection of cells from oxidative stress (Liu & Dudley, 2020; Mathew & Panonnummal, 2021). Magnesium is also essential for the bone development and the mineralization; it stimulates the activity of osteoblasts and phosphatase enzymes involved in bone formation. Hypomagnesemia leads to a decrease in bone density and the development of osteoporosis, parathyroid gland dysfunction, and low $1,25(OH)_2D$ levels (Ciosek et al., 2021). It is known that low serum magnesium levels correlate with a decrease in the bone mineral density, as well as changes in the metabolic activity of bone cells, which may indicate an increased risk of fractures (Orchad et al., 2014; Mederle et al., 2022).

Ascorbic acid (vitamin C) is a powerful antioxidant and an essential factor in the bone formation. In experiments on mice undergoing ovariectomy, vitamin C administration increased the bone density, normalized the alkaline phosphatase activity and free radical load (Deyhim et al., 2018). There is also evidence that serum vitamin C concentrations are inversely proportional to the increased risk of bone fractures (Rondanelli et al., 2021).

Zinc is a cofactor of a number of metalloenzymes, participates in longitudinal bone growth, endocrine regulation and intracellular signal transduction in osteogenesis, stimulates osteoblasts to the protein synthesis and prevents their apoptosis, regulates extracellular matrix mineralization, affects the expression of bone marker genes encoding proteins such as osteopontin and osteocalcin, increases the expression of bone alkaline phosphatase and bone sialoprotein (Alghadir et al., 2016; O'Connor et al., 2020; Westhauser et al., 2020).

Copper is a cofactor of lysyloxidase, an enzyme that catalyses the bonding of elastin and collagen fibres in the organic component of bone tissue. Copper exhibits pro-angiogenic effects, stimulates osteogenic differentiation of osteoblast progenitor cells and promotes the mineralization of the extracellular matrix. This element is also a cofactor of a number of antioxidant enzymes: cytochrome c-oxidase, superoxide dismutase, ceruloplasmin (Westhauser et al., 2020; Chen et al., 2023).

Given that, in addition to increasing oxidative stress and suppressing the antioxidant defense system, pyrethroid pesticides interfere with the processes controlled by female sex hormones both directly and indirectly through the metabolic transformations, a possible solution to the problem of complications resulting from prolonged exposure of these compounds would be the use of flavonoids such as quercetin. Quercetin has an estrogen-like effect, it is able to exert a regulatory effect on the functions of the reproductive system, including folliculogenesis, egg maturation, and ovulation; this flavonoid is a powerful antioxidant that protects cells from the damaging effects of reactive oxygen species; at the same time, long-term use of quercetin is practically not accompanied by significant side effects (Rashidi et al., 2020).

In view of the above, the aim of the study was to investigate the toxic effects of α-cypermethrin in relation to the reproductive, musculoskeletal

systems and liver condition of female rats, as well as to establish the preventive effectiveness of an adaptogenic complex based on the bioflavanoid quercetin, calcium citrate, vitamins, macro- and microelements.

Materials and methods

Laboratory rats were kept in standard vivarium conditions in compliance with the bioethical norms stipulated by the rules of the World Medical Association statement on animal use in biomedical research and the European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes (Strasbourg, March 18, 1986, ETS No. 123). Animals were housed in cages, two individuals per cage, with access to drinking water and food. The rats were fed a complete combined diet, daily food and water intake was not measured. Temperature and humidity were controlled according to the recommendations of the Guide for the Care and Use of Laboratory Animals (www.ncbi.nlm.nih.gov/books/NBK54039). The animals were removed from the experiment by bleeding from the heart under thiopental anesthesia (20 mg/kg).

The research was carried out on the basis of the biological faculty of the Odessa I. I. Mechnikov National University and of the State Establishment "The Institute of Stomatology and Maxillofacial Surgery of the National Academy of Sciences of Ukraine" from December 2022 to April 2023. Twenty-eight female rats, aged three months and weighing an average of 104.8 ± 6.9 g at the start of the experiment, were used.

The animals were divided into three groups: 1 – an intact group (10 animals), 2 – a group of animals treated with α-cypermethrin (8 animals), 3 – a group of animals that received the prophylactic complex against the background of α-cypermethrin use (10 animals). α-Cypermethrin of the trade mark "Fas", manufactured LLC "Factory of Agrochemicals", Cherkasy, manufactured to the order of LLC "Ukravit Agro", Kyiv, elaborated on August 8, 2022 (expiry date 3 years), concentration of an active substance 100 g/L in this experiment used. The insecticide was administered at a dose of 10 mg/kg body weight to rats orally via an enterogastric tube together with corn oil daily in the morning.

The control group of animals received drinking water via an enterogastric tube. The prophylactic adaptogenic complex was administered orally every morning at a dose of 50 mg/100 g of animal body weight per day in the form of a suspension. The preventive complex was composed as follows: calcium citrate from oyster shells (the sample was made using our own technology) – 11 mg; vitamin C ("Kyiv Vitamin Plant" PJSC, Kyiv, Ukraine) – 3.7 mg; magnesium ("Magnesium active", LLC "Elit-Pharm", Ukraine, Dnipro) – 11 mg; quercetin ("Quertin" tablets, Borshchagiv Chemical and Pharmaceutical Plant, Ukraine) – 3.7 mg; manganese ("Active manganese", LLC "Elit-Pharm", Dnipro, Ukraine) – 5.5 mg; vitamin D_3 ("Olidetrim Kids oral drops" Medana Farma JSC, Poland) – 2.2 IU; selenium ("Active Selenium", LLC "Elit-Pharm", Dnipro, Ukraine) – 3.7 mg; zinc ("Active Zinc", LLC "Elit-Pharm", Dnipro, Ukraine) – 3.7 mg; copper ("Active Copper", LLC "Elit-Pharm", Dnipro, Ukraine) – 5.5 mg. Doses of vitamins and minerals are correlated with the daily human need, taking into account the mass and surface area of a human and a rat body.

The experiment lasted 4 months. During the second month, the study of the duration of the estrous cycle of rats was carried out using vaginal smears with the determination of the proestrus phases (mostly epithelial cells can be identified in the smear), estrus (keratinized epithelial cells can be observed in the smear), metestrus (intact and keratinized epithelial cells are present in the smear as well as leukocytes) and diestrus (leukocytes and mucus are present in the smear) (Ajayi et al., 2020).

After removing the animals from the experiment, blood serum was collected. The level of estradiol, markers of hepatotoxicity (activity of alanine aminotransferase, alkaline phosphatase), activity of elastase, catalase, and malondialdehyde were determined in blood serum. The ovaries were isolated to determine the organ index. The femur and vertebrae were isolated, in which tissue density, the content of the mineral-organic complex, and the content of mineral and organic components were determined. The bone of the alveolar process of the lower jaw was isolated to determine the degree of atrophy. In the alveolar bone homogenate (75 mg/mL of 0.1 M citrate buffer, pH 6.1), the activity of acid and alkaline phosphatases, elastase, catalase and the content of malondialdehyde were determined. The activity of elastase, acid phosphatase, catalase and the content of malondialdehyde were determined in the liver homogenate (50 mg/mL of 0.05 M Tris-HCl buffer, pH 7.5) (Makarenko et al., 2022).

Atrophy of the alveolar process, which characterizes the development of the dystrophic process in the periodontium, was determined by the degree of molar root exposure and expressed as a percentage. The volume of femur and vertebrae was determined based on the measurement of the Archimedean force acting on the bone when it is completely immersed in water, by weighing the bone in air and in water. The value of the Archimedean force as the difference between the weights of the bone in air and when it is immersed in water, taking into account the acceleration of gravity and the density of water, was translated into the volume of water displaced by the bone, which is equal to the volume of the bone. Bone density was calculated as the ratio of wet bone mass to its volume. The relative content of mineral and organic components of bones was determined using constant values of the density of these components (Makarenko et al., 2022). The level of estradiol in the blood serum of rats was determined by chemiluminescence immunoassay using the IMMULATE 2000 Estradiol reagent kit (Siemens Healthcare Diagnostics Products Ltd., UK) and expressed in 1 pg/mL.

Elastase activity was assessed by the degree of hydrolysis of the synthetic substrate N-t-BOC-L-alanine-p-nitrophenyl ester ("Sigma", USA) according to the Visser-Blouf method and expressed in microcaths (mccats) per 1 kg or per 1 liter of the investigated tissue. Elastase activity, which catalyses the cleavage of 1 n-nitrophenol in 1 s, was taken as 1 catal (Makarenko et al., 2022).

The activity of alanine aminotransferase in the blood serum of rats was determined by the Reitman-Frenkel method. The determination of activity was based on the measurement of the optical density of 2,4-dinitrophenylhydrazones of 2-oxoglutaric and pyruvic acids (LLC RPE "Filisit-Diagnostika", Ukraine, Dnipro) in an environment that has an alkaline reaction. The optical density of the experimental sample was measured against the blank at a wavelength of 530 nm. Enzyme activity was calculated according to the calibration graph and expressed in μkat/L. The activity of acid and alkaline phosphatase was determined on the basis of the hydrolysis of the substrate disodium 4-nitrophenyl phosphate hexahydrate (Sigma, USA) by the Bessey-Lowry-Brock method and was expressed in μcat per 1 kg or per 1 litre of the investigated tissue (Makarenko et al., 2022). Our study of the catalase enzyme activity was based on the ability of hydrogen peroxide to form a stable coloured complex with the absorption maximum at 410 nm with ammonium molybdate (PE "Simesta" Ukraine, Odessa). Catalase activity was assessed by the intensity of the solution colour and expressed in μcat per 1 kg or 1 L of tissue (Makarenko et al., 2022). The determination of the malondialdehyde content was based on the principle that at high temperature in an environment that has an acidic reaction, malondialdehyde reacts with 2-thiobarbituric acid (PE "Simesta", Ukraine, Odesa), forming a coloured trimethyl complex which has an absorption maximum at waves of 532 nm. The amount of malondialdehyde was expressed in mmol/kg or mmol/L tissue (Makarenko et al., 2022). The data in the paper are presented as mean ± standard error (x ± SE). Significance of differences between samples was calculated using one-way analysis of variance with Bonferroni's correction and Tukey's multiple comparison procedure. P value of less than 0.05 was considered statistically significant.

Results

The study of the estrous cycle duration showed that long-term administration of α-cypermethrin to female rats caused a significant lengthening of the total cycle by 30.2%, mainly due to an increase in the time of the progesterone-dependent stage – diestrus (Table 1). Established violations are one of the signs of gonadotoxic action typical to pyrethroids. In rats that were treated with the adaptogen together with α-cypermethrin, the indicator of the total duration of the estrous cycle was 26.8% less than in the group of animals with intoxication ($F_{0.05}$ = 3.39, F = 6.84, P = $3.9*10^{-3}$). Each of the stages of the cycle in rats receiving the adaptogen lasted approximately the same period of time, which may indicate the normalization of the course of the estrous cycle in animals using the proposed complex in the conditions of pesticide load. The correction of the

cycle in rats that received the adaptogenic complex occurred due to a decrease in the duration of diestrus ($F = 7.42$, $P = 2.71*10^{-3}$) and estrus ($F = 4.33$, $P = 1.5*10^{-2}$, Table 1).

A decrease in the organ index of the ovaries in rats by 21.9% was established, and although these changes are unreliable, one can see a certain tendency to decrease in the mass of the gonads, which arose as a result of the initiation of a number of pathological changes in the cells in these organs caused by the action of the pesticide. At the same time, the level of estradiol in the rat serum under the condition of using α-cypermethrin increased by 103.5%. This can be another sign of the destructive effect of the insecticide on the ovary state and interference with the processes of hormonal regulation of sexual functions. Administration the adaptogen to rats against the background of receiving α-cypermethrin contributed to a significant increase in the organ index by 52.0% ($F = 3.69$, $P = 3.93*10^{-2}$) and also reduced the level of estradiol in the blood to normal values ($F = 5.07$, $P = 3.35*10^{-2}$). The obtained results testify to the powerful protective potential of the proposed adaptogen in maintaining the hormonal balance in the body and the state of the reproductive organs (Table 2).

Table 1

Duration of estrous cycle in female rats exposed to chronic intoxication with α-cypermethrin and rats treated with the adaptogen to prevent its complications ($x \pm SE$, $n = 28$)

Group	Total duration of the estrous cycle, days	Duration of individual phases of the estrous cycle, days			
		proestrous	estrous	metestrous	diestrous
Control	4.3 ± 0.1^{a}	1.01 ± 0.01^{a}	1.03 ± 0.03^{ab}	1.11 ± 0.02^{a}	1.24 ± 0.03^{a}
Cypermethrin	5.6 ± 0.2^{b}	1.06 ± 0.03^{a}	1.29 ± 0.01^{b}	1.06 ± 0.02^{a}	2.14 ± 0.05^{b}
Adaptogen	4.1 ± 0.1^{a}	1.03 ± 0.02^{a}	1.03 ± 0.03^{a}	1.01 ± 0.01^{a}	1.03 ± 0.01^{c}

Notes: values with different superscripts in each column are significantly different by Tukey's multiple comparison procedure ($P < 0.05$); adaptogen – preventive adaptogenic complex, which consisted of quercetin (3.7 mg), Ca (11.0 mg), D_3 (2.2 IU), Se (3.7 mg), Cu (5.5 mg), Zn (3.7 mg), Mg (11.0 mg), Mn (5.5 mg), vitamin C (3.7 mg).

Considering the fact that there was no decrease in estradiol levels in the blood serum in rats under the action of the insecticide, it would be imprudent to talk about the development of the ovarian exhaustion. But, summarizing the data presented in Tables 1 and 2, it can be concluded that α-cypermethrin definitely has a toxic effect on the reproductive system, which is expressed in abnormal changes in the duration of the estrous cycle and the level of estradiol in blood serum, a decrease in the ovarian organ index, which is a sign violation of the regulation of the homeostasis of sex hormones and the function of the gonads, which in turn creates a risk of the possible development of the primary ovarian insufficiency.

Since one of the main symptoms of premature ovarian failure, including due to the influence of endocrine disruptors, is the activation of osteoresorptive processes, the study of the morphometric parameters of the femur, lumbar vertebrae and alveolar process was conducted. It was found that under the condition of the insecticide application, the femur density significantly decreased by 3.3% ($F = 3.53$, $P = 4.46*10^{-2}$), the content of the mineral-organic complex decreased by 5.2% ($F = 4.04$, $P = 3.01*10^{-2}$), while the content of the mineral component in the thigh, which decreased by 8.4%, the content of the organic component, mass and volume of the bone did not undergo statistically significant changes (Table 3).

The density of the lumbar vertebrae during intoxication decreased by 3.2% ($F = 5.62$, $P = 9.6*10^{-3}$), the content of the mineral-organic complex – by 5.7% ($F = 3.72$, $P = 3.85*10^{-2}$), the content of the mineral component became significantly smaller by 10.2% ($F = 6.36$, $P = 5.85*10^{-3}$), the content of the organic component, as in the case of the femur, remained within the control values, while the mass and volume indicators in the vertebrae also did not undergo statistically significant changes (Table 4).

Table 2

Ovarian organ index and estradiol level in blood serum in rats exposed to chronic intoxication with α-cypermethrin and in rats given the adaptogen to prevent its complications ($x \pm SE$, $n = 28$)

Group	Ovarian mass index, mg/g	Serum estradiol concentration, pg/mL
Control	0.322 ± 0.021^{ab}	22.53 ± 4.53^{a}
Cypermethrin	0.253 ± 0.042^{a}	45.85 ± 9.63^{b}
Adaptogen	0.382 ± 0.040^{b}	27.55 ± 6.42^{a}

Note: see Table 1.

Table 3

Morphometric parameters of femurs in female rats exposed to chronic intoxication with α-cypermethrin and in rats given the adaptogen to prevent its complications ($x \pm SE$, $n = 28$)

Group	Density, mg/mm^3	Mass, mg	Volume, mm^3	Mineral-organic complex content, % (weight fraction)	Mineral component content, % (weight fraction)	Organic component content, % (weight fraction)
Control	1.526 ± 0.014^{a}	472 ± 30^{a}	310.0 ± 19.6^{a}	65.08 ± 0.79^{a}	40.13 ± 1.07^{a}	24.95 ± 0.75^{a}
Cypermethrin	1.475 ± 0.018^{b}	507 ± 30^{a}	343.6 ± 19.1^{a}	61.72 ± 1.35^{b}	36.76 ± 1.18^{a}	24.96 ± 0.62^{a}
Adaptogen	1.529 ± 0.015^{a}	501 ± 14^{a}	327.6 ± 7.7^{a}	65.19 ± 0.73^{a}	40.41 ± 1.14^{a}	24.78 ± 0.58^{a}

Note: see Table 1.

Table 4

Morphometric parameters of lumbar vertebrae in female rats exposed to chronic intoxication with α-cypermethrin and in rats given the adaptogen to prevent its complications ($x \pm SE$, $n = 28$)

Group	Density, mg/mm^3	Mass, mg	Volume, mm^3	Mineral-organic complex content, % (weight fraction)	Mineral component content, % (weight fraction)	Organic component content, % (weight fraction)
Control	1.433 ± 0.009^{a}	121.3 ± 9.3^{a}	84.5 ± 6.3^{a}	60.23 ± 0.52^{a}	33.01 ± 0.79^{a}	27.22 ± 0.64^{a}
Cypermethrin	1.387 ± 0.015^{b}	113.7 ± 10.2^{a}	81.7 ± 6.9^{a}	56.80 ± 1.62^{b}	29.63 ± 0.85^{b}	27.17 ± 0.95^{a}
Adaptogen	1.439 ± 0.011^{a}	126.4 ± 6.3^{a}	87.8 ± 4.3^{a}	60.01 ± 0.68^{ab}	33.84 ± 0.90^{a}	26.17 ± 0.31^{a}

Note: see Table 1.

Thus, the content of the mineral-organic complex, the density of the femur and vertebrae in rats under the influence of α-cypermethrin decreased primarily due to a decrease in the mineral component content in the bone. The use of the adaptogenic complex effectively prevented the demineralization of the protein matrix in both types of the studied bones, which in turn contributed to the reliable normalization of the morphometric parameters of bone tissue compared to the indicators in the group with pyrethroid intoxication. Thus, the femur density in animals that used the adaptogen increased significantly by 3.7%; the content of the mineral component – by 9.3%, which contributed to the increase in the content of the mineral-organic complex by 5.7%. In the vertebrae, the growth of the specified parameters was 14.2% for the density indicator and 3.8% for the content of the mineral component. It should be noted that the use of the adaptogen against the background of the toxic insecticide load did not significantly affect the content of the organic component (Tables 3 and 4).

The alveolar process of the mandible is very sensitive to bone resorption, as evidenced by an increase in the degree of its atrophy in the presence of pathology. In the group affected by the toxic insecticide effects,

there was a significant increase in the degree of the alveolar process atrophy by 10.8% (Table 5). Based on this result, it can be assumed that resorptive processes develop in the bones in the maxillofacial area and there is a risk of periodontitis under the condition of prolonged exposure to α-cypermethrin and its metabolites. The administration of the adaptogenic complex to rats against the background of intoxication reduced the atrophy rate by 16.9 %, which indicates the prevention of bone destruction ($F = 6.74$, $P = 4.5*10^{-3}$).

The osteoresorptive effect of α-cypermethrin intoxication and the osteotropic effect of the proposed adaptogen were also confirmed by biochemical changes in the bone tissue of the alveolar process in rats. Thus, the activity of acid phosphatase (an osteoresorption marker) in the tissue of animals with pesticide intoxication increased by 29.8%, and when the prophylactic complex was used, the activity of this enzyme decreased to the values of the intact group. It should be noted although these changes were not statistically significant, in rats with pathology a characteristic tendency to increase in the activity of the enzyme, which is the main marker of osteoclasts, is clearly observed. At the same time, in the jaws of rats of the intoxication group, there was an increase in the activity of bone alkaline phosphatase by 36.2% with a high degree of confidence, which may indicate a compensatory activation of the osteoblastogenesis processes against the background of increased destruction of periodontal bone tissue caused by pesticide load. The administration of the adaptogenic complex to rats treated with α-cypermethrin contributed to a statistically significant decrease in the activity of bone alkaline phosphatase by 40.3% ($F = 15.24$, $P = 2.21*10^{-5}$). Moreover, the level of this indicator had no statistical difference from the value of the intact group (Table 5).

Also, under conditions of prolonged exposure to α-cypermethrin in the bone tissue of the jaws of animals, there was a significant increase in the activity of the proteolytic enzyme elastase by 201.1% ($F = 41.23$, $P = 3.31*10^{-5}$), the activity of the antioxidant enzyme catalase – by 39.6% ($F = 9.00$, $P = 1.11*10^{-3}$) and the end product of lipid peroxidation – malondialdehyde – by 38.9% ($F = 8.29$, $P = 1.70*10^{-3}$). The use of the adaptogenic complex contributed to the normalisation of these parameters to the values in the jaws of intact rats. However, it should be noted that in the case of elastase activity, the result was significantly higher than in the jaws of the intact group, although there was a decrease in the activity of this enzyme by 30.8% compared to the group without prophylaxis. Since the activity of proteolytic enzymes and the state of the antioxidant-prooxidant system, with the predominance of the formation of excessive amounts of reactive oxygen species and lipid peroxidation products, are of great importance in the pathogenesis of bone destruction, the obtained data may serve as evidence of the osteoprotective and antioxidant effect of the proposed adaptogenic complex (Table 5).

Table 5

Biochemical indicators of osteogenesis, osteoresorption and the states of antioxidant and prooxidant systems in the mandible alveolar process in female rats exposed to chronic intoxication with α-cypermethrin and in rats given the adaptogen to prevent its complications ($x \pm SE$, $n = 28$)

Group	Alveolar process atrophy, %	Acid phosphatase activity, μkat/kg	Alkaline phosphatase activity, μkat/kg	Elastase activity, μkat/kg	Malondialdehyde content, mmol/kg	Catalase activity, μkat/kg
Control	30.5 ± 0.8^{ab}	4.66 ± 0.44^{a}	28.35 ± 2.33^{a}	6.48 ± 0.52^{a}	4.91 ± 0.43^{a}	1.061 ± 0.072^{a}
Cypermethrin	33.8 ± 1.2^{b}	6.05 ± 0.52^{a}	38.60 ± 1.84^{b}	19.51 ± 1.48^{b}	6.82 ± 0.48^{b}	1.484 ± 0.081^{b}
Adaptogen	28.1 ± 1.2^{a}	4.81 ± 0.48^{a}	23.03 ± 1.56^{a}	13.51 ± 0.97^{c}	4.79 ± 0.21^{a}	1.091 ± 0.074^{a}

Note: see Table 1.

In the processes of detoxification of xenobiotics, the liver is necessarily involved, biochemical changes in which will signal the nature of the toxic effect of one or another substance, as well as the effectiveness of measures aimed at preventing complications. In the study of the liver, we found that α-cypermethrin intoxication is accompanied by a suppression of the antioxidant defence system in this organ, which was expressed in a significant decrease in catalase activity by 18.4% ($F = 16.63$, $P = 4.25*10^{-6}$). At the same time, the content of malondialdehyde, which is an indicator of lipid peroxidation in liver homogenate increased by 72.3% ($F = 9.71$, $P = 8.0*10^{-4}$). The intensification of free radical processes in the liver was accompanied by the development of inflammation, as evidenced by an increase in the activity of the lysosomal enzyme acid phosphatase by 41.9% ($F = 29.16$, $P = 5.4*10^{-5}$). However, in the liver of rats under the influence of α-cypermethrin, the activity of elastase, which is also a marker of inflammatory phenomena, did not undergo statistically significant changes (Table 6). The use of the prophylactic complex against the background of α-cypermethrin intoxication led to a significant decrease in the acid phosphatase activity by 26.0% in the rat liver. Catalase activity increased by 20.1%, while the content of malondialdehyde in the liver homogenates decreased by 26.5% (Table 6). Such data can be interpreted as a manifestation of the anti-inflammatory and antioxidant effect of the complex of vitamins and minerals (Table 6).

It is worth noting that despite the presence of signs of inflammation and cell lysis in the liver of α-cypermethrin-treated rats, there were no significant changes in serum alanine aminotransferase activity. As for another serum marker of liver function, alkaline phosphatase activity, a significant decrease of 9.3% was observed (Table 7).

The use of the complex contributed to a decrease in the alkaline phosphatase activity in blood serum by 15.5% compared to the intact group ($F = 9.51$, $P = 8.0*10^{-4}$), this indicator was also lower by 6.8% compared to the indicator in the group without prophylaxis, but it is not reliable. Statistically significant changes were also not observed in the change in the indicator of the alanine aminotransferase activity under the condition of prophylaxis (Table 7).

Table 6

Biochemical indicators of inflammation and the states of antioxidant and prooxidant systems in the liver homogenates of female rats exposed to chronic intoxication with α-cypermethrin and in rats receiving the adaptogen to prevent its complications ($x \pm SE$, $n = 28$)

Group	Acid phosphatase activity, μkat/kg	Elastase activity, μkat/kg	Malondialdehyde content, mmol/kg	Catalase activity, μkat/kg
Control	94.40 ± 2.84^{a}	480.02 ± 25.49^{a}	23.71 ± 2.26^{a}	6.034 ± 0.101^{a}
Cypermethrin	133.97 ± 5.68^{b}	474.17 ± 25.62^{a}	40.83 ± 3.15^{b}	4.922 ± 0.182^{b}
Adaptogen	99.14 ± 3.17^{a}	422.93 ± 21.37^{a}	30.01 ± 2.74^{a}	5.912 ± 0.150^{a}

Note: see Table 1.

Table 7

Biochemical indicators of inflammation, the state of antioxidant-prooxidant system and hepatotoxicity in the blood serum of female rats exposed to chronic intoxication with α-cypermethrin and in rats receiving the adaptogen to prevent its complications ($x \pm SE$, $n = 28$)

Group	Alanine aminotransferase activity, μcat/L	Alkaline phosphatase activity, μkat/kg	Elastase activity, μkat/kg	Malondialdehyde content, mmol/kg	Catalase activity, μkat/kg
Control	0.23 ± 0.02^{a}	4.85 ± 0.14^{a}	124.30 ± 3.86^{a}	0.69 ± 0.04^{a}	0.181 ± 0.009^{a}
Cypermethrin	0.22 ± 0.02^{a}	4.40 ± 0.11^{b}	165.87 ± 4.68^{b}	0.87 ± 0.04^{b}	0.158 ± 0.006^{a}
Adaptogen	0.20 ± 0.02^{a}	4.10 ± 0.12^{b}	130.68 ± 2.58^{a}	0.70 ± 0.04^{a}	0.169 ± 0.007^{a}

Note: see Table 1.

Catalase activity decreased by 12.7%, but not without a sufficient level of an statistical significance, but at the same time, an increase in malondialdehyde content by 25.6% ($F = 5.81$, $P = 8.57*10^{-3}$) in the blood serum of rats under α-cypermethrin intoxication is additional evidence that this insecticide promotes the development of prooxidative processes against the background of inhibition of the antioxidant defense. The increase in elastase activity by 33.4% ($F = 33.03$, $P = 3.6*10^{-6}$) in the blood serum of rats with pesticide load indicates the development of generalized inflammation. The use of an adaptogenic agent contributed to the normalization of biochemical parameters of inflammation, antioxidant defence and lipid peroxidation in the blood serum of rats, which was expressed in a decrease in elastase activity by 21.2%, malondialdehyde content by 19.5% and an increase in catalase activity by 7.0%, but not statistically significant (Table 7).

Discussion

Effects of α-cypermethrin and quercetin on the reproductive system of female rats. In our study, the increase in the duration of the estrous cycle in rats was accompanied by an increase in the concentration of estradiol in the blood serum and a decrease in the ovarian organ index, which may be related to the hormone-like activity of the pesticide α-cypermethrin and the destruction of the proportion of follicular cells and oocytes, confirming the presence of the gonadotoxic action of α-cypermethrin. The use of preventive measures contributed to the normalization of the studied parameters of the state of the reproductive system in female rats under the conditions of pesticide intoxication and testifies to the effectiveness of a complex of preparations based on the flavonoid quercetin, calcium citrate and a complex of vitamins, macro- and microelements in protecting the function of the gonads in conditions of pesticide load.

The data obtained mostly coincide with the results of other authors whose studies examined the toxicity of cypermethrin and its isomers, but there are also some differences. In general, α-cypermethrin is believed to be the most dangerous among the stereoisomers of cypermethrin, and this applies to both its acute toxicity and endocrine disruption from chronic exposure (Zhang et al., 2021).

In a study on mice, it was found that the estrous cycle was prolonged with the use of cypermethrin at doses of 25 and 50 mg/kg/day, the proportions of primordial, primary and antral follicles were reduced in a dose-dependent manner, and apoptosis of ovarian granulosa cells and mitochondria was increased. According to the authors, pyrethroids exert this effect by increasing the concentration of reactive oxygen species in cells during their metabolism. However, these changes were not accompanied by a significant decrease in ovarian weight (Wang et al., 2019).

Khatab et al. (2018) studied the effect of α-cypermethrin at a dose of 50 mg/kg for 2 months on the reproductive system of female rabbits. It was found that the toxic effect of the insecticide was accompanied by follicular atresia and a decrease in their volume, a decrease in ovarian weight, lower levels of estradiol, increased levels of malondialdehyde in the blood serum, and a decrease in the activity of antioxidant enzymes. These pathological phenomena were significantly reduced or disappeared with the use of propolis, which contains a large set of flavonoids, including quercetin.

Oral administration of β-cypermethrin to female mice at doses of 1.38, 2.76 and 5.52 mg/kg body weight for 6 months resulted in a decrease in ovarian and uterine weight, pregnancy problems, and an increase in serum estradiol and follicle-stimulating hormone levels in a dose-dependent manner (Zhou et al., 2018). A study by Li et al. (2006) also noted an increase in serum estradiol levels after exposure to high doses of cypermethrin. There are mixed views among researchers on the interaction of cypermethrin, its isomers and metabolites with estrogen receptors, which is one of the factors that causes the gonadotoxic effects of this compound. A number of studies have reported estrogen-like and antiandrogenic effects of cypermethrin and α-cypermethrin.

In the study by Chen et al. (2002) on the effect of pyrethroid and organophosphate pesticides on proliferation in the human breast carcinoma cell line MCF-7, it was found that pyrethroids are partial agonists of estradiol receptors and were ranked according to their estrogenic activity in the following order permethrin > fenvalerate > cypermethrin > deltamethrin, with fenvalerate and cypermethrin being the most effective of all the pesticides studied in competitively binding the estrogen receptor to estradiol. Jin et al. (2010) found that in MCF-7 cells, β-cypermethrin and 3-PBA exhibit estrogenic effects; the authors emphasise that when assessing the risk of using synthetic pyrethroids, it is necessary to take into account the toxicological effects, including those of the endocrine system, of both the parent compounds and their metabolites.

In an analysis of reporter genes mediated by hERα (human estradiol receptor alpha) and rERα (rat estradiol receptor alpha), it has been shown that some pyrethroid insecticides (fenvalerate > cypermethrin > permethrin) exhibit the estrogenic potential, which was expressed in the induction of luciferase expression, while the metabolite of these pyrethroids 3-PBA exhibited anti-estrogenic effect (Sun et al., 2014). This result is in line with the findings of Tyler et al. (2000) and McCarthy et al. (2006), who demonstrated the anti-estrogenic activity of 3-PBA in modified yeast cells and the blocking of estradiol binding to the receptor in these cells.

It is also known that α-cypermethrin can bind to estrogen receptors, exhibiting antagonistic activity, preventing binding to estradiol (Brander et al., 2016). In the experiments of Zhang et al. (2021) the authors studied the endocrine toxicity of cypermethrin stereoisomers: α-, β-, and θ-cypermethrin, by analysing dual luciferase reporter genes in danio fish cells. The results showed that α-cypermethrin acted as an ERα antagonist, binding to this receptor more strongly than other isomers, and exhibited the greatest endocrine toxicity.

In their study, Du et al. (2010) tested the activity of several pyrethroids and their metabolites through their effects on estrogen, androgen and thyroid hormone receptors. It was found that such synthetic pyrethroids as cyhalothrin, deltamethrin, fenvalerate, permethrin have weak agonist activity on estrogen receptors; 3-PBA and DCCA – dimethylcyclopropanecarboxylic acid (a specific metabolite of cypermethrin and its isomers) had a pronounced anti-estrogenic effect; in the same study, cypermethrin had an antagonistic effect on androgen receptors.

These differences can be explained by the different types of cell cultures used in the experiments, the impossibility of accurately predicting the metabolic transformations of insecticides and the interaction of their metabolites with hormone receptors *in vitro*, especially in the study of pesticide isomers, since having similar physical and chemical properties, their biological activity may differ due to enantioselectivity, in addition, they have different degrees of toxicity, participation in metabolism and bioaccumulation (Ji et al., 2019; Zhang et al., 2021). The studies of the same pesticides *in vivo* and *in vitro* can also give different results, for example, permethrin and bifethrin show estrogenic activity *in vivo* and anti-estrogenic activity *in vitro* (Brander et al., 2012). The dosage of the substance is also important, as endocrine disruptors are characterised by a non-monotonic response, in which low doses of a toxicant can more effectively affect changes in certain indicators than their high concentrations; the duration of the experiment should also be taken into account (Shepelska et al., 2021b).

Taking into account the possibility of antagonism of α-cypermethrin and its metabolites to estrogen receptors, it should be noted that the antagonistic effect results in blocking and lack of activation of these receptors, which entails increased production of gonadotropin-releasing hormone by the hypothalamus, as well as luteinising and follicle-stimulating hormones by the pituitary gland. However, due to disruption of negative feedback mechanisms, serum estradiol levels will also increase. The blocking of receptors in estrogen-dependent tissues leads to disruption of neuroendocrine regulation, which can be expressed in an increase in the duration of the estrous cycle (Bretveld et al., 2006).

The prolongation of the estrous cycle due to the diestrus stage, as in the case of our study, is generally a sign characteristic of hypoestrogenism. For example, in ovariectomised rats vaginal smears contain only leukocytes and mucus, and no keratinisation of cells is observed (Ghorbel et al., 2020). However, it should be noted that in the study by Shepelska et al. (2021), α-cypermethrin caused an increase in the duration of the estrous cycle due to the estrogen-dependent stage – proestrus.

The protective role of our proposed complex in supporting the endocrine function of the ovaries is primarily associated with the presence of quercetin, which enhances the degree of antioxidant protection of the ovaries and has estrogen-like properties, which consist in the ability to bind to α- and β-receptors of estrogens, resulting in the activation of transcription

processes that regulate the expression of genes that determine the development and functioning of the reproductive system (Sligohua et al., 2023). Quercetin is able to affect the axis "hypothalamus – hypophysis – gonads", reducing the level of follicle-stimulating and luteinizing hormones in the blood serum (Cao et al., 2014; Rashidi et al., 2021). The study by Shu et al. (2011) shows that quercetin via oral administration can affect body weight, follicle development and ovarian hormone secretion in the prepubertal mice. In aging rats, quercetin and other polyphenols can support ovarian function by affecting the state of the follicular reserve (Chen et al., 2010). In the above study by Ghorbel et al. (2020), the use of 17-β-estradiol or sage leaf extract, which contains a wide range complex of flavonoids, including quercetin, increased the activity of a number of antioxidant enzymes in the liver and in uterine homogenates of ovariectomized rats. At the same time, epithelial cells in vaginal smears became keratinized, indicating that flavonoids are similar to female sex hormones and have the ability to normalize the estrous cycle.

Osteoresorptive effect of α-cypermethrin intoxication and osteoprotective effect of the developed adaptogen. In our study, the long-term effect of α-cypermethrin on the body is accompanied by a violation of the bone tissue remodeling process with a predominance of the bone destruction processes due to its demineralization, an increase in markers of inflammation and proteolysis with a simultaneous increase in the end products of lipid peroxidation, which is a sign of increased pro-oxidative processes against the background of intoxication. Similar changes in biochemical indicators occurred in the blood serum: an increase in elastase activity and malondialdehyde content, while catalase activity also significantly decreased. Such data can be interpreted as evidence of the generalised toxic effects of α-cypermethrin, as a result of which the body as a whole undergoes damage.

Increased content of malondialdehyde in liver tissue and blood serum is the main indicator by which it can be argued that α-cypermethrin leads to activation of the lipid peroxidation processes, which results in damage to cells and organs. Since catalase is an enzyme that destroys hydrogen peroxide (which is formed after the inactivation of superoxide anion by superoxide dismutase) by splitting it into oxygen and water, inhibition of its activity due to excessive load on the antioxidant system creates conditions for the inhibition of the defense links aimed at scavenging reactive oxygen species (Sankar et al., 2012).

Acid phosphatase is an enzyme from the hydrolase group, the increased activity of which is a sign of the cell membrane destructions, both external and internal. The increase in its activity occurs in the initial stages of the inflammatory process, which in turn stimulates the production of mediators involved in the pathogenesis of subsequent inflammation links. Elastase is a marker of leukocyte accumulation in damaged tissues, so the degree of increase in its activity can also serve as a sign by which to characterize the severity of inflammation, but in addition, elastase due to its proteolytic action is also responsible for the destruction of elastic fibers that leads to tissue damages.

It is well known from the scientific literature that insecticides in general and pyrethroids in particular cause liver tissue damage with subsequent impairment of its functions, and the protective role of flavonoids in these conditions is also well studied. Thus, oral administration of cypermethrin to rats at a dose of 25 mg/kg resulted in increased levels of malondialdehyde, alanine aminotransferase and alkaline phosphatase in the blood serum, increased concentrations of lipid peroxidation markers in the kidneys, liver and brain, and inhibition of enzymatic and non-enzymatic antioxidant defence systems. These pathological changes were effectively prevented by the use of curcumin (Sankar et al., 2012). In a study by Afolabi et al. (2019), cypermethrin at a dose of 25 mg/kg caused a decrease in glutathione and the overall ability of the kidneys and liver to neutralize the reactive oxygen species in rats, an increase in IL-8, TNF-α, and an increase in malondialdehyde. The established violations were not detected when rats were treated by a complex of green tea polyphenols. The study by Hamed (2017) investigated the hepatoprotective role of quercetin in λ-cyhalothrin intoxication. Quercetin prevented mitochondrial damages in liver cells by activating the antioxidant defence systems and restoring the mitochondrial functions, which was accompanied by an increase in glutathione content, ATPase and NADH dehydrogenase activity. Quercetin prophylaxis reduced markers of hepatotoxicity, malondialdehyde content, and increased the antioxidant defence indicators in the blood serum in fenvalerate intoxication (Waheed & Mohammed, 2012). There is information on the hepatoprotective effect of quercetin in case of intoxication by other types of insecticides, not only pyrethroids (Miltonprabu et al., 2016).

It should be noted that pathological changes in the liver can occur not only due to the interaction of hepatocytes with reactive oxygen species, but also due to diseases of the gonads, and these two causes are closely related to each other (Li et al., 2015). The liver is involved in the metabolism of estrogens, which take part in the maintenance of hepatocytes, stimulate their division in case of damage and suppress the proliferation of Kupffer cells, preventing the development of fibrosis in liver tissue. During menopause and other ovarian dysfunctions of various etiologies, there is suppression of cellular and humoral immunity, a decrease in the activity of antioxidant enzymes (superoxide dismutase, catalase, glutathione-S-transferase), an increase in the level of interleukins and pro-inflammatory cytokines (which are produced in large quantities by Kupffer cells under conditions of oxidative stress), decrease in the function of Na^+K^+ATPase and Ca^{2+}ATPase. The listed factors lead to an increase in free radical processes and lipid peroxidation, changes in signaling pathways and functions of cells in various tissues and organs, including creating conditions that intensify hepatocyte apoptosis and contribute to the development of pathological changes in the liver. There is evidence that estradiol prevents the development of liver fibrosis in ovariectomized rats, as well as the development of non-alcoholic fatty liver disease in mice fed a high-fat diet. (Brady, 2015; Yu et al., 2021; Tkachenko et al., 2023).

In the present study, high estradiol levels induced by α-cypermethrin did not have a positive effect on inflammation and lipid peroxidation in the liver, which is a consequence of a significant free radical load on the liver, and this can also be interpreted as a manifestation of the antagonistic effect of α-cypermethrin in relation to estradiol, which did not fulfill its physiological role in these conditions. However, the use of the prophylactic complex suppressed the development of oxidative stress and inflammation in liver tissue, again due to the presence of quercetin in its composition. According to our previous study, in ovariectomized rats, the malondialdehyde content in the liver homogenate increased and the catalase activity decreased, and the use of quercetin in a complex with minerals and vitamins normalized these indicators (Sidletskyi & Makarenko, 2023).

The antioxidant activity of flavonoids is associated with the destruction of peroxidation products in the cell, maintaining the integrity of the double lipid membrane of cells, and in addition increasing the activity of the body's own antioxidant defence mechanisms. Quercetin inhibits the cyclooxygenase and lipoxygenase activities, which mediate the formation of eicosanoids – the oxidized derivatives of polyunsaturated fatty acids, and it is also able to inhibit xanthine oxidase – the main enzyme of superoxide anion radical formation, it indirectly affects the synthesis of glutathione, which is a necessary hydrogen donor for the conversion of hydrogen peroxide into water by catalase, it increases the level of antioxidant enzymes' expressions, stimulating both catalase and superoxide dismutase activities, as well as modulating the action of antioxidant system enzymes, affecting signaling pathways. Due to its phenolic structure, quercetin interacts with free radicals, which helps to reduce the content of malondialdehyde, as the main negative factor in lipid peroxidation. It is also worth noting that the antioxidant properties of quercetin in complex with metal ions Cu, Mg, Co, Ca, Fe are significantly enhanced; ascorbic acid also makes a powerful antioxidant contribution (Xu et al., 2019; Demkovych, 2020).

It should be noted that in the present study, an increase in markers of inflammation and lipid peroxidation in the liver was not accompanied by an increase in serum alanine aminotransferase and alkaline phosphatase, which may be explained by the dose of α-cypermethrin for oral administration of 10 mg/kg chosen by us. Since our goal was to model the bone pathology caused by the gonadotoxic effect of this pyrethroid, the analysis of the literature allowed us to choose the optimal dose of α-cypermethrin, which provided the possibility of long-term administration of the insecticide, namely for 4 months, taking into account the duration of bone remodeling cycles (Baron et al., 1984). It can be assumed that an increase in the activity of hepatic markers will occur at higher doses, even with a shorter duration of the experiment. A significant decrease in the alkaline phosphatase activity in the rat blood serum after administration of the

prophylactic complex can be explained by both the individual sensitivity of animals to the pesticide and the components of the complex and the peculiarities of the interaction between them. That requires more detailed study.

Conclusion

Chronic intoxication with α-cypermethrin in rats led to an increase in the duration of the estrous cycle due to the diestrus stage and a decrease in the ovarian organ index, while there was a twofold increase in serum estradiol, indicating a significant gonadotoxic effect of this insecticide and interference with the processes mediated by female sex hormones. The consequence of impaired the endocrine function of the ovaries and the initiation of oxidative stress was an increase in the degree of atrophy of the alveolar process in the jaws, a decrease in the densities of the femurs and lumbar vertebrae, due to a decrease in the mineral component of the bone tissue. An increase in biochemical markers was observed in the bone tissue of the alveolar process, indicating an increase in the processes of osteoresorption, inflammation, lipid peroxidation with the compensatory activation of osteogenesis markers and suppression of the antioxidant system. The toxic effect of α-cypermethrin was also manifested in an increase in markers of inflammation and oxidative stress in the liver, but there was no increase in such markers of hepatotoxicity as alanine aminotransferase and alkaline phosphatase in the blood serum.

The use of an adaptogen based on quercetin, vitamins D and C, micro- (Mn, Zn, Cu, Se) and macro elements (Ca, Mg) against the background of the insecticide intoxication contributed to the regular course of the estrous cycle, each of the stages of which lasted approximately the same time. There was also an increase in the organ index of the ovaries and a normalization of the estradiol concentration in the blood serum. These positive changes in the reproductive system can be explained by the antioxidant and cytoprotective potential of the complex as a whole and its regulation of the sex hormone levels due to the presence of quercetin in the supplement. The use of the adaptogen prevented atrophy of the alveolar process, normalized the morphometric parameters of the femurs and lumbar vertebrae by increasing the mineral component in the bone, reduced the content and activity of markers of osteoresorption, inflammation and lipid peroxidation in bone tissue. The adaptogenic complex contributed to the balanced functioning of the antioxidant-prooxidant system and reduced the activity of elastase in the blood serum of rats and had a pronounced hepatoprotective effect.

The results obtained allow us to conclude that the developed adaptogenic complex has a powerful preventive effect, which is expressed in the protection of the endocrine function of the female reproductive system, and also has osteoprotective and hepatoprotective effects under conditions of prolonged toxic effects of the pyrethroid insecticide α-cypermethrin.

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All authors declare that they have no conflicts of interests.

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