Зараз особливе місце серед вчених займають питання впливу на організм наноформ важких металів, в тому числі, і свинцю. В сучасних умовах в різних джерелах води господарського, питного і культурно-побутового водокористування крім важких металів у значних кількостях знаходяться і поверхнево-активні речовини, до яких відносяться стеарати натрію і калію. Метою дослідження було вивчити дію НЧ свинцю на тлі вживання тваринами води з вмістом стеарату натрію і стеарату калію на кістковий мозок і периферичну кров білих щурів. Таким чином, при комбінованому впливі НЧ свинцю в дозі 70 мг / кг і стеаратів натрію і калію на організм піддослідних щурів відзначалося більш значне підвищення в кістковому мозку кількості промієлоцитів, паличкоядерних і сегментоядерних нейтрофілів, лімфоцитів, нормоцитів і більш значне зменшення мієлоцитів і метамієлоцитів, ніж при роздільному впливі НЧ свинцю. НЧ свинцю на тлі вживання питної води різного складу викликали збільшення кількості паличкоядерних нейтрофілів, еозинофілів, моноцитів, лімфоцитів і зменшення кількості сегментоядерних нейтрофілів в крові піддослідних тварин.

Ключові слова: наночастинки свинцю, питна вода, стеарат натрію, стеарат калію, кістковий мозок, периферична кров.

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особое место среди ученых занимают вопросы воздействия на организм наноформ тяжелых металлов, в том числе, и свинца. В современных условиях в различных источниках воды хозяйственного, питьевого и культурно-бытового водопользования кроме тяжелых металлов в значительных количествах находятся и поверхностно-активные вещества, к которым относятся стеараты натрия и калия. Целью исследования было изучить действие НЧ свинца на фоне употребления животными воды с содержанием стеарата натрия и стеарата калия на костный мозг и периферическую кровь белых крыс. Таким образом, при комбинированном воздействии НЧ свинца в дозе 70 мг/кг и стеаратов натрия и калия на организм подопытных крыс отмечалось более значительное повышение в костном мозге количества промиелоцитов, палочкоядерных и сегментоядерных нейтрофилов, лимфоцитов, нормоцитов и более значительное уменьшение миелоцитов и метамиелоцитив, чем при раздельном воздействии НЧ свинца. НЧ свинца на фоне употребления питьевой воды различного состава вызвали увеличение количества палочкоядерных нейтрофилов, эозинофилов, моноцитов, лимфоцитов и уменьшение количества сегментоядерных нейтрофилов в крови подопытных животных.

Ключевые слова: наночастицы свинца, питьевая вода, стеарат натрия, стеарат калия, костный мозг, периферическая кровь.

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POSITIVE INFLUENCE OF ARTICHOKE EXTRACT ON STRUCTURAL AND METABOLIC PROCESSES IN BONE TISSUE OF RATS CONDITIONED UPON CADMIUM-NITRIC INTOXICATION

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The purpose of the study was to investigate the changes in the histological structure, bioelement compositionand markers of bone metabolism in the blood of white male rats with experimental cadmium-nitrite intoxication and the use of artichoke extract for correction of disturbances that arose under the influence of toxicants. It has been established that on the background of combined action of $CdCl_2$and $NaNO_2$in animal femoral bones, osteoclastic resorption processes over osteosynthesis predominate and ash content of the most important osteotropic elements (Ca, Zn and Cu significantly decrease), while cadmium content increases greatly. Indicators of phosphorous-calcium metabolism change in blood plasma, the activity of acid phosphatase increases and oxyproline concentration increases, alkaline phosphatase activity decreases. Artichoke extract positively influences on reparative processes in bones. With the administration of artichoke extract the balance of macro- and micronutrients is restored in the femur of the animals, cadmium is significantly reduced. Biochemical markers of bone metabolism are normal in the blood plasma, the values of which do not significantly differ from those of intact ones (with the exception of the concentration of oxyproline that remains higher) to the end of the experiment.

Key words: artichoke extract, cadmium-nitrite intoxication, histological structure of femoral bones, markers of bone metabolism.

The work is a fragment of the research project "Optimization of medical and preventive measures to reduce the level of dental morbidity of the rural population of Prykarpattia, which inhabits anthropogenically burdened territories", state registration No. 0117U000946.

According to publications devoted to environmental monitoring [1,10],cadmium (Cd), nitrates and nitrites are considered to be the most common pollutants, and their content in the environment is constantly increasing.Sources and ways of receiving these xenobiotic in the body are well known [1, 8]. The cumulative properties of Cd [10] and, conversely, the rapid metabolism of nitrates and nitrites with the formation of more toxic metabolites have been proved [8]. Cd is a thiol poison: it binds to -SH groups of proteins, causes changes in their structure, blocks active centers of enzymes, which suppresses their catalytic action [2, 4]. Nitrites cause gemic hypoxia; when exposed to oxyhemoglobin, free radicals are generated, which activates free radical oxidation and damage to cell membranes [8].

It is known that chronic xenogeneic intoxication can lead to toxic osteopathy [2, 3, 11]. Such data prompt to search effective, accessible and safe means of correction of metabolic and structural disturbances arising from the combined effect of Cd compounds and nitrites, in bone tissue (BT) in particular. Our attention was paid to a well-known food and medicinal plant Artichoke (*Cynara scolymus*), which was introduced into the culture even in classical times. Modern studies have confirmed and expanded the pharmacological properties of artichoke extracts, among which the antioxidant, membrane-stabilizing and detoxifying effect, which increases the excretion of toxins from the body (including nitrocompounds and heavy metal salts) [5]. While manufacturingthe medication "Artichoke Extract-Health" (EA), a unique technology is used to avoid drying artichoke leaf juice, and therefore ensures the preservation of a complex of active substances that are contained by a fresh plant: flavonoidal glycosides cinnarine and cyarozid in combination with phenolocarboxylic acids and bioflavonoids, as well as inulin, ascorbic acid, carotene, vitamins B_1 and B_2, which contribute to the normalization of metabolic processes. New studies have shown good chelating effect of artichoke leaf extracts as for the Lead [5]. However, in literature there is no data on the influence of EA on the state of BT in the conditions of cadmium-nitrite intoxication. The most common model for research is white laboratory rats, which provide an opportunity to explore various pathological conditions and mechanisms for their development, followed by extrapolation to humans [6].

The purpose of the study was to investigate changes in histological structure,bioelement composition and markers of metabolic processes in BT in animals with experimental cadmium-nitrite intoxication and also conditions of artichoke extract (EA) applicationon the background of the combined action of Cadmium chloride ($CdCl_2$) and Sodium nitrite ($NaNO_2$).

Materials and methods. The study was conducted on 52 individuals of white, sexually mature male rats weighing 180-220 g. The animals were kept and manipulated with the requirements of bioethics [4]. The animal were divided into intact (control, 12 animals) and two experimental groups: 1st– 24 animals, 2nd group had 16 animals. Intoxication was performed during 10 days with administration of 1/10 LD_{50} xenobiotics (0.12 mg $CdCl_2$ and 0.21 mg $NaNO_2$ per 100 g of body weight) daily once a day. After completing the introduction of toxicants, animals of the 1st group were with drawn from the experiment on 1st, 14th and 28th day, and animals of the 2nd group with the purpose of correction were injected EA. The mice of the 2nd group were with drawn from the experiment on 14th and 28th days as mentioned above. Blood and femoral bones were removed, which were cleaned from soft tissues. A part of the bone was prepared for histological studies. Decalcification was carried out by Wilens (1950). The sections were stained with hematoxylin and eosin. Lumam P8 and Axioskop Microscopes, the IS-capture software (V.1.0) were used for micro-photography. The bioelement composition of the femur was determined by the atomic absorption method on a spectrophotometer C-115PK. Determination of bone metabolism in blood plasma was carried out in a biochemical laboratory based on the Center of Bioelemetology of Ivano-Frankivsk National Medical University according to standardized methods using reagent kits: "Phyllisit", "Simko" (Ukraine); "Vital" (Russia), "Lachema" (Check Republic). Statistical processing was conducted on a PC using Microsoft Excel and STATISTICA (StatSoft, Inc., 2010), the results were considered to be reliable if $p < 0,05$. Correlation matrices were calculated using the Pearson method to estimate the relationship between the studied parameters.

Results of the study and their discussion. Histological studies of different regions of femur bones of the animals of the 1st group showed that on the 14th day after the ten-day injection of toxicants in a compact BT diaphysis of the femoral bones, defects of the structure of all layers were determined: the disorganization of the collagen fibers of the organic matrix and the proper placement of bone plates. In the osteonic layer the phenomenon of osteoporosis with the presence of multiple cavities filled with connective tissue, osteoclasts and osteoblasts is highlighted (fig. 1).

The massive destruction of bone trabeculae leads to a violation of the characteristic pattern of spongy bone, it loses cellular appearance. On the 28th day, the amount and volume of osteoporotic cavities in the osteonic layer decrease with the activation of organic matrix recovery processes. At the same time, in some animals, multiple phenomena of smooth and axillary resorption of BT and the increased activity of osteoblasts are observed. At the same time, neo-osteogenesis takes place, but the fibers of the organic matrix are hypochromic andchaoticallyoriented. More vivid changes are visualized in the spongiform BT in epiphysis. Unevenly thinned bone beams, some with many usurums, are defined near the articular surface, with the distance from the femoral head, their density decreases. Observed phenomena of smooth and

sometimes lacunar resorption of BT with the dominance of osteomalacia without activation of cells of the monocyte-osteoclastic series.

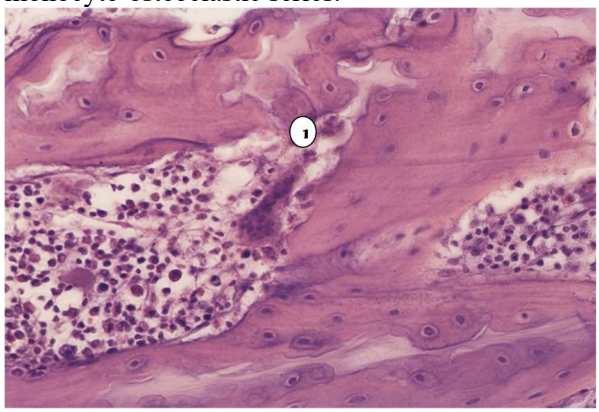

Fig. 1.Osteoclast (1) insponge bone tissue of femur epiphysis of rats on the 14th day after $CdCl_2$ and $NaNO_2$ administration. Hematoxylin and eosin staining. Photomicrography. Zooming: × 430.

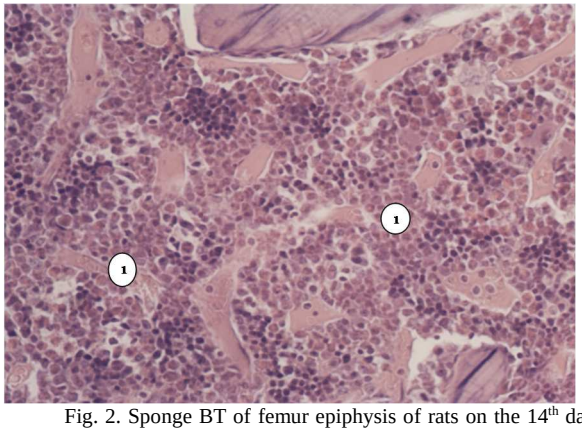

Fig. 2. Sponge BT of femur epiphysis of rats on the 14th day after $CdCl_2$, $NaNO_2$ and artichoke extract administration. 1 – neoplasms of simple axillary beams. Hematoxylin and eosin staining. Photomicrography. Zooming: × 430.

EA administration to animals of the 2nd group reduces the severity of the processes of destruction of the compact bone in the diaphyses of the tubular bones, reduces the disproportion level between the processes of osteomalacia and reparative regeneration, although it does not completely eliminate the adverse effects of the toxicant on BT (14th day). This is manifested by the insignificant severity of neoplasms and more homogeneous structure of the osteon layer. The preparations visualize only single deformed, sharply thinned bone beams. The fibers of the organic matrix of the osteonic layer equally perceived the dye, the bonding lines are directed mainly along the long axis of the bone. On the surface it is possible to form a thin layer of osteoid in the form of a homogeneous oxyphilic mass, in which single osteocytes are deposited. Changes in spongy BT are somewhat different in different animals in this group. In most of them, the structure of BT is generally preserved. Bone trabeculae are mostly represented by plates that are closely adjacent to each other, there are some osteocytes with elongated coresbetween them. The surface of the beams is smooth. At the same time, in some animals, in which the phenomena of resorption of BT are determined, the enhanced activity of osteoblasts with pericellular deposition of a newly created osteoid is determined in the thickness of beams or on the surface (fig. 2). On the 28th day of EA administration in animals of the 2nd group, signs of restructuring of compact and spongy BT of animals are quite polymorphic.

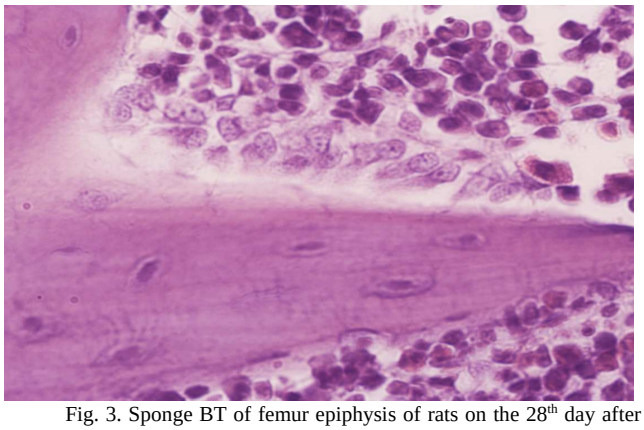

Fig. 3. Sponge BT of femur epiphysis of rats on the 28th day after $CdCl_2$, $NaNO_2$ and artichoke extract administration. 1 – osteoblasts proliferation. Hematoxylin and eosin staining. Photomicrography. Zooming: × 430.

In particular, the diaphysis of the femur of the rats does not determine the thinned areas, but there are areas with a large number of enlarged bone canals, the phenomena of fiberoxyfilia, which coincides with the histological picture that is observed in intact animals. The fundamentally different feature of the restructuring of the BT of the diaphysis of the femur in rats of the 2nd group receiving EA is the presence of sites of enhanced neocologenesis. They are characterized, first of all, by a larger thickness and more homogeneous nature of the colour of the fibers of the organic matrix. This indicates a sufficient maturity of the newly formed BT with signs of osteon structure.

Alongside with this, in the osteonic layer, foci of neocolagenogenesis with a relatively chaotic structure are noted, which is determined by the features of the angioarchitectonics of the newly created BT, although the number of bone canals is relatively small. Neocolagogenogenesis has a focal character, which gives the osteonic layer a mosaic form. Correctionof the pathological condition caused by the toxicants administration with the help of EA has a significant effect on the nature of changes in spongy BT in epiphyses of the femur bones. There is a vivid proliferation of osteoblastic cells in the areas of bone remodeling (fig. 3). Bioelement composition of femur bones ash of rats is given in table 1. The content of Ca, main component of the mineral matrix BT decreased by 6-14 % ($p < 0,05$), and Mg increased by 21-35 % in the animals of the 1st group in comparison with the intact. Zink (Zn) and Copper

(Cu) are important osteotropic trace elements, their content in BT of animals of the 1st group decreased with respect to intact indices: Zn - by 19-45%, Cu - by 25-27% (p <0,001).

Table 1

Element composition of femur bones ash of rats affected by $CdCl_2$and $NaNO_2$ followed by artichoke extract correction

Indices (chemical elements)	Groups of animals	1th day	14th day	28th day
Calcium (mg/g ash)	Intact animals	330.9 ± 6.2		
	1st experimental	311.1 ± 4.3*	305.7 ± 3.3*	285.1 ± 3.5**
	2nd experimental	-	334.6 ± 7.3 #	339.9 ± 6.4#
Magnesium (mg/g ash)	Intact animals	38.1 ± 1.4		
	1st experimental	51.8±2.1**	34.6 ± 1.8*	45.6 ± 2.5 *
	2nd experimental	-	42.6 ± 1.0# *	38.2 ± 2.2 #
Zinc (µg/g ash)	Intact animals	458.6 ± 37.2		
	1st experimental	314.2± 25.1*	252.9 ±32.8**	369.6 ± 29.3*
	2nd experimental	-	423.4 ± 10.7#	454.1 ± 14.5 #
Copper (µg/g ash)	Intact animals	17.9 ± 0.9		
	1st experimental	13.6 ± 0.7 *	13.1 ± 1.2 *	16.7 ± 1.1
	2nd experimental	-	14.3 ± 1.1 #*	17.3 ± 1.4
Cadmium (µg/g ash)	Intact animals	2.10 ± 0.26		
	1st experimental	8.18±0.43*	8.85 ± 0.52**	37.08±1.02**
	2nd experimental	-	5.73 ±0.38# **	2.51 ± 0.29*

Note: here and in the table 2: 1) * – p < 0.05, ** – p < 0.01 – the degree of probable changes compared with the indicators of intact animals group; 2) # – p < 0.05 – degree of probable changes between the 2nd and 1st experimental groups of animals.

However, on the 28th day of EA correction, the content of the studied bioelements did not significantly differ from the control values of the intact rats. As the authors [7] note the ability of Cd to cumulate in BT and its competitive relationship with essential double-valent metals, it was important to determine whether the biologically active substances of EA influence the level of accumulation of Cd in the bones.Investigation of the content of this heavy metal in the bone ash of animals of the 1st group showed that it gradually increased and on the 28th day exceeded the intact indicesin 17,7 times (p<0.001). Under conditions of EA administration to animals, content of Cd decreased by 14.8 times in comparison with the animals of the first group, exceeding the value of intact animals by only 19 %.

Table 2

Biochemical parameters of blood plasma of rats affected by $CdCl_2$ and $NaNO_2$and with subsequent correction of artichoke extract

Indices	Groups of animals	1th day	14th day	28th day
Calcium (mmol/L)	Intact animals	2.34±0.08		
	1st experimental	2.68±0.13*	2.83±0.14**	2.08±0.17*
	2nd experimental	-	2.72±0.11 *	2.49±0.12 *#
Phosphates (mmol/L)	Intact animals	1.33±0.05		
	1st experimental	2.16±0.24*	1.42±0.08*	1.76±0.15*
	2nd experimental	-	1.58±0.10*	1.52±0.07*#
Magnesium (mmol/L)	Intact animals	0.72± 0.08		
	1st experimental	0.43±0.02**	0.31±0.03**	0.33±0.03**
	2nd experimental	-	0.69 ±0.06#	0.94±0.04#*
Oxyproline (mmol/L)	Intact animals	28.31±2.79		
	1st experimental	71.4±3.23**	74.56±1.39**	99.70±2.94**
	2nd experimental	-	47.13±3.15#*	45.25 ±2.41#*
Activity of alkaline phosphatase (µmol/s·L)	Intact animals	15.07±0.08		
	1st experimental	10.84±2.28*	9.30±1.23**	7.10 ±1.95**
	2nd experimental	-	16.09±1.77#	18.13±0.58#*
Activity of acid phosphatase µmol/s·L	Intact animals	0.93±0.23		
	1st experimental	1.88±0.18**	2.47 ±0.13**	4.17± 0.48**
	2nd experimental	-	1.53± 0.12#*	1.29± 0.04# *
The ratio of activity of alkaline and acid phosphatase	Intact animals	16.20±0.35		
	1st experimental	5.77±0.21*	3.76±0.28**	1.70± 0.19**
	2nd experimental	-	10.49±0.16#*	14.05±0.54# *

Biochemical studies of markers of bone metabolism in blood plasma confirm the positive effect of EA on metabolic processes in BT of animals with cadmium-nitrite intoxication. In particular, animals of the first experimental group showed an increase in the concentration of total calcium (Ca) during the first 14 days, and on the 28th day – a decrease by 11 % compared with intact animals. With the use of EA, Ca concentration did not significantly differ from the control values of intact animals (table 2).

The concentration of Phosphates in the blood (62 %) greatly increases on the first day after a ten-day intoxication in the 1st experimental group compared with intact animals. In the 2nd experimental group of animals with an EA administration this index was 14-19 % higher, but had no sufficient fluctuations. The activity of phosphatase significantly changed in Group 1: AlPh, which is a marker of osteoblast activity, gradually decreased by 28-53 %, while AcPh, which characterizes osteoclast function, increased by 2.0-4.5 times. It can be assumed that early phosphatemia is associated with an increase in AcPh activity, which already on the first day almost doubled the intact ones. Activation of this enzyme may be caused by the development of subcompensated metabolic acidosis, which is observed with cadmium intoxication [10]. The decrease in AlPh activity may be due to the substitution of Zn^{2+} та Mg^{2+} ions in the active center of enzyme by Cadmium since the ion radii of Zn^{2+}, Mg^{2+} та Cd^{2+}cations are close. Similar results regarding the content of Ca in blood plasma and the activity of AlPh in the context of the influence of heavy metals have been obtained by other researchers [9]. Concentrations of Mg^{2+} in blood plasma of animals of group 1 were significantly lower by 40-54 %, while the concentration of oxyproline (OP) increased by 2.5-3.5 times, indicating that the collagen bone matrix was destroyed by the combined action of $CdCl_2$ and $NaNO_2$. In the 2nd experimental animal group, the activity of AlPh was close to that of intact animals, and AcPh was significantly lower, and by the end of the experiment, the intact rates were 39% higher. The reflection of the balance of osteosynthesis and bone resorption processes is the ratio of AlPh/AcPh activity. In the 1st group, this index was lower 9.5 times, and in the 2nd only by 13 % compared to the indicator of intact animals (on the 28th day of observation). Such data make it possible to state that the use of EA against the combined effects of $CdCl_2$ and $NaNO_2$ significantly improves the metabolic processes in BT of animals, which is confirmed by the biochemical parameters of blood plasma (table 2).

Correlation analysis of the obtained results allowed to estimate the degree of interconnection of the studied indicators, in particular between the content of Cd in BT and the OP concentration in blood plasma ($r = +0.86$; $p < 0.05$), Mg ($r = -0.76$; $p < 0.01$), AcPh activity ($r = +0.85$; $p < 0.001$). Also, strong, reliable correlations were detected between the activity of AcPh and OP concentration in plasma ($r = +0.84$; $p < 0.001$), AlPh activity ($r = -0.62$; $p < 0.05$), Zn content in femur bone ash ($r = -0.79$; $p < 0.001$). Consequently, the OP concentration and the activity of the AcPh in blood plasma may be markers of the depth of structural and metabolic disturbances in BT under the conditions of these toxicants and the effectiveness of corrective factors applying.

The positive effect of EA in this study can be explained by high antioxidant, membrane-stabilizing and detoxifying properties, which are provided by the unique multi-component artichoke and drug composition on its basis [5].

Conclusions

1. Histological studies confirm structural changes in BT developing in the process of experimental cadmium-nitrite intoxication. In particular, the phenomenon of osteoporosisin the osteonoid layer of compact boneand numerous erosions in the spongy BT have been determined. Such data prove the predominance of osteoclastic resorption processes over osteosynthesis in BT. In the ash of the femur of experimental animals, the content of osteotropic bioelements Ca, Zn and Cu decreases against the background of significant accumulation of toxic heavy metal Cd. Simultaneously, there is an increase in 2.5-3.5 times in the concentration of oxyproline, a marker amino acid of collagen catabolism and a 2.0-4.5-time increase in the activity of the AcPh, the marker of osteoclast functioning. Phosphoric-calcium exchange rates have changed.

2. Artichoke extract positively influences reparative processes in the bone (presence of sites of enhanced neocologenesis, proliferation of osteoblastic cells,the balance of macro- and microelements in the BT is restored, while the Cd content is reduced in 14.8 times.The artichoke extract administration normalizes the biochemical markers of bone metabolism in animals with EA administration, with the exception of Oxyproline concentration that remains (at 60% higher).

Prospects for further research lie in the fact that the obtained data open the possibility of studying the clinical application of EA for the correction of structural and metabolic disturbances in BT, due to the action of these xenobiotics.

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Реферати

ПОЗИТИВНИЙ ВПЛИВ ЕКСТРАКТУ АРТИШОКУ НА СТРУКТУРНО-МЕТАБОЛІЧНІ ПРОЦЕСИ У КІСТКОВІЙ ТКАНИНІ ЩУРІВ ЗА УМОВ КАДМІЄВО-НІТРИТНОЇ ІНТОКСИКАЦІЇ

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Проведено дослідження гістологічної структури, біоелементного складута маркерів кісткового метаболізму у крові щурів-самців з кадмієво-нітритною інтоксикацією та за умов введення екстракту артишоку. Встановлено, що на тлі комбінованої дії $CdCl_2$ та $NaNO_2$ у стегнових кістках тварин переважають процеси остеокластичної резорбції над остеосинтезом, достовірно знижується вміст у золі остеотропних елементів (Ca, Zn та Cu), зростає вміст кадмію. У плазмі крові змінюються показники фосфорно-кальцієвого обміну, зростає активність кислої фосфатази та концентрації оксіпроліну, знижується активність лужної фосфатази. Введення екстракту артишоку позитивно впливає на репаративні процеси у КТ, про що свідчить наявність ділянок посиленого неоколагенезу, проліферація клітин остеобластичного ряду. Одночасновідновлюється баланс макро- та мікроелементів, достовірно знижується вміст кадмію у КТ. У плазмі крові нормалізуються біохімічні маркери кісткового метаболізму, значення яких до кінця експерименту достовірно не відрізняються від показників інтактних (за винятком концентрації оксіпроліну, яка залишається вищою).

Ключові слова: екстракт артишоку, кадмієво-нітритна інтоксикація, гістологічна структура стегнових кісток, маркери кісткового метаболізму.

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Проведено исследование гистологической структуры, биоэлементного состава и маркеров костного метаболизма в крови крыс-самцов с кадмиево-нитритной интоксикацией, а также на фоне применения экстракта артишока. Установлено, что на фоне комбинированного действия $CdCl_2$ та $NaNO_2$в бедренных костях животных преобладают процессы остеокластической резорбции над остеосинтезом, достоверно снижается содержание в золе важнейших остеотропных элементов (Ca, Zn и Cu), возрастает содержание кадмия. В плазме крови изменяются показатели фосфорно-кальциевого обмена, возрастает активность кислой фосфатазы и концентрации оксипролина, снижается активность щелочной фосфатазы. Экстракт артишока положительно влияет на репаративные процессы в кости, о чем свидетельствует наличие участков усиленного неоколагенеза, пролиферация клеток остеобластичного ряда,восстанавливается баланс макро- и микроэлементов, достоверно снижается содержание кадмия. В плазме крови нормализуются биохимические маркеры костного метаболизма, значение которых до конца эксперимента достоверно не отличаются от показателей интактных (за исключением концентрации оксипролина, которая остается выше).

Ключевые слова: экстракт артишока, кадмиево-нитритная интоксикация, гистологическая структура бедренных костей, маркеры костного метаболизма.

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