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Abstract

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CHEMICAL COMPOSITION OF THE TESTES OF IMMATURE RATS IN CONDITIONS OF ELEVATED INTAKE OF HEAVY METALS SALTS IN THE BODY AND ITS CORRECTION

The research presents the results of the study of seminal gland tissue of 128 immature rats treated with salts of zinc, copper, iron, manganese, chromium and lead from drinking water for 60 days. The aim of the study was to investigate the accumulation of zinc, copper, lead, manganese, chromium and iron in the tissue of the testes during their alimentary intake and under conditions of L-carnitine correction. Using spectrophotometry in atomic absorption mode, it was found out that the level of zinc reduced and the content of other analyzed trace elements increased. Accumulation of copper, iron, manganese, chromium and lead in seminal gland tissue was mostly expressed in the period of 60 days; iron, lead and chromium showed the largest organ tropism. The low rate of HMC accumulation in the testes of rats of breast and suckling age periods could be explained by the limited intake of xenobiotics via maternal milk. Loss of zinc by testis tissue was caused by antagonistic interactions between chemical elements that came into the body of rats in excessive quantities. Interaction of chemical elements at different levels could lead to secondary violations of chemical and structural homeostases of the organ causing further inhibition of its function.

The results of chemical analyses of testes of immature rats, obtained by SEM, indicated the dependence of accumulation of micro elements of heavy metals from the morphofunctional activity of the histological structure of the studied organ. Heavy metals mainly accumulated in the functionally active spermatogenic epithelium.

Key words: testes, immature rats, heavy metals, chemical composition, atomic absorption spectroscopy, scanning electron microscopy with microanalysis.

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ХІМІЧНИЙ СКЛАД СІМ'ЯНИКІВ СТАТЕВОНЕЗРІЛИХ ЩУРІВ В УМОВАХ ВПЛИВУ ПІДВИЩЕНОГО НАДХОДЖЕННЯ СОЛЕЙ ВАЖКИХ МЕТАЛІВ В ОРГАНІЗМ ТА ЙОГО КОРЕКЦІЇ

У роботі представлені результати дослідження тканини сім'яних залоз 128 статевонезрілих щурів, які протягом 60 днів отримували з питною водою солі цинку, міді, заліза, марганцю, хрому та свинцю. Метою дослідження було вивчення накопичення цинку, міді, свинцю, марганцю, хрому та заліза в тканині сім'яників при їх аліментарному надходженні та в умовах корекції L-карнітином. За допомогою спектрофотометрії в атомно-

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абсорбційному режимі встановлено, що рівень цинку знижується, а вміст інших досліджуваних мікроелементів зростає. Накопичення міді, заліза, марганцю, хрому та свинцю у тканині сім'яних залоз найбільш виражене у терміні 60 діб, найбільшу тропність до органа проявляють залізо, свинець та хром. Невисокий темп накопичення СВМ у сім'яниках щурів грудного і підсисного вікового періоду пояснюється обмеженим надходженням ксенобіотиків із молоком. Втрата цинку тканиною сім'яників обумовлюється антагоністичними взаємодіями між хімічними елементами, що надходять до організму щурів у надлишкових кількостях. Взаємодія хімічних елементів на різних рівнях може призводити до вторинного порушення хімічного та структурного гомеостазу органа, викликаючи у подальшому пригнічення його функції.

Результати хімічного аналізу, отримані за допомогою РЕММА, сім'яників статевонезрілих тварин вказують на залежність накопичення мікроелементів – важких металів від морфофункціональної активності гістологічних структур досліджуваного органа. Сполуки важких металів переважно накопичуються в більш функціонально активному сперматогенному епітелії.

Ключові слова: сім'яники, статевонезрілі щури, сполуки важких металів, хімічний склад, атомно-абсорбційна спектроскопія, растрова електронна мікроскопія з мікроаналізом.

Резюме

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ХИМИЧЕСКИЙ СОСТАВ СЕМЕННИКОВ НЕПОЛОВОЗРЕЛЫХ КРЫС В УСЛОВИЯХ ВЛИЯНИЯ ПОВЫШЕННОГО ПОСТУПЛЕНИЯ СОЛЕЙ ТЯЖЕЛЫХ МЕТАЛЛОВ В ОРГАНИЗМ И ЕГО КОРРЕКЦИИ

В работе представлены результаты исследования ткани семенных желез 128 неполовозрелых крыс, которые в течение 60 дней получали с питьевой водой соли цинка, меди, железа, марганца, хрома и свинца. Целью исследования было изучение накопления цинка, меди, свинца, марганца, хрома и железа в ткани семенников при их алиментарном поступлении и в условиях коррекции L-карнитином. С помощью спектрофотометрии в атомно-абсорбционном режиме установлено, что уровень цинка снижается, а содержание других исследуемых микроэлементов увеличивается. Накопление меди, железа, марганца, хрома и свинца в ткани семенных желез наиболее выражено в сроке 60 дней, наибольшую тропность к органу проявляют железо, свинец и хром. Невысокий темп накопления СВМ в семенниках крыс грудного и подсосного возрастного периода объясняется ограниченным поступлением ксенобиотиков с молоком.

Потеря цинка тканью семенников обусловливается антагонистическими взаимодействиями между химическими элементами, которые поступают в организм крыс в избыточных количествах. Взаимодействие химических элементов на разных уровнях может приводить к вторичному нарушению химического и структурного гомеостаза органа, вызывая в дальнейшем подавление его функции.

Результаты химического анализа, полученные с помощью растровой электронной микроскопии с микроанализом, семенников неполовозрелых животных указывают на зависимость накопления микроэлементов – тяжелых металлов от морфофункциональной активности гистологических структур исследуемого органа. Соединения тяжелых металлов преимущественно накапливаются в более функционально активном сперматогенном эпителии.

Ключевые слова: семенники, неполовозрелые крысы, соединения тяжелых металлов, химический состав, атомно-абсорбционная спектроскопия, растровая электронная микроскопия с микроанализом.

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Introduction

Nowadays in Ukraine the demographic situation is rather complicated: the decline in population is among the highest in Europe – 0.9–1.1 % per year, the birth rate compensates for mortality by about 51 %. However, from 8 to 20 % of married couples across the country are infertile [1; 2]. The structure of the male factor of an infertile marriage takes from 30 to 60 %, with 50 % of detected testicular form of infertility [2].

One reason for the prevalence of infertility in men is high sensitivity of the male reproductive system to adverse exogenous and endogenous factors in the prenatal and earlypostnatal periods [3]. One of the most common and dangerous pollutants for the reproductive system is the heavy metals compounds (HMC), which have both direct and indirect effects on the development of reproductive system and the mechanisms regulating the formation of hormones and germ cells [4; 5].

Different medications were used to reduce the negative impact of HMC on the body. The choice of L-carnitine drug for protection of testicular tissue from HMC effects is due to the fact that under conditions of influence of lead and other heavy metals mitochondria sustentacular cells are mostly affected, spermatogenic cells are affected somewhat less [4]. L-carnitine is a medication of domestic production, the main pharmacological effect is the normalization of energy metabolism by transport of fatty acids into the mitochondria [6]. Therefore to explore the capability of L-carnitine drug as a corrector of this pathological condition is appropriate. Materials relating to the usage of L-carnitine for correction of the HMC negative impact on the testes of immature specimens are absent.

Thus, the study of the chemical composition of immature rat testes under the influence of HMC is relevant, because the mechanisms of male infertility require clarification and further study, both in clinical and experimental practice.

Our **aim** was to investigate the intensity of accumulation of zinc, copper, lead, manganese, chromium and iron in tissues and structures of immature rat testes under their alimentary intake and under conditions of L-carnitine correction.

Materials and methods

The experiment was conducted on 128 white lab immature male rats (from birth to 60 days of life). During the experiment, laboratory animals were maintained in accordance with regulations adopted by the European Convention for the Protection of vertebrate animals used for experimental and scientific tasks (Strasbourg, 1986), "General ethical rules of experiments on animals" and approved by the National Congress on Bioethics (Kyiv, 2001) and the Law of Ukraine "On protection of animals from cruelty" № 3477-IV of 21.02.2006 [7].

All animals were divided into two series – control and experimental. Control series consisted of a group of intact animals and a group of animals using L-carnitine. Experimental series was divided into the group of HMC exposure and the group of L-carnitine correction of HMS solts exposure. Each group consisted of 8 animals.

Experimental animals were divided into subgroups based on the resulting set of xenobiotics. The rats of the first group of control series received distilled water. The second group of animals received L-carnitine at a dose of 50 mg/kg/day with the help of the probe. Animals of the third group received distilled water with a combination of HMS: zinc ($ZnSO_4$ x $7H_2O$) – 5 mg/l (hereinafter, in terms of metal); copper ($CuSO_4$ x $5H_2O$) – 1 mg/l; iron ($FeSO_4$) – 10 mg/l; manganese ($MnSO_4$ x $5H_2O$) – 0.1 mg/l; lead ($Pb(NO_3)_2$) – 0.1 mg/l and chromium ($K_2Cr_2O_7$) – 0.1 mg/l. In the fourth group, on the background of the exposure of above

mentioned metals combination, the rats received L-carnitine at a dose of 50 mg/kg with the help of the probe.

Experimental animals were sacrified on the 5th, 15th, 30th and 60th day by decapitation under ether anesthesia, after which their testes were removed. Such terms corresponded to different periods of rat puberty – breast, suckling, infantile and juvenile age [8].

Chemical analysis was carried out by atomic absorption. Weighted testes were dried in a drying oven at a temperature of 105 ºC to constant weight. Then the cloth was burned in porcelain crucibles in a muffle furnace at 450 ºC for 48 hours. The obtained ash was dissolved in 10 % hydrochloric and nitric acids and adjusted to the required volume by bidistilled water [9]. In atomic absorption spectrophotometer C-115M1 the quantities of zinc (wavelength – 213.9 nm), copper (wavelength – 324.7 nm), lead (wavelength – 283.3 nm), manganese (wavelength – 279,5 nm), chromium (wavelength – 357.9 nm), and iron (wavelength – 248.3 nm) were determined by the conventional method. To determine Zn, Cu, and Fe atomization in the flame, identification of Pb, Mn and Cr were carried out using electrothermal atomization in a graphite cuvette by means of "Graphite 1".

The material of testes was prepared by the standard method for the scanning electron microscopy (SEM) [10]. The specimens were viewed under the electron microscope SEM-102 (JSC "SELMI", Ukraine), which is a device that combines the functions of scanning electron microscope and X-ray microanalyzer. To prevent the accumulation of surface charge in electron-probe experiment dielectric samples were covered with a thin layer (30–50 nm) of aluminum or carbon in the vacuum system VUP-5M (JSC "SELMI", Ukraine).

By means of atomic absorption spectroscopy (AAS) the trace element composition was determined – the accumulation of chemical elements coming into the body in excess amounts. The same set of chemical elements, heavy metals in the testes of immature rats, was studied by the method of scanning electron microscopy with microanalysis (SEM), and also the same method additionally determined the contents of some trace elements (Na, K, P, Ca, Cl), the set of which was defined by the biological significance and technical capacity of the equipment.

Comparison between the groups was performed using the Student's t-test; statistical significance was $p < 0.05$. Statistical analysis was processed using the computer programs Microsoft Office Excel-2003.

Results

Determining the trace element compositions of testes tissue of the intact rats group by atomic absorption spectroscopy, it was found that the content of Zn in lactate period was 89.16 ± 8.4 mg/g; in suckperiod – 83.63 ± 8.34 mg/g; in infantile period – 76.19 ± 7.5 mg/g and in juvenile period – 67.37 ± 7.6 mg/g. Thus, during the development and growth of the testes a slight decrease in Zn occured. The contents of Cu, Fe, Mn, Cr and Pb in the tissue of seminal glands throughout the immature age also fluctuated within a small statistically insignificant difference. Thus, Cu ranged 1.53–1.6 mg/g; Fe – 38.47–42.28 mg/g; Mn – 1.55–1.6 mg/g; Cr – 1.46–1.5 mg/g, and Pb – 0.08–0.1 mg/g, respectively.

The study of the chemical composition of the tissue of the rat testes treated with L- carnitine alone showed that the contents of Cu, Fe, Mn, Cr and Pb during puberty were defined at the level of 1.56–1.59 mg/g; 42.9–44.66 mg/g; 1.58–1.6 mg/g; 1.48–1.51 mg/g and 0.09–0.1 mg/g, respectively. In the testes of animals that received only L-carnitine, zinc content in breast period was 90.26 ± 7.6 mg/g; in suck period – 85.24 ± 7.04 mg/g; in the infantile period – 79.78 ± 8.34 mg/g and in the juvenile period – 72.9 ± 7.13 mg/g. The results were not significantly statistically different from the content of trace elements of the intact group.

The accumulation of chemical elements that came into the bodies of experimental rats during the experiment revealed progressive accumulation of most of them (except zinc) in the tissue of rat testes. During the milk feeding (breast and sucking age – the 5th and 15th day of the experiment) the accumulation of chemical elements was not intense. Thus, the content of Cu increased in testes tissue relative to intact rats on the 5th day to 6.1 % ($P > 0.05$), on the 15th day the increase was 9.6% ($p>0.05$); the content of Fe on the 5th day increased by 4.75 % ($P > 0.05$), on 15th day – by 41.1% ($p > 0.05$); the increase in Mn content was 5 % ($p > 0.05$) and 17.4 % ($p > 0.05$), respectively; the increase of the content of Cr – 8.57 % ($p > 0.05$) and 18.49 % ($p < 0.05$). Pb, unlike other trace elements, showed a high ability to accumulate in the tissue of the organ, which is also confirmed by the reports of other authors [4; 5]. After 5 days the content of Pb in the testes increased by 20 %

($p > 0.05$), on 15th day it grew more than twice – by 209.09 % ($p < 0.05$). The general tendency in the total accumulation of chemical elements did not include Zn, because during the study, the decrease occurred in the content of trace elements. In the period of breast feeding the reduction of Zn was statistically insignificant – 8.03 % ($p > 0.05$) and 9.89 % ($p > 0.05$).

During puberty (infantile and juvenile age – 30 and 60 days, respectively) unidirectional change in the dynamics of accumulation of chemical elements was observed. So, on the 30th and 60th days of experiment Cu content in testes tissues increased by 30.21 % ($P < 0.05$) and 36.11 % ($P < 0.05$), respectively; Fe – by 80.54 % ($p < 0.05$) and 113.47 % ($p < 0.05$); Mn – by 44.65 % ($p < 0.05$) and 59.87 % ($p < 0.05$); Cr – by 87 16 % ($p < 0.05$) and 154.11 % ($p <0.05$); Pb – by 281.82 % ($p < 0.01$) and 390.91 % ($p < 0.05$) compared with the results of the control group. Unlike other metals, Zn levels in testes tissue reduced by 12.77 % ($P > 0.05$) on the 30th day and by 22.61 % ($P < 0.05$) on the 60th day of observation, despite its excessive intake in the body (Fig. 1).

When we used L-carnitine for correcting HMC impact on the structure of the testes, we obtained the the following results. After 5 days of observation the accumulation of analyzed chemical elements decreased relative to the series, in which the animals were influenced by HMC. For example, accumulation of Cu decreased by 5.74 % ($p > 0.05$); Fe – by 0.86 % ($p > 0.05$); Mn – by 1.19 % ($p > 0.05$); Cr – by 4 29 % ($p > 0.05$) and Pb – by 16.67 % ($p > 0.05$). Loss of zinc influenced by the corrector after 5 days of observation decreased by 3.7 % ($p > 0.05$). On the 15th day of the research, in animals, which received L-carnitine on the background of HMC combination, accumulation of Cu compounds decreased by 8.76 % ($p > 0.05$); Fe – by 13.14 % ($p > 0.05$); Mn – by 6.96 % ($p > 0.05$); Cr – by 6.36 % ($p > 0.05$) and Pb – by 35.29 % ($p > 0.05$). Zn was by 4.46 % ($p > 0.05$) higher in the tissue of rat testis under conditions of correction of HMC effect by L-carnitine comparing with animals who received only HMC.

After 30th and 60th days of experiment the dynamics of chemical composition was: accumulation of Cu compounds decreased by 23.2 % ($p < 0.05$) and 26.53 % ($p < 0,05$); Fe – by 26.29 % ($p < 0.05$) and 26.83 % ($p < 0.05$); Mn – by 15.62 % ($p < 0.05$) and 17.13 % ($p < 0.05$); Cr – by 26.71 % ($p < 0.05$) and 32.61 % ($p < 0.05$); Pb – by 38.1 % ($p < 0.05$) and 42.59 % ($p < 0.05$). At the same time, the amount of Zn increased by 15.32 % ($P > 0.05$) and 30.53 % ($P < 0.05$), respectively (Fig. 2)

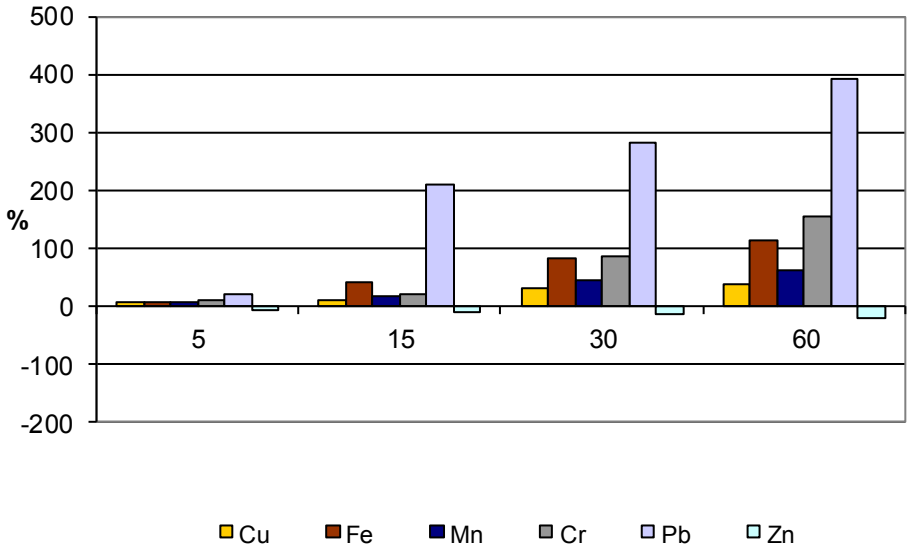


Fig. 1. Dynamics of chemical elements accumulation in the tissue of immature rat testes

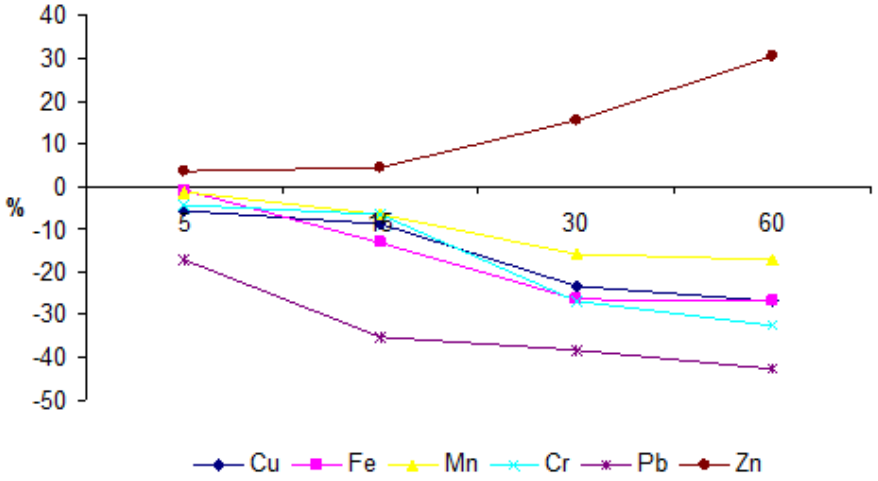
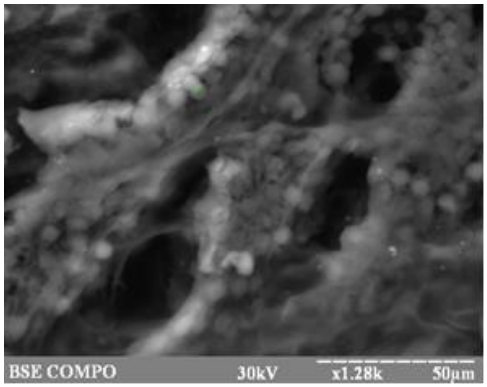


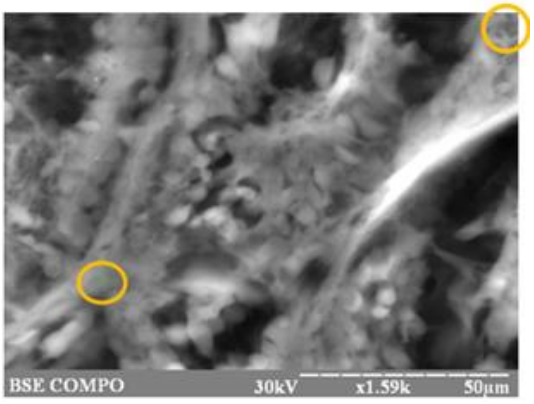
Fig. 2. Changes of HMC chemical elements in testes tissue under conditions of L-carnitine correction

In addition to determining the levels of accumulation of chemical elements by atomic absorption spectroscopy, the relative shares of chemical elements in testes tissue were determined by means of SEM microanalysis. Comparison of results of chemical composition obtained by the method of atomic absorption spectroscopy and by SEM allowed estimationg the selective accumulation of the analyzed elements in certain structures of the testes. Technically SEM microanalysis is carried out on a particular point or plane, while during spectroscopy of atomic absorption mode the whole tissue is analysed in total, without defining a specific localtion. The comparison of these methods allowed detecting certain tendencies in the dynamics of changes in the chemical composition of tissues and their structures.

On the 60th day of the experiment to identify the distribution of heavy metals accumulation in immature rat testes their content was determined in the spermatogenic epithelium of convoluted tubules (A), in interstitial tissue (B) and in albuginea (C) by means of SEM (Fig. 3).



A



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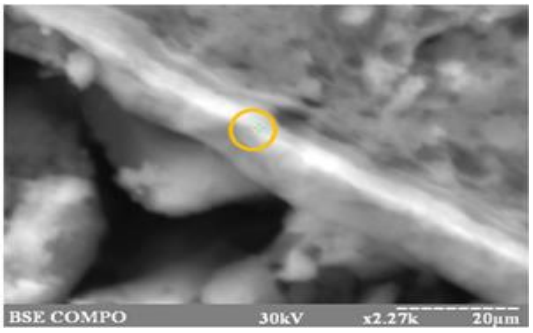

C

Fig. 3. Scanning electron microscopy of immature rat testis (on the 60th day, HMC impact); A – spermatogenic epithelium, x1280; B – interstitium, x1590; C – albuginea, x2270. The point of determining chemical composition is marked by the green cross

Determination of the chemical composition of testes tissue by SEM in the area of projection of spermatogenic epithelium showed that under the influence of HMC combination the content of analyzed elements decreased. Thus, relative to the control group Na content decreased by 2.9 % ($p > 0.05$); the content of K – by 15.4 % ($p < 0.05$); P – by 2.44% ($p > 0.05$); Ca – by 15.24 % ($p > 0.05$) and Cl – by 34.64 % ($p < 0.05$).

The contents of analyzed trace elements, except zinc, increased in the structure of spermatogenic epithelium. For example, the content of Fe increased by 47.25 % ($P < 0.05$), Mn content increased by 140.0 % ($p < 0.05$); Cu – by 76.47 % ($p < 0.05$), the content of Cr and Pb increased more than five (512.5 % ($p < 0.05$)) and fifteen (1587.5 % ($p < 0.05$)) times, respectively. On the background of HMC intake Zn content decreased by 21.52 % ($P < 0.05$). Such changes in the content of trace elements are consistent with the results of atomic absorption spectroscopy of testes of immature rats described above.

In terms of L-carnitine correction of HMC impact on testes, the following changes were observed. Compared with the group of animals treated with HMC combination in spermatogenic epithelium the insignificant increase in macronutrients contents was observed: Na increased by 1.38 % ($p > 0.05$); K – by 6.4% ($p > 0.05$); P – by 0.16 % ($p > 0.05$); Ca – by 10.18 % ($p > 0.05$) and Cl – by 36.47 % ($p < 0.05$). Comparing with the group of HMC impact on the background of correction in the testes tissue accumulation of trace elements ingested in excessive quantities decreased: the content of Fe decreased by 11.62 % ($p < 0.05$); Mn – by 36.11 % ($p < 0.05$); Cu – by 18.89 % ($p > 0.05$); Cr – by 62.96 % ($p < 0.05$) and Pb – by 34.69 % ($p < 0.05$); Zn loss decreased by 11.29 % ($p > 0.05$).

Changes in the chemical composition of the testes tissue in the area of interstitium projection also showed the reduction of macroelements relative to the control group of rats. Na content reduced by 3.02 % ($p > 0.05$); the content of K – by 15.1 % ($p < 0.05$); P – by 1.59 % ($p > 0.05$); Ca – by 14.31 % ($p > 0.05$) and Cl – by 34.1 % ($p < 0.05$). During the investigation of elements contents the increase of Fe was by 47.41 % ($p < 0.05$); Mn content increased by 88.89 % ($p < 0.05$); Cu – by 125.0 % ($p < 0.05$); contents of Cr and Pb increased three (300 % ($p < 0.05$)) and eleven (1.100% ($p < 0.05$)) times, respectively. On the background of HMC combination intake Zn content decreased by 22.77 % ($P < 0.05$).

Under conditions of L-carnitine correction of HMC impact on testes the growth of macronutrients content in the interstitium was also observed. Na contents is increased by 0.39 % ($p > 0.05$); K – by 5.74 % ($p > 0.05$); P – by 0.65 % ($p > 0.05$); Ca – by 6.5 % ($p > 0.05$); Cl – by 23.35% ($p < 0.05$). After correction, tissue Fe content in testes decreased by 15.17 % ($p < 0.05$); Mn – by 38.24 % ($p < 0.05$); Cu – by 37.08 % ($p < 0.05$); Cr – by 59.26 % ($p < 0.05$) and Pb – by 40.63% ($p < 0.05$). Zn content increased by 15.38 % ($P > 0.05$) (Fig. 6).

The chemical composition of the tunica albuginea testis under the influence of HMC changed relative to the control group of rats. In protein shell of experimental rats Na content decreased by 6.37% ($p > 0.05$); K – by 16.26 % ($p < 0.05$); P – by 0.74% ($p > 0.05$); Ca – by 3.94 % ($p > 0.05$) and Cl – by 25.38 % ($p < 0.05$). The content analyses of trace elements in the protein shell showed the increase in Fe content by 88.99 % ($p < 0.05$); Mn – by 15.0 % ($p > 0.05$) and Cu – by 134.29 % ($p < 0.05$). As in intact animals in the protein shell of testis Cr and Pb were not detected, we presented the average values of their contents, which amounted to 0.53 ± 0.07% and 0.23 ± 0.03% respectively. Under the background of HMC combination in the protein shell Zn content decreased by 45.0 % ($p < 0.05$).

Under conditions of L-carnitine correction of the negative impact of HMC protein shell of testis retained more macrocells. Na content increased by 1.97% ($p > 0.05$); K – by 5.99 % ($p > 0.05$); P – by 0.19 % ($p > 0.05$); Ca – by 0.71 % ($p > 0.05$) and Cl – by 16.42 % ($p > 0.05$). After correction Fe content in testis tissue decreased by 20.99 % ($p < 0.05$); Mn – by 13.04 % ($p < 0.05$); Cu – by 29.27 % ($p < 0.05$); Cr – by 24.53 % ($p < 0.05$) and Pb – by 26.09 % ($p < 0.05$), Zn content increased by 51.52 % ($p < 0.05$) (Fig. 7).

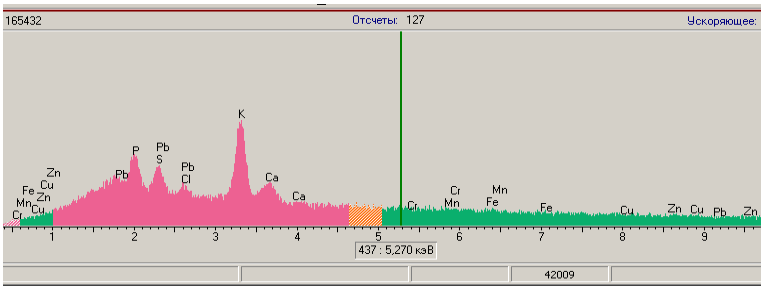

Fig. 4. A typical SEM spectrum of the tissue of immature rat testis.

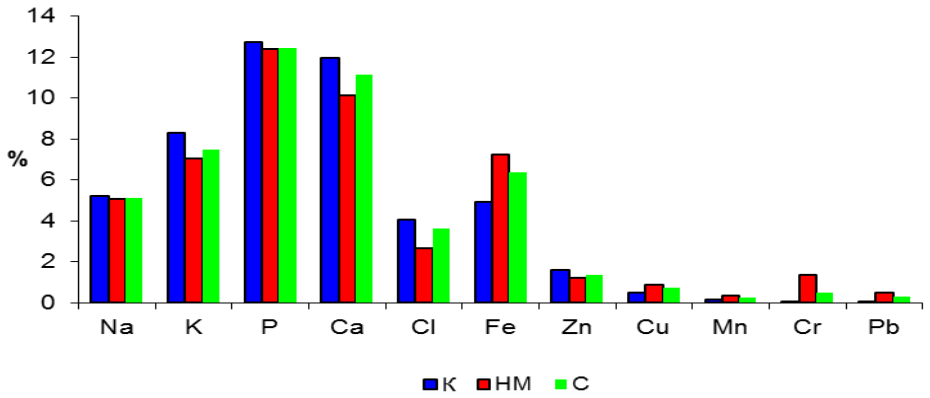

Fig. 5. The contents of chemical elements in the spermatogenic epithelium of testes of immature rats after 60 days of the experiment. SEM method. K – control, HM - under the influence of heavy metals, C – under the influence of L-carnitine correction

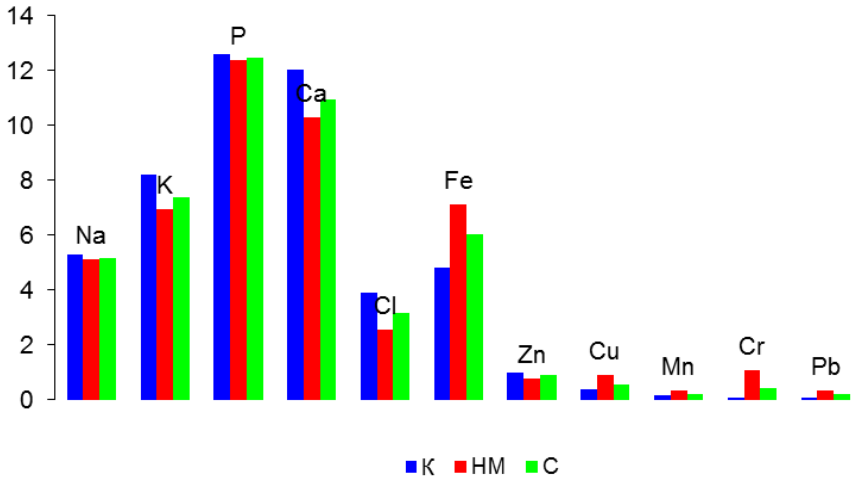

Fig. 6. The ratio of chemical elements in the interstitial tissue of testes of immature rats after 60 days of the experiment. SEM method. K – control, HM – under the influence of heavy metals, C – under the influence of L-carnitine correction

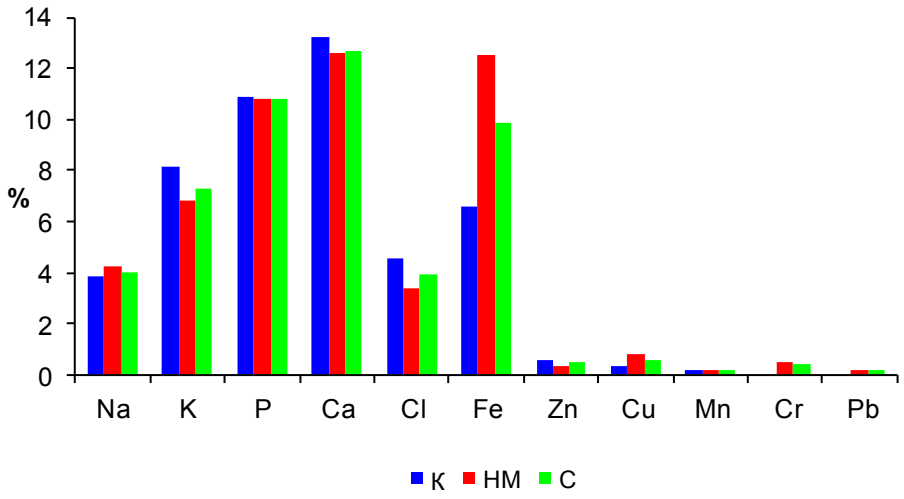

Fig . 7. The ratio of chemical elements in the albuginea of testes of immature rats after 60 days of the experiment. K – control, HM – under the influenceof heavy metals, C – under the influence of L-carnitine correction

Discussion

Analyzing the dynamics of accumulation of Zn, Fe, Cu, Mn, Cr and Pb in the tissue of immature rat testes, it is necessary to point on some physiological characteristics of nutritional intake of chemical elements. In breast and partly suckling periods, the intake of surplus quantities of heavy metals occured via maternal milk, which reduced the possibility of accumulation in tissues of immature rat testes. In juvenile and infantile ages the rate of HMC accumulation in the testes significantly increased, which can be seen in the diagram (Fig. 1).

During the experiment the contents of Fe, Cu, Mn, Cr and Pb in the tissue of the testes increased in direct proportion to the timing of impact of their excessive intake. Compounds of Fe, Cr and Pb exhibited more affinity to testes, their accumulation level exceeded targets two or three times. The most significant changes in trace element homeostasis in the analyzed organ were found after 60 days, confirming the cumulativeness of HMC. Observing changes in the content of Zn in the tissue of the testes, the decrease of its level by 22.61 % ($p < 0.05$) from the control rate after 60 days of the experiment should be noted. Similar results were obtained in the study of the influence of microelementosis on the chemical composition of mature rat testes [11]. The explanation of the results is the antagonistic relationship between the analyzed chemical metal elements [12; 13]. Moreover, the interaction between these compounds can occur at different levels – absorption in the gastrointestinal tract, transport proteins, tissue and cellular levels. According to the literature, Fe, Cu, and Pb exhibit the antagonistic effect relative to Zn among ME under study at almost all levels. Cr has synergism at the level of absorption in the gastrointestinal tract [12]. By the way, antagonistic relations of Zn ions with other "metallic" ions are used to reduce gonadotoxism. For example using zinc sulfate (dose 50 mg/kg) reduces the negative effect of aluminum sulfate (dose of 50 mg/kg) on the rats testes in the 45–day experiment [14]. Many scientific studies show Zn essention to the testes and the reproductive system of vertebrates [15]. Thus, the reduction of Zn can cause deeper damage of tissue of the investigated organs and can lead to secondary negative effects. For example, between bivalent metal ions there is a competition for binding to testicular hialuronidase, which is involved in the formation of connective tissue. Zn ions reduce its activity, Cu and Mn ions – reinforce it [16]. It can be concluded that the functional failure of Zn, due to the antagonism with other trace elements, under conditions of microelementosis promotes the process of sclerosation in seminal glands and reduction of its functional activity.

In the scientific literature the fact of HMC promotion of processes of free radical oxidation (FRO), mainly of membrane lipid peroxidation (LPO), which is one of the most important factors in the pathogenesis of male infertility, is extensively highlighted [17]. However, FRO can exert influence on the chemical composition of testis tissue by activating or inhibiting the expression of metal containing enzymes. For example, simulation of oxidative stress in the study of Schlorff E. C. (1999) led to the decrease in Zn-Cu-dependent superoxide dismutase (SOD), the reduction of the level of iron-dependent catalase and the increase of Mn-dependent SOD [18]. Thus, excessive intake of HMC into the body launches a rather complex chain of biochemical and morphological changes in the testes of immature rats that subsequently may cause infertility of such animals.

Despite the lower sensitivity of SEM method in soft tissues and different units of measurement, the data generally reflect the dynamic of changes in the chemical composition of the testes, obtained by AAS. During the study of the chemical composition of spermatogenic epithelium, interstitium (intracanalicular stroma, vessels and aits of Leydig cells) and protein shell, it is found out that the content of macronutrients in experimental conditions is subject to minor, in the vast majority – statistically unreliable fluctuations. The exception to general tendency are K and Cl, the contents of which decreased significantly in all structures of the testis, which may be associated with excessive accumulation of trace elements. Heavy metals trace elements most definitely accumulated in spermatogenic epithelium, less accumulated in the interstitial tissue and the least – in the protein shell, which obviously depends on the morphofunctional activity of the histological structure of the body.

Conclusion

1. In determining the trace element composition of the testes tissue of immature rats of control series (the intact group and the group of isolated

impact of carnitine) it was found out that the content of zinc, copper, iron, manganese, chromium and lead remained at a stable level, without significant difference for each observation period.

2. The contents of Fe, Cu, Mn, Cr and Pb in the tissue of the testes during the experiment increased in direct proportion to the timing of the impact of their excessive intake. Compounds of Fe, Cr and Pb exhibited more affinity to testes, their accumulation level exceeded targets two or three times. The most significant changes in trace element homeostasis in the analyzed organ were found after 60 days, confirming the cumulativeness of HMC.
3. The low rate of HMC accumulation in the testes of rats of breast and suckling age period is explained by the limited intake of xenobiotics via maternal milk.
4. Loss of zinc by testis tissue is caused by antagonistic interactions between the chemical elements that come into the rats' organisms in excessive quantities. Interaction of chemical elements at different levels can lead to secondary violations of chemical and structural homeostasis of the organ, causing further inhibition of its function.
5. The results of chemical analysis of immature animals' testes, obtained by SEM, indicate the dependence of accumulation of micro elements of heavy metals from the morphofunctional activity of the histological structure of the analyzed organ. Heavy metals mainly accumulated in a functionally active spermatogenic epithelium.

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