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Features in the dynamics of morphometric parameters in rats after long-term intake of vegetable products, grown in industrial regions of the Karaganda region

The article is devoted to the influence of accumulation of some heavy metals on the change of basic biometric indicators, such as body weight and mass of internal organs. A brief literary analysis was made on the effect of copper and its salts on biochemical processes that affect the dynamics of the increase in mass and index indices of the body. The article presents the results of a study on the effect of plant products grown in suburban areas in various industrial regions of Kazakhstan, on the morphometric parameters of the body and internal organs. Average statistical indicators characterizing the general development of internal organs and the animal as a whole were determined. The obtained results showed that the body weight of experimental animals varies depending on the duration of metal exposure and the amount of Fe^{3+}, Cu^{2+}, Zn^{2+} in the vegetables. It has been established that the increase in body weight and liver weight in animals is associated with a high amount of copper in vegetable products. The results of the study also showed the selective response of internal organs to the effect of heavy metals in the case of a change in mass.

Keywords: heavy metals, vegetables, industrial regions, copper, morphometry, body weight, liver.

Currently, one of the actively developing areas in toxicology is the study of the heavy metals effects on morpho-functional indicators and biochemical processes in living organisms. This is due to the increase of technogenic load, which poses a threat to environmental safety and public health. One of the most widespread and important microelements in nature is copper (Cu^{2+}). It is a part of the nitrate reductase complex in nitrogen exchange. Stimulates the maturation of reticulocytes to erythrocytes, promotes the assimilation of proteins and carbohydrates, increases the activity of insulin [1–3].

Recently, a large research material on the toxicity of high doses of copper for plants, animals and microorganisms has been accumulated. So, the content of copper sulfate in water at a concentration of 0.5 mg/l causes profound morphological changes in the liver, causing destructive hepatitis, characterized by acute dystrophy and hepatocyte necrosis, combined with pronounced cellular infiltration [4]. Studies on the impact of technogenic water pollution by heavy metals on morpho-physiological parameters in the lake frog have shown that the copper concentration in water equal to the MPC 21 and the zinc concentration is equal to MPC 38, leads to an increase of liver index [5]. Along with this, arises the question of the possible negative effects of iron and copper salts, as the most common in nature, as endocrine disruptors. It was also found that the use of copper and iron orally, at a dose level of 1 MPC, potentiates the adipogenic effect of a high-fat diet, which affects the increase in both absolute and relative fat content [6]. At the same time, the question of the effect of the intake of heavy metals, from plant products, on general morphometric parameters in animals, remains poorly studied.Proceeding from all above-mentioned, the purpose of our research was to study the general morphometric parameters of the body and organs in experimental animals fed with plant products grown in the zone of influence of industrial enterprises.

Materials and methods

The studies were conducted in the laboratory of ecological and genetic studies of Biology and Geography Faculty of the Ye.A. Buketov Karaganda State University. During the research, the ethical principles set out in the European Community Directive (2010/63/EC) and the requirements of the World Society for the Protection of Animals (WSPA) were observed.

The experiments were carried out on 40 white random-bred male rats. The animals were of the same age and were kept in standard vivarium conditions with free access to food and water. The feed ration of the tested animals consisted of solid grain feed, vegetable supplements and water in a ratio of 10×30×20 g.

Four groups of animals were formed. The first group of specimens received a standard diet of fodder grown in an environmentally clean region of Karkaralinsk. The second group is specimens, whose diet included vegetables grown in the area of influence of industrial enterprises in Temirtau (steelmaking plant). The third group is the animals, in the diet of which were vegetables grown in the area of influence of industrial enterprises in Balkhash (copper-smelting plant). The fourth group consisted of rats whose diet included vegetables obtained in the area of influence of industrial enterprises in Zhezkazgan (copper-smelting plant).

The accumulation and content of heavy metals in the samples of vegetables of the study groups 2–4 was recorded by the authors in earlier publications [7].

The duration of the experiment was four months. Weighing of animals was carried out once a month. At the end of the experiment, the control weighing was conducted, followed by decapitation and organ harvesting (liver, kidney, spleen). All organs were weighed and placed in formalin solution, for further studies. The statistical processing of the results was carried out using the Microsoft Excel 2007 application package. The reliability of differences in weight values was determined by the Wilcoxon-Mann-Whitney test. Differences in the dynamics of the weight increase were calculated by comparing the average values using the NA Plokhinsky table.

Results and discussion

From the data presented in Table 1 it can be seen that at the end of the experiment in all groups of animals a positive dynamics of weight gain was observed. Thus, the body weight of the animals in the control group increased by 46 % at the end of the experiment. The most significant increase in body weight, at the end of the experiment, was observed in experimental animals of group 3 (52 %), and the smallest increase in body weight in the experimental animals of group 2 (40.5 %). In animals of the 4th experimental group, the average body weight increased by 47 %.

Table 1

Dynamics of monthly average body weight indexes of experimental animals

Period	Control, $n = 6$		Temirtau, $n = 6$		Zhezkazgan, $n = 6$		Balkhash, $n = 7$	
	ΔMass (g)	P	ΔMass (g)	P	ΔMass (g)	P	ΔMass (g)	P
0 month	162±17.72		201±16.44		197±20.14		176±10.19	
1 month	192±29.79	0.05	216±35.51	0.05	231±27.80	0.05	187±11.64	> 0.05
2 month	182±24.34	> 0.05	218±15.85	> 0.05	216±45.40	> 0.05	193±15.14	> 0.05
3 month	194±21.55	> 0.05	220±16.24	> 0.05	238±21.08	> 0.05	213±10.58	< 0.01
4 month	236±31.56	< 0.01	282±25.66	< 0.01	291±27.95	0.05	268±41.00	< 0.01

Note. P — differences reliability criterion.

Calculation of the reliability of differences in weight increase in each group showed that in the 1st, 2nd and 4th groups, a significant change in weight was observed at the 1st and 4th months of the experiment, and in the third group at the 3rd and 4th months of the experiment. In other regions it was not statistically reliable. It can be noted that, in all groups, there is no significant increase in body weight at the 2nd month of the experiment.

An analysis of the dynamics of weight gain (Fig.) showed that animals receiving vegetables from the Temirtau and Balkhash industrial regions showed a constant positive dynamics of weight gain. In the 2nd and 4th groups, in 1st, 3rd and 4th months of the experiment, there was also a positive dynamics of increase in body weight, and in the second month of the experiment both groups showed a negative dynamics.

At the same time, there is a decrease in the relative increase in the body weight of the control group when compared with the body weight of the experimental groups. If in the first month of the experiment in

the control group the highest coefficient of weight gain (18.5 %) is observed, then for the fourth month thelow coefficient of body weight increase is 25 % compared to the experimental groups.

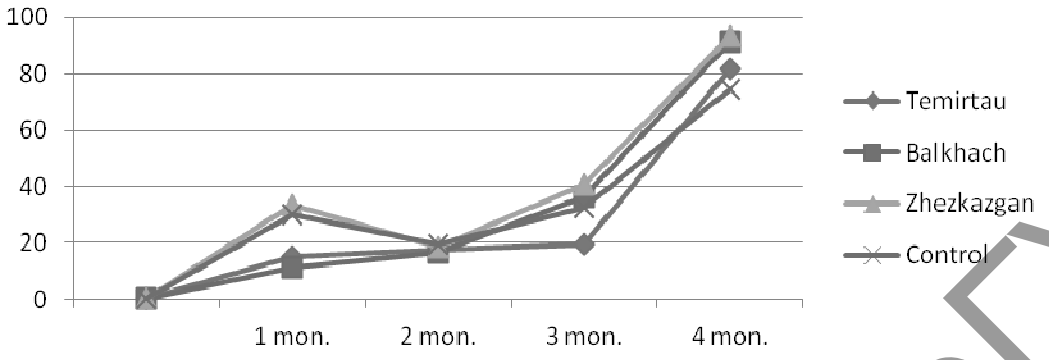

Figure. Dynamics of monthly weight gain in animals of various groups (g)

The reverse tendency of dynamics is shown by the third group of experimental animals. In the beginning of the experiment had the lowest body weight gain coefficient of 6.32 % to it, and at the end of experiments, the highest of 31.2 %. Such indicators of body weight increase in groups 3 and 4 can be explained by the adipogenic attribute of copper. The animals of these groups were fed vegetables that contained copper exceeding the MPC limit by 12.8 times. Analysis of the literature showed that the accumulation of copper in the body leads to increased adipogenesis process and increase in body weight [6].

Analysis of the increase in body weight in rats in the experimental groups as compared to the control group shows that the third group in the first and second month has a statistically significant difference in the monthly increase in body weight. The fourth group has a statistically significant increase only in the 3rd month of the experiment with respect to the control group. And the second group has a significant increase in the 3rd and 4th month of the experiment (Table 2).

Table 2

Comparison of the monthly dynamics of the increase in the average body weight of rats

Period	Control, *n* = 6	Temirtau, *n* = 6		Zhezkazgan, *n* = 6		Balkhash, *n* = 7	
	M (±m)	M (±m)	P	M (±m)	P	M (±m)	P
1 month	30.20 (±7.75)	15.00 (±11.02)	> 0.05	33.50 (±0.86)	> 0.05	11.14(±2.47)	0.05
2 month	–10.6 (±3.44)	2.67 (±11.16)	> 0.05	–14.83 (±1.44)	> 0.05	5.29 (±1.91)	0.001
3 month	12.80 (±1.98)	2.00 (±1.83)	0.001	21.83 (±0.62)	0.001	19.86 (±3.03)	> 0.05
4 month	42.00 (±5.39)	61.83 (±6.09)	0.05	53.00 (±1.28)	> 0.05	55.14 (±12.63)	> 0.05

Note. P — differences reliability criterion (according to N.A. Plokhinsky).

Representativity error calculation showed that in the second group the reaction of animals to the presence of metal ions in the vegetables, which were fed, was individual in comparison with groups 3 and 4. In these groups, the effect of metal ions has more general group character.

Table 3

The indicators of the increase in the average body weight of rats in comparison with the initial one

Period	Control, *n* = 6	Temirtau, *n* = 6		Zhezkazgan, *n* = 6		Balkhash, *n* = 7	
	M±m	M±m	P	M±m	P	M±m	P
1 month	30.20±15.48	15.00±24.63	> 0.05	33.50±12.71	> 0.05	11.14±6.06	0.05
2 month	19.60±12.50	17.67±3.30	> 0.05	18.67±26.27	> 0.05	16.43±6.90	> 0.05
3 month	32.40±9.53	19.67±7.32	<0.01	40.50±22.81	> 0.05	36.29±3.24	> 0.05
4 month	74.40±15.72	81.50±12.39	> 0.05	93.50±18.01	> 0.05	91.43±31.13	> 0.05

Note. P — differences reliability criterion.

As can be seen from Table 3, the total increase in body weight in the animals of the experimental groups and the control group, during the whole experiment, does not have statistically significant deviations in most cases. Separately, we can distinguish a significant change in the increase in body weight between the control group and the group that received vegetables obtained in the Temirtau industrial region.

After studying the mass and mass ratios of internal organs (liver, spleen, kidneys), it was revealed that the average both absolute and relative parameters of the liver mass of the control group were lower than in the experimental groups. The reverse tendency is observed with the mean and relative mass of the spleen, where the average mass and the mass ratio of the spleen in the control group are higher than in all experimental groups (Table 4).

Table 4

Indicators of mass and mass ratios of internal organs in experimental animals

Region	Liver		Kidneys		Spleen	
	Organ mass	Mass ratio, %	Organ mass	Mass ratio, %	Organ mass	Mass ratio, %
Control	7.1±0.93	3.00±0.39	1.71±0.03	0.72±0.01	0.98±0.08	0.42±0.03
Temirtau	9.74±1.39	3.45±0.49	1.82±0.20	0.65±0.07	0.92±0.13	0.33±0.05
Zhezkazgan	11.23±0.42	3.86±0.14	1.83±0.03	0.63±0.01	0.86±0.01	0.30±0.002
Balkhash	10.75±1.62	4.01±0.61	1.67±0.4	0.62±0.15	0.70±0.01	0.26±0.004

An analysis of organ mass variations showed that the greatest deviation in variations were observed in group 2, where the animals received vegetables grown in the suburban areas of the Temirtau industrial region. Also, a wide variational series is observed for the mass of the liver and kidneys in Group 3 animals, which received vegetables obtained from the suburban areas of the Balkhash industrial complex. The analysis of the literature data has shown that plants of the test arrays suburban regions contain metal ions of these metals exceeding MPC in vegetables several times [7]. Especially it is necessary to allocate the high amount of zinc, lead and manganese in vegetables from Temirtau region and zinc and copper in the Balkhash region.The fourth group of experimental animals has the least wide variation in the mass of organs. The vegetable feed of this group contained a large amount of copper and zinc and small concentrations of lead. Presumably, their joint presence causes an increase in the toxic effect on the target organs.

Conclusion

Thus, it was found that the increase in body weight and liver mass in animals consuming vegetable products with a high copper content, which apparently affects the intensification of adipogenesis processes, which in turn leads to an increase in body weight and liver mass. The effect of this metal is cumulative and is strengthened with the duration of nutrition by vegetables containing a large amount of copper. The mechanism of amplification of adipogenesis processes can be caused by activation of peroxidation in hepatocytes, which is accompanied by inhibition of the enzyme oxidation of cholesterol to bile acids of 7-b-cholesterol hydroxylase. That in turn may lead to lower fracture rate of cholesterol to bile acids [8].

The presence in vegetable forages of copper and zinc and absence of lead, leads to decrease in biometric parameters of kidneys and a spleen.

References

1 Ноздрюхина Л.Р. Биологическая роль микроэлементов в организме животных и человека / Л.Р. Ноздрюхина. — М.: Наука, 1987. — 184 с.

2 Бала Ю.М. Микроэлементы в гематологии и кардиологии / Ю.М. Бала, В.М. Лифшиц. — М.: Медицина, 1975. — 861 с.

3 Коломийцева М.Г. Микроэлементы в медицине / М.Г. Коломийцева, Р.Д. Габович. — М.: Медицина, 1981. — 1632 с.

4 Федорова Н.Н. Развитие патологии внутренних органов карпа в зависимости от концентрации сульфата меди / Н.Н. Федорова, Г.Ф. Журавлева, Г.В. Земков // Вестн. Астраханск. гос. ун-та. — 2004. — № 2(21). — С. 156–161.

5 Файзулин А.И. Влияние техногенного загрязнения тяжелыми металлами и урбанизации на морфофизиологические показатели популяции озерной лягушки *Rana Ridibunda* / А.И. Файзулин, Ф.Ф. Зарипова // Вестн. Томск. гос. ун-та. — 2013. — № 18(3). — С. 834–837.

6 Тиньков А.А. Влияние перорального поступления солей железа и меди на инициацию адипогенеза и атерогенеза у крыс линии Wistar / А.А. Тиньков, А.А. Никоноров // Вестн. Оренбургск. гос. ун-та. — 2013. — № 12(161). — С. 173–177.

7 Конкабаева А.Е. Оценка накопления тяжелых металлов в почве, воде и растениях промышленных регионов Карагандинской области: монография / А.Е. Конкабаева, М.Ю. Ишмуратова. — Караганда: Полиграфист, 2016. — 112 с.

8 Меерсон Ф.З. Роль подавления активности 7-б-гидроксилазы холестерина печени в возникновении стрессовой дислипопротеидемии / Ф.З. Меерсон, В.П. Твердохлиб, А.А. Никоноров // Кардиология. — 1988. — № 9. — С. 85–87.

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Қарағанды облысының өнеркәсіптік аймақтарында өсірілген өсімдік өнімдерімен ұзақмерзімді қоректенген егеуқұйрықтардың морфометриялық динамика көрсеткіштерінің ерекшеліктері

Мақала дене салмағы және ішкі ағзаның массасы сияқты негізгі биометриялық көрсеткіштердің өзгеруіне кейбір ауыр металдардың жинақталуының әсер етуіне арналған. Дененің массалық және индекс көрсеткіштерінің өсу динамикасына әсер ететін биохимиялық процестерге мыс тұздарының ықпалы туралы қысқаша әдеби талдау жүргізілді. Авторлар Қазақстанның әртүрлі өнеркәсіптік аймақтарындағы саяжай массивтерінде өсірілген өсімдіктердің дененің және ішкі органдардың морфометриялық көрсеткіштеріне әсері туралы зерттеудің нәтижелері келтірілген. Жалпы алғанда, жануарлардың және ішкі ағзаларының дамуын сипаттайтын орташа статистикалық көрсеткіштер анықталған. Алынған нәтижелер эксперименталдық жануарлардың дене салмағының өзгеруін жануарлар ағзасына металдардың түсу ұзақтығына және көкөністердегі Fe^{3+}, Cu^{2+}, Zn^{2+} мөлшеріне байланысты екенін көрсетті. Жануарлардың бауыры мен дене салмағының жоғарылауы көкөніс өнімдеріндегі мыс құрамының жоғары болуымен байланысты екендігі анықталды. Сонымен қатар зерттеу нәтижелері ішкі органдары массасының өзгеруі кезінде ауыр металдардың әсеріне олардың селективті реакциясын көрсетті.

Кілт сөздер: ауыр металдар, көкөністер, өндірістік аймақтар, мыс, морфометрия, дененің салмағы, бауыр.

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Особенности динамики морфометрических показателей у крыс, длительно употреблявших растительную продукцию, выращенную в промышленных регионах Карагандинской области

Статья посвящена влиянию накопления некоторых тяжелых металлов на изменение основных биометрических показателей у крыс, таких как масса тела и масса внутренних органов. Был проведен краткий литературный анализ по влиянию меди, её солей на биохимические процессы, которые влияют на динамику увеличения массовых и индексовых показателей тела. В статье представлены результаты исследования по влиянию растительной продукции, выращенной в дачных массивах различных промышленных регионов Казахстана, на морфометрические показатели тела и внутренних органов. Были определены среднестатистические показатели, характеризующие общее развитие внутренних органов и животного в целом. Полученные результаты показали, что масса тела у экспериментальных животных изменялась в зависимости от длительности поступления металлов в организм животных и содержания Fe^{3+}, Cu^{2+}, Zn^{2+} в овощах. Установлено, что увеличение массы тела и массы печени у животных связано с большим содержанием меди в овощной продукции. Результаты исследования также показали избирательную реакцию внутренних органов на воздействие тяжелых металлов при изменении их массы.

Ключевые слова: тяжелые металлы, овощи, промышленные регионы, медь, морфометрия, масса тела, печень.

References

1 Nozdryukhina, L.R. (1987). *Biolohicheskaia rol mikroelementov v orhanizme zhivotnykh i cheloveka [Biological role of microelements in animals and humans]*. Moscow: Nauka [in Russian].

2 Bala, Yu.M., & Lifshits, V.M. (1975). *Mikroelementy v hematolohii i kardiolohii [Microelements in haematology and cardiology]*. Moscow: Meditsina [in Russian].

3 Kolomiitseva, M.G., & Gabovich, R.D. (1981). Mikroelementy v meditsine [Microelements in medicine]. Moscow: Meditsina [in Russian].

4 Fedorova, N.N., Zhuravleva, G.F., & Zemkov G.V. (2004). Razvitie patolohii vnutrennikh orhanov karpa v zavisimosti ot kontsentratsii sulfata medi [Pathologic development in internal organs of carp depending on the concentration of copper sulfate]. *Vestnik Astrakhanskoho hosudarstvennoho universiteta — Bulletin of Astrakhan State University, 2(21)*, 156–161 [in Russian].

5 Faizulin, A.I., & Zaripova, F.F. (2013). Vliianie tekhnohennoho zahriazneniia tiazhelymi metallami i urbanizatsii na morfofiziolohicheskie pokazateli populiatsii ozernoi liahushki *Rana Ridibunda* [The influence of anthropogenic heavy metal pollution and urbanization on the morphophysiological indicators of the population of the lake frog *Rana Ridibunda*]. *Vestnik Tomskoho hosudarstvennoho universiteta — Bulletin of Tomsk State University, 18(3)*, 834–837 [in Russian].

6 Tinkov, A.A., & Nikonorov, A.A. (2013). Vliianie peroralnoho postupleniia solei zheleza i medi na initsiatsiiu adipoheneza i ateroheneza u krys linii Wistar [Effect of oral intake of iron and copper salts on the initiation of adipogenesis and atherogenesis in Wistar rats]. *Vestnik Orenburhskoho hosudarstvennoho universiteta — Bulletin of Orenburg State University, 12(161)*, 173–177 [in Russian].

7 Konkabaeva, A.E., & Ishmuratova, M.Yu. (2016). *Otsenka nakopleniia tiazhelykh metallov v pochve, vode i rasteniiakh promyshlennykh rehionov Karahandinskoi oblasti [Assessment of the accumulation of heavy metals in soil, water and plants in the industrial regions of the Karaganda region]*. Karaganda: Poligrafist [in Russian].

8 Meerson, F.Z., Tverdokhlib, V.P., & Nikonorov, A.A. (1988). Rol podavleniia aktivnosti 7-b-hidroksilazy kholesterina pecheni v vozniknovenii stressovoi dislipoproteidemii [The role of suppressing the activity of 7-b-hydroxylase in liver cholesterol in the onset of stressful dyslipoproteidemiia]. *Kardiolohiia — Cardiology, 9*, 85–87 [in Russian].