DOI 10.29254/2077-4214-2022-3-166-115-119
UDC 612.3:591.39:661.852:661.782-092.9

[1]Tymchuk K. M., [2]Abramov S. V., [2]Kryzhanovsky D. G., [2]Fedchenko M. P., [2]Filipenko V. V., [2]Chernenko G. P., [2]Myakushko V. A.

EMBRYOTOXIC EFFECT OF CADMIUM CHLORIDE AND CUPRUM DURING THE ENTIRE PREGNANCY PERIOD IN WHITE RATS

[1]Dnipro State Medical University (Dnipro, Ukraine)
[2]Dnipro Medical Institute of Traditional and Non-Traditional Medicine (Dnipro, Ukraine)
rudenkohomerudenko@gmail.com

Cadmium (Cd) is one of the most common and harmful transition metals present in our environment. Recently, there has been increased interest all over the world in studying the impact of habitat quality on the growth of non-infectious diseases. A whole series of mass human and animal diseases is associated with technogenic geochemical anomalies in the environment, which develop against the background of an immunodeficient state of the body. The main sources of the chemical factor entering the human body are drinking water, food products and atmospheric air. The toxic effect of heavy metals is related to their absorption in the gastrointestinal tract. Pregnancy is a special physiological state, and prenatal development is a critical period in the etiology of pathological changes in embryogenesis. Therefore, the study of the influence of cadmium compounds on embryonic development is relevant today. Teratogenic drugs act during certain critical periods of ontogenesis, especially during embryogenesis.

The purpose of the work is to determine the effect of cadmium (2,0 mg/kg) and copper (0,1 mg/kg) on the general course of embryogenesis in white rats with combined introduction and throughout the entire period of pregnancy.

The intrauterine survival rate was the highest in the control group on the 19th day of embryogenesis (95,29%), and the lowest in the group exposed to cadmium chloride on the 19th day (75,07%). As the analysis of the obtained results showed, there is a pronounced embryotoxic effect of cadmium chloride on the processes of embryogenesis, which is manifested by a significant increase in the indicators of total embryonic mortality, pre-implantation and post-implantation mortality compared to the control group at all studied terms of embryogenesis.

Key words: *cadmium, copper, embryogenesis, embryotoxicity, embryos.*

Connection of the publication with planned research works. The experimental study was carried out as part of the research work of the Department of Medical Biology, Pharmacognosy, Botany and Histology of the Dnipro State Medical University "Biological bases of morphogenesis of organs and animals under the influence of microelements and ultramicroelements in the experiment" (state registration number 0118U006635).

Introduction. Recently, there has been increased interest all over the world in studying the impact of habitat quality on the growth of non-infectious diseases. It is known that a number of mass human and animal diseases are associated with man-made geochemical anomalies in the environment, which develop against the background of an immunodeficiency state of the body [1]. Cadmium is a ubiquitous environmental pollutant of worldwide concern. The main sources of a chemical factor entering the human body are food products, drinking water, and atmospheric air. The range of toxic effects is quite wide. The result of acute intoxication with cadmium compounds is damage to the kidneys, liver, digestive system, lungs, and reproductive system. With the growth of urbanization, there is a complication of the ecological situation on transport highways, areas occupied by industrial enterprises, as well as in the territories adjacent to them. The toxic effect of chemical elements is related to their absorption in the gastrointestinal tract. Cadmium belongs to the elements for which the root system of plants is not an obstacle. Food crops that are grown on cadmium-containing soils or on soils naturally rich in this metal and smoking are the main sources of non-professional exposure to this metal [2]. Heavy metals remain one of the main groups of pollutants with local, regional and global distribution. The toxicity of heavy metals depends on the concentration, duration of action, temperature, water saturation with oxygen and many other factors [3]. Characteristic features of the toxic effect of heavy metals are the universality of their effect on living organisms, both as systemic poisons and the ability to form complexes with cell components, proteins, amino acids, and other radicals. One of the main reasons for this is the excessive content of trace elements in the body, which have an embryotoxic effect. Such substances include cadmium, which causes the development of various pathologies [4].

Cadmium (Cd) is a heavy metal assigned to the second class of danger, which has a tendency to accumulate in the body. Cd poisoning occurs when it enters the digestive system or by inhalation. Changes in the intensity of free radical reactions are at the basis of the cytotoxic effect of cadmium, lipid peroxidation with disruption of DNA replication. Large doses of heavy metal can cause embryotoxic and gonadotoxic effects, and as a result affect reproductive function. According to the data of many studies, the indicators of the reproductive function of women living in polluted cities differ significantly from the control indicators. In them, there is an increase in the number of complications during pregnancy, childbirth and the postpartum period, the number of congenital developmental defects, and the deterioration of the health of the offspring. All these changes lead to a decrease in the birth rate, as well as to the birth of sick children with physical and intellectual disabilities [5]. When supplied through the gastrointestinal tract, the average absorption of cadmium is 5%, while a change in the composition of the intestinal flora is noted. Literary sources also mention the vital need for cadmium in non-toxic concentrations, namely: regulation of blood sugar levels, stimulation of animal growth, deficiency of the heavy metal in the diet provokes growth retardation and puberty. According to the requirements of the World Health Organization

(WHO), the level of Cd entering the human body from all sources should not exceed 400–500 μg/week. The main role in terms of the natural biological barrier is played by the intestinal epithelium, which reflects the body's ability to resist the action of various exotoxicants, including Cd [6]. Children are one of the risk groups for the accumulation of cadmium in the body, and in connection with this, there is a need to determine the effect of heavy metal compounds on organ systems and the course of embryogenesis, and to search for possible antagonists of their action, which is relevant from the point of view of theoretical biology , and practical medicine.

The aim of the study. Experimentally determine the effect of low doses of cadmium (2,0 mg/kg) and copper (0,1 mg/kg) when administered in combination during the entire period of pregnancy in white rats.

Object and methods of research. The study was conducted on 32 rats of the Wistar line (Dali 2000 kennel, Kyiv), weighing 180-300 g. The choice of rats as the object of research is based on the fact that they have a low level of spontaneous developmental defects (0,02-0,85%) compared to rabbits (0,74-4,2%) and mice (0,04-15,7%). In accordance with the conditions and requirements of conducting embryonic experiments, we provided a complete food ration, drinking water and careful care of experimental animals. At the preparatory stage, the estrous cycle of females was studied by the method of vaginal smears, which made it possible to determine the duration of the cycle and individual phases, the presence of four phases of the cycle and the rhythmicity of their alternation in each female. For further investigation of possible embryotoxicity, females with a steady rhythm of the estrous cycle of proestrus and estrus stages were taken and mated with intact males according to the 2:1 scheme. Thus, detecting spermatozoa in vaginal swabs indicated the first day of pregnancy in females. Before the start of the experiment, the animals were weighed, the required amount of cadmium chloride was calculated, and the amount of solution of the required concentration was prepared.

According to the generally accepted instructions for conducting the experiment, the solutions were administered to females intragastrically, through a probe once a day, at the same time: Group I – control, Group II – administration of a solution of cadmium chloride at a dose of 2,0 mg/kg – group of isolated administration of cadmium, and III – group of combined administration of cadmium chloride, at a dose of 2,0 mg/kg, together with copper succinate at a dose of 0,1 mg/kg. The cadmium chloride solution was of ionic form, and the copper succinate solution was of nanoaquachelate form. Female rats were exposed to the solution daily from the 1st to the 19th day of pregnancy. During the experiment, the condition and behavior of female rats, rectal temperature, body weight dynamics, and the duration of pregnancy were recorded. On the 13th and 19th days of pregnancy, females were slaughtered. Rat pups were removed from the uterus, a macroscopic examination was performed to detect external anomalies, the number of corpora lutea of pregnancy was counted in the ovaries of females and their correspondence to the number of embryos in both horns of the uterus. For the experiment, we chose a low dose of cadmium chloride, which reproduces the real concentration in the daily rations of women, including pregnant women from industrial regions.

The possible negative effect of the studied substances on embryonic development was determined by the ability to increase the level of embryonic mortality, the overall development of fetuses was assessed according to the tables of normal embryonic development according to Hamburger and Hamilton [5]. The experiments were conducted in accordance with the Council of Europe Convention on the Protection of Vertebrate Animals Used for Scientific Purposes (Strasbourg, 1985). Indicators of embryotoxicity are generally accepted criteria: pre-implantation (DIM), post-implantation embryonic mortality (PIM), total embryonic mortality (TEM), indicators of intrauterine survival, morphological (anatomical) malformations, as well as general retardation of fetal development, which were calculated according to well-known formulas:

1. Total embryonic mortality, TEM $= \frac{B-A}{B} \times 100\%$,

where A is the number of live fetuses, B is the number of corpora lutea of pregnancy.

2. Pre-implantation mortality, DIM $= \frac{C-(A+B)}{C}$,

where A is the number of live fruits, B is the number of dead (resorbed) fetus,

C – the number of corpora lutea of pregnancy.

3. Post-implantation mortality, PIM $= \frac{B}{A+B}$,

where A is the number of live fruits, B is the number of dead (resorbed) fetus.

The obtained results were processed by the method of variational statistics. The probability assessment of statistical studies was carried out using the Student's t-test, the obtained data were considered to be significantly significant at $p<0.05$.

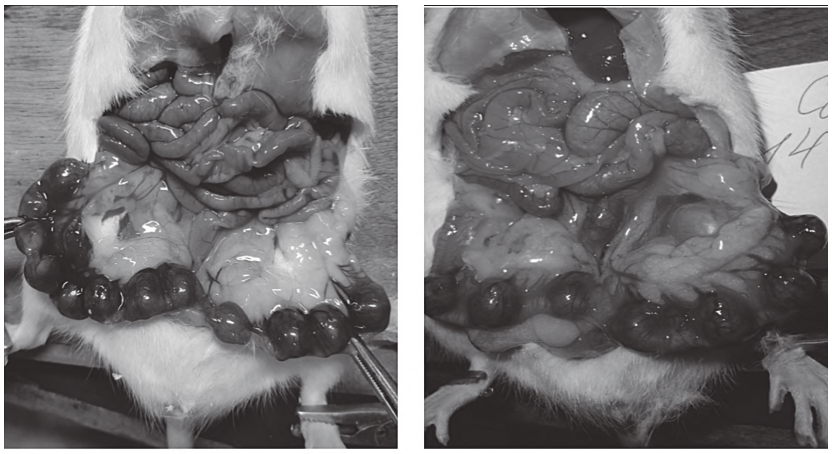
Research was carried out in accordance with the principles of the Declaration of Helsinki, adopted by the General Assembly of the World Medical Association (2000), the Convention of the Council of Europe on Human Rights and Biomedicine (1997), the relevant provisions of the WHO, the International Council of Medical Scientific Societies, the International Code of Medical Ethics (1983), «General ethical principles of experiments on animals» approved by the First National Congress on Bioethics (Kyiv, 2001) in accordance with the provisions of the «European Convention for the Protection of Vertebrate Animals Used in Experiments and Other Educational Purposes» (Strasbourg, March 18, 1986).

Research results and their discussion. During the surgical removal of embryos on the 13th day of embryogenesis, a decrease in the number of embryos in the uteri of female rats was noted **(fig. 1).** The rat uterus has two horns, in which implantation and development of embryos take place. In the control group, both horns of the female's uterus were evenly filled with embryos and their number was significantly higher compared to the group exposed to cadmium chloride. In the group exposed to cadmium, embryos were located at intervals, which indicates the presence of pre-implantation mortality or resorption of embryos after implantation **(fig. 1).**

The average indicator of the number of embryos in the group exposed to cadmium chloride was 9,25±0.72, which is 1,3 times less than the similar indicator of the control values (12.13±0.87). The obtained results indicate the embryotoxic effect of cadmium on the embryonic development of white rats.

Analysis of experimental studies revealed a negative effect of cadmium compounds in both tested doses on embryogenesis indicators on both the 13th and 19th days of pregnancy **(table)**. The study showed that the fertility index in the experimental groups did not differ from the available literature data.

An increase in TEM indicators was shown in the groups exposed to cadmium chloride on the 13th day and 19th day of embryonic development, compared to the control group, by 4,6 times and 5,0 times. In the group treated with cadmium chloride and copper succinate, a 2,2 times decrease in this indicator was noted on the 13th day, and 3,4 times on the 19th day of embryonic development. Thus, the positive dynamics of reducing the influence of cadmium chloride under the influence of copper succinate by 52,17% and 32%, respectively, were recorded.



A **B**

Figure 1 – Photo of the bicornuate uterus of a female rat with embryos on the 13th day of pregnancy during surgical removal: A – control group; B – group of exposure to cadmium chloride. A decrease in the number of embryos in the uterus is noticeable in the group exposed to cadmium chloride.

Table – Indicators of embryogenesis of rats in the norm and in the experimental study, under the influence of cadmium chloride and copper succinate on the 13th and 19th days of embryogenesis, (M±m)

Indicator/ groups	Control		№1 Cadmium chloride 2.0 mg/kg		№2 Cadmium chloride + copper 0.1 mg/kg	
Era embryogenesis	13	19	13	19	13	19
The number of corpora lutea	12,75± 0,92	13,13± 0,91	11,63± 0,49	11,88± 0,24	12,38± 0,45	12,13± 0,62
Total embryonic mortality, (TEM)	0,05± 0,02	0,05± 0,02	0,23± 0,04***	0,25± 0,07**	0,11± 0,02*##	0,17± 0,04*
Pre-implantation mortality, (DIM)	0,02 ± 0,01	0,01± 0,01	0,08± 0,02*	0,15± 0,07*	0,08 ± 0,02*	0,10 ± 0,03**
Post-implantation mortality, (PIM)	0,03 ± 0,01	0,04± 0,02	0,15± 0,04**	0,11± 0,05	0,03 ± 0,02##	0,08 ± 0,03
Index of intrauterine survival rate, %	95,16± 1,65	95,29± 0,95	77,23± 3,57	75,07± 6,86	83,19± 2,80	83,19± 2,80

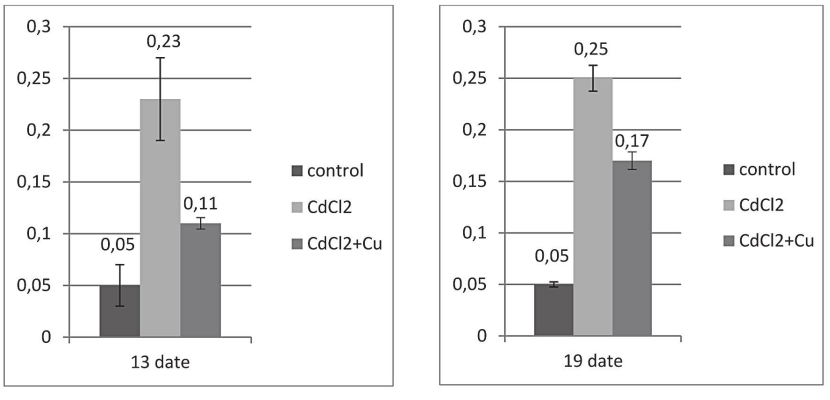
Notes:
①* -p<0,05,** – p<0,01; *** – p<0,001 relative to the control group
②#- p<0,05, ## – p<0,01; ### – p<0,001 with respect to the cadmium chloride group
③o- p<0,05, oo – p<0,01; ooo – p<0,001 in relation to the cadmium + copper group

The analysis of the obtained results indicates a pronounced embryotoxic effect of cadmium compounds at a dose of 2,0 mg/kg on the processes of embryogenesis, which is manifested by a significant increase in total embryonic mortality (TEM) compared to the control group, and positive dynamics are observed in the group of combined administration of cadmium chloride with copper succinates **(fig. 2)**.

A comparison of indicators of the effect of heavy metals on the body indicates a pronounced embryotoxic effect of cadmium compounds at a dose of 2,0 mg/kg on the processes of embryogenesis, which is a significant increase in total TEM compared to the control group, and positive changes were recorded in the group of combined administration of cadmium chloride with succinates copper.

There is a 4,0 times increase in DIM indicators in the cadmium chloride groups on the 13th day of pregnancy, and they remain unchanged when copper succinates are administered, compared to the control group, and increase by 15 times on the 19th day of embryonic development, and in the group of combined introduction of cadmium chloride with copper decreased by 33,3%, compared to the cadmium chloride group **(fig. 3)**.

Postimplantation mortality increased in the group exposed to cadmium chloride, compared to control values: 5,0 times on the 13th day of embryogenesis, and decreased by 80% in the group of cadmium chloride and copper succinate, compared to the group with independent administration of cadmium chloride. On the 19th day of pregnancy, there is a 2.8 times increase in cadmium chloride indicators, and an improvement in indicators by 27,3%, in the group with the combined administration of cadmium chloride and copper



A **B**

Figure 2 – Total embryological mortality (TEM): A – 13th day of embryonic development; B – the 19th day of embryonic development, in the control and experimental groups.

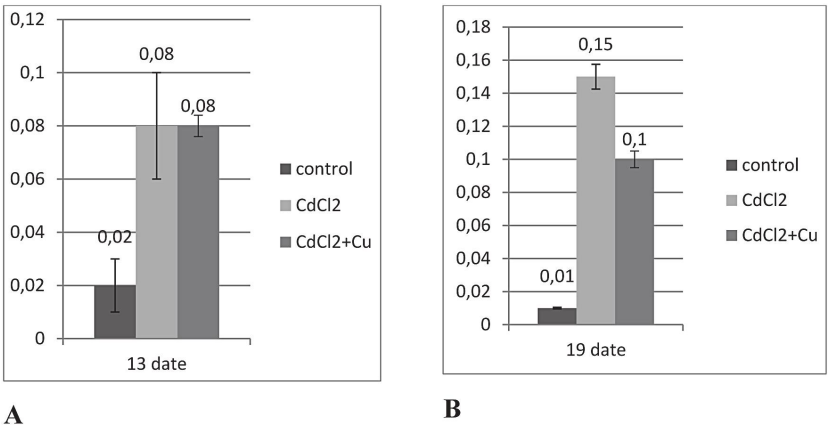


Figure 3 – Pre-implantation (DIM) mortality: A – the 13th day of embryonic development; B – the 19th day of embryonic development, in the control and experimental groups.

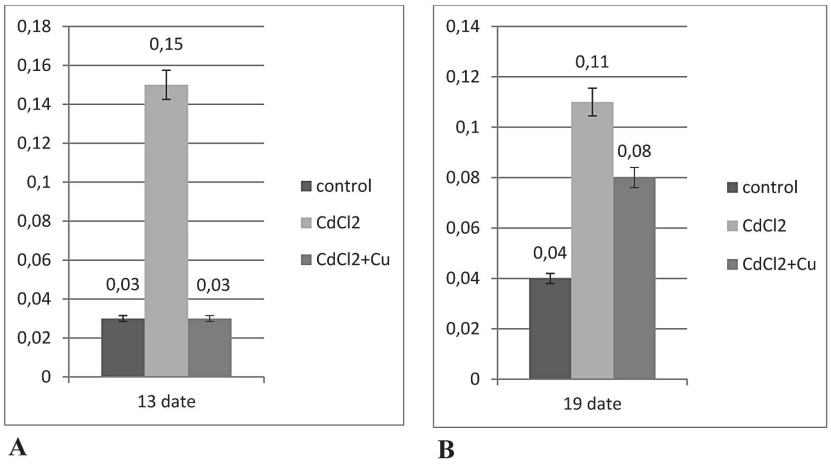


Figure 4 – Postimplantation mortality (PIM): A – the 13th day of embryonic development; B – the 19th day of embryonic development, in the control and experimental groups.

(fig. 4). The results of the experiment show that under the influence of negative factors during pregnancy, abortion occurs in the pre-implantation period, which does not contradict the available literature data.

Indicators of the number of yellow bodies in the group with the independent introduction of cadmium chloride decreased by 1,1 times on the 13th day of embryonic development, compared to the control, and an improvement was noted in the group with the combined introduction of cadmium chloride and copper succinate by 0,9 times compared to cadmium chloride group. On the 19th day of pregnancy in white rats, a 1,1 times decrease in the number of corpora lutea was recorded in cadmium chloride, and in the group with a combined administration of cadmium chloride and cuprum, the indicators improved by 0,9 times.

The intrauterine survival rate was the highest in the control group on the 19th day of embryogenesis (95,29%), and the lowest in the group exposed to cadmium chloride at a dose of 2,0 mg/kg on the 19th day (75,07%).

Conclusions. The analysis of the obtained data indicates a pronounced embryotoxic effect of cadmium chloride on the processes of embryogenesis, which is manifested by a significant increase in the indicators of total embryonic mortality, pre-implantation and post-implantation mortality compared to the control group at all studied terms of embryogenesis.

Prospects for further research. In our opinion, it is promising to identify and compare the degree of accumulation of cadmium in the organs of embryos by the method of polyelement analysis and to conduct histological studies of the small intestine of embryos, which will help to highlight changes at the tissue level, which will make it possible to explain the level of embryonic mortality.

References

1. Khopta NS, Yerstenyuk AM. Metabolíchní zmíni v kístkoví tkaniní tvarin dlya umov yeksperimental'nogo kadmíozu. ScienceRise. Biologicheskaya nauka. 2018;5:31-35. [in Ukrainian].
2. Hao R, Ge J, Ren Y, Song X, Jiang Y, Sun-Waterhouse D, et al. Caffeic acid phenethyl ester mitigates cadmium-induced hepatotoxicity in mice: Role of miR-182-5p/TLR4 axis. Ecotoxicol Environ Saf. 2021;207:111578.
3. Arustamyan OM, Tkachishin VS, Aleksíychuk OYu. Vpliv spoluk kadmíyu na organízm lyudini. Meditsina neotlozhnykh sostoyaniy. 2016;7:109-114. [in Ukrainian].
4. Lynch S, Horgan K, White B, Walls D. Selenium source impacts protection of porcine jejunal epi-thelial cells from cadmium-induced DNA damage, with maximum protection exhibited with yeast-derived selenium compounds. Biol. Trace Elem. Res. 2017;176(2):311-20.
5. Skal'nyy AV, Zaytseva IP, Tin'kov AA. Mikroelementy i sport. Personalizirovannaya korrektsiya elementnogo statusa sportsmenov. M.: Sport; 2018. 288 s.
6. Kolosova II, Rudenko KM, Shatorna VF. Kadmiy – zahroza dlya zhyvykh orhanizmiv (ohlyad literatury). Abstracts of V International Scientific and Practical Conference Perspectives of world science and education; 29-31 Jan 2020; Osaka, Japan; p. 433-442.

ЕМБРІОТОКСИЧНА ДІЯ ХЛОРИДУ КАДМІЮ ТА КУПРУМУ ПРИ ВНУТРІШНЬОШЛУНКОВОМУ ВВЕДЕННІ ВПРОДОВЖ ВСЬОГО ПЕРІОДУ ВАГІТНОСТІ У БІЛИХ ЩУРІВ

Тимчук К. М., Абрамов С. В., Крижановський Д. Г., Федченко М.П., Філіпенко В. В., Черненко Г. П., Мякушко В. А.

Резюме. Кадмій (Cd) – один з найбільш поширених і шкідливих перехідних металів, присутніх в нашому довкіллі. У сучасному світі на організм мешканців урбанізованих територій одночасно впливають безліч шкідливих хімічних сполук і є тератогенними, тобто ушкоджувальними для плода людини та тварин. Вагітність – це особливий фізіологічний стан, а пренатальний розвиток є критичним періодом в етіології патологічних змін в ембріогенезі. Тому актуальним на сьогоднішній день є вивчення впливу сполук кадмію на ембріональний розвиток. Тератогенні чиники діють упродовж певних критичних періодів онтогенезу, особливо під час ембріогенезу.

Метою роботи є визначення впливу низьких доз кадмію (2,0 мг/кг) та міді (0,1 мг/кг) на загальний хід ембріогенезу щурів при комбінованому введенні, впродовж всього періоду вагітності білих щурів.

Для проведення досліджень отримували самиць з датованим терміном вагітності, для цього ми досліджували естральний цикл самиць методом піхвових мазків. Розчини вводили через зонд, внутрішньошлунково, щоденно (раз на добу) з першого дня вагітності: перша група – контроль, друга група – введення розчину кадмій хлориду в дозі 2,0 мг/кг – група ізольованого введення кадмію, та третя – група комбінованого введення хлориду кадмію, у дозі 2,0 мг/кг, разом з сукцинатом міді у дозі 0,1 мг/кг. Розчин хлориду кадмію був іонної форми, а розчин сукцинату міді – наноаквахелатної.

Показник внурішньоутробної виживаності найвищим був у групі контролю на 19-ту добу ембріогенезу (95,29%), та найнижчим у групі впливу кадмію хлориду на 19-ту добі (75,07%). Як показав аналіз отриманих результатів, спостерігається виражений ембріотоксичний вплив хлориду кадмію на процеси ембріогенезу, що виявляється достовірним підвищенням показників загальної ембріональної смертності, доімплантаційної та постімплантаційної смертності порівняно з контрольною групою на всіх досліджуваних термінах ембріогенезу.

Ключові слова: кадмій, купрум, ембріогенез, ембріотоксичність, ембріони.

EMBRYOTOXIC EFFECT OF CADMIUM CHLORIDE AND CUPRUM DURING THE ENTIRE PREGNANCY PERIOD IN WHITE RATS

Tymchuk K. M., Abramov S. V., Kryzhanovsky D. G., Fedchenko M. P., Filipenko V. V., Chernenko G. P., Myakushko V. A.

Abstract. Cadmium (Cd) is one of the most common and harmful transition metals present in our environment. In the modern world, the bodies of residents of urban areas are simultaneously affected by many harmful chemical compounds that are teratogenic, that is, harmful to human and animal fetuses. Pregnancy is a special physiological state, and prenatal development is a critical period in the etiology of pathological changes in embryogenesis. Therefore, the study of the influence of cadmium compounds on embryonic development is relevant today. Teratogenic drugs act during certain critical periods of ontogenesis, especially during embryogenesis.

The purpose of the work is to determine the effect of low doses of cadmium (2,0 mg/kg) and copper (0,1 mg/kg) on the general course of embryogenesis in rats with combined administration throughout the entire period of pregnancy of white rats.

To carry out the research, females with a dated pregnancy were obtained, for this we studied the estrous cycle of females by the method of vaginal smears. The solutions were administered through a probe, intragastrically, daily (once a day) from the first day of pregnancy: the first group – control, the second group – administration of a cadmium chloride solution at a dose of 2,0 mg/kg – the group of isolated cadmium administration, and the third – the group of combined administration cadmium chloride, at a dose of 2,0 mg/kg, together with copper succinate at a dose of 0.1 mg/kg. The cadmium chloride solution was of ionic form, and the copper succinate solution was of nanoaquachelate form.

The intrauterine survival rate was the highest in the control group on the 19th day of embryogenesis (95,29%), and the lowest in the group exposed to cadmium chloride on the 19th day (75,07%). As the analysis of the obtained results showed, there is a pronounced embryotoxic effect of cadmium chloride on the processes of embryogenesis, which is manifested by a significant increase in the indicators of total embryonic mortality, pre-implantation and post-implantation mortality compared to the control group at all studied terms of embryogenesis.

Key words: cadmium, copper, embryogenesis, embryotoxicity, embryos.

ORCID and contributionship:
Tymchuk K. M.: 0000-0003-0117-9033 [BDF]
Abramov S. V.: 0000-0002-7088-1865 [E]
Kryzhanovsky D. G.: 0000-0002-1595-9447 [D]
Fedchenko M. P.: 0000-0002-1655-2366 [E]
Filipenko V. V.: 0000-0003-2825-8137 [A]
Chernenko H. P.: 0000-0002-0667-7376 [C]
Myakushko V. A.: 0000-0002-3516-0106 [B]

Conflict of interest:
The Authors declare no conflict of interest.

Corresponding author
Tymchuk Kateryna Mykolaivna
Dnipro State Medical University
Ukraine, 49044, Dnipro, 9 V. Vernadsky str.
Tel: +380954001655
E-mail: rudenkohomerudenko@gmail.com

A – Work concept and design, **B** – Data collection and analysis, **C** – Responsibility for statistical analysis, **D** – Writing the article, **E** – Critical review, **F** – Final approval of the article.

Received 06.03.2022
Accepted 12.09.2022