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EXPERIMENTAL STUDY OF COPPER SUCCINATE ON EMBRYOLETHALITY OF CADMIUM CHLORIDE IN WHITE RATS

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In recent years, in connection with the intensive development of industry, there has been an increase in the content of heavy metals in the environment, which has a detrimental effect on the human body, animals and plants. One of the most dangerous ecotoxicants in the group of heavy metals is cadmium, which, due to its high migration capacity, tendency to bioaccumulation and polytropic action, poses a threat to living organisms, especially in regions with developed industry, which includes the Dnipropetrovsk region, because man-made accumulation in the environment is progressing at a high pace. Cd enters the body of humans and animals mainly in the following ways: through the gastrointestinal tract (alimentary), respiratory organs (inhalation) and the surface of the body (transcutaneous), from where this element is absorbed into the blood, and, having high cumulative properties, up to 20% accumulates in the small intestine.

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.

Females with a dated gestation period were obtained for the research, and the estrous cycle of the females was studied by the method of vaginal smears. The solutions were administered through a tube, intragastrically, daily (once a day) from the first day of pregnancy: Group I – control, Group II – administration of cadmium chloride solution at a dose of 2,0 mg/kg – group of isolated administration of cadmium, and Group III – 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. On the 13th and 19th days of pregnancy, females were slaughtered. 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.

Key words: *cadmium, copper, embryogenesis, embryos, small intestine.*

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 and Botany 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.

In recent years, in connection with the intensive development of industry, there has been an increase in the content of heavy metals in the environment, which has a detrimental effect on the human body, animals and plants [1]. One of the most dangerous ecotoxicants in the group of heavy metals is cadmium, which, due to its high migration capacity, tendency to bioaccumulation and polytropic action, poses a threat to living organisms, especially in regions with developed industry, which includes the Dnipropetrovsk region, because man-made accumulation in the environment is progressing at a high pace.

Cadmium (Cd) is the most widespread pollutant of the environment and has a toxic effect in small concentrations, has an acute and chronic effect on health. It accumulates throughout life, and has a long biological half-life in the human body from 10 to 30 years [2].

Cd enters the body of humans and animals mainly in the following ways: through the gastrointestinal tract (alimentary), respiratory organs (inhalation) and the body surface (transcutaneous), from where this element is absorbed into the blood, and, having high cumulative properties, up to 20% accumulates in the small intestine [3]. When entering the body, it has a high rate of penetration into various organs, is characterized by a polytropic toxic effect, biochemical activity and the ability to accumulate in a number of organs and tissues, disrupts metabolic processes and physiological functions, inducing processes of carcinogenesis, and is an antagonist of a number of vital micro- and macroelements. A feature of the harmful effects of this heavy metal is its rapid assimilation by the body and slow removal from it [2]. Cd also affects the transmembrane transmission of hormonal signals in cells and suppresses the hormonal function of the body. 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 mg/week.

Copper (Cu) is present in almost all food products, but in varying concentrations. According to WHO, the recommended dose for humans is 2-3 mg of copper per day (normally, about 30% of it is absorbed in the small intestine). Toxic doses of soluble copper salts when ingested are in the range of 100-500 mg (3-8 mg/kg of body weight). Absorption of copper from the gastrointestinal tract is carried out in ionic or amino acid-bound form. The processes of Cu absorption are interconnected by competitive dependence with Cd and Zn, which depend on the form of absorbed copper and the composition of the daily diet of humans or animals. Copper has pronounced

anti-inflammatory properties and bacteriostatic effect [4].

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 cadmium. Children are one of the risk groups for Cd accumulation in the body [5]. Therefore, an urgent task of modern morphological and medical research is to determine the effect of heavy metal compounds on organ systems and the course of embryogenesis and search for possible antagonists of their action.

The aim of the study.

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.

Object and research methods.

The experiment was carried out on 48 female rats of the Wistar line (Dali 2000 nursery, Kyiv), which were kept in a vivarium on a standard diet, weighing 180-300 g. Females with a dated gestation period were obtained for the research, and the estrous cycle was studied for this females by the method of vaginal smears. At the stage of estrus and proestrus, they were mated with intact males according to the 2:1 scheme. The presence of spermatozoa in vaginal smears indicates the first day of pregnancy in females. They were weighed to calculate the required amount of cadmium chloride injection.

The solutions were administered through a tube, intragastrically, daily (once a day) from the first day of pregnancy: Group I – control, Group II – administration of cadmium chloride solution at a dose of 2,0 mg/kg – group of isolated administration of cadmium, and Group III – 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. On the 13th and 19th days of pregnancy, females were slaughtered. The rat pups were removed from the uterus, they were weighed, their development was determined (up to the normal stage of development), a macroscopic examination was performed to detect external anomalies, they were photographed and fixed in a 10% formalin solution for further histological examination, and the number of corpora lutea of pregnancy was counted in the ovaries. The experiments were conducted in accordance with the Council of Europe Convention on the Protection of Vertebrate Animals Used for Scientific Purposes (Strasbourg, March 18, 1986). 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, $\mathrm{TEM} = \frac{B-A}{B} \times 100\%$,

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

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

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, $\mathrm{PIM} = \frac{B}{A+B}$,

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

The results of the research were processed by the method of variational statistics, their reliability was evaluated using the Student's test (t), the obtained data were considered reliably significant at $p<0,05$.

Research results and their discussion.

During the experiment, a negative effect of Cd compounds was found in both studied groups on the indicators of embryogenesis, on the 13th and 19th day of pregnancy. The conducted study showed that the fertility index in the experimental groups did not differ from the available literature data. Embryos of the control group met the criteria of embryonic development according to Hamburger and Hamilton, and corresponded to 16 stages. Massometric indicators of embryos of the studied period also demonstrated a delay in embryonic development, which did not have a significant difference from the control.

The total embryonic mortality (TEM) is a significant indicator in determining the embryotoxicity of substances. We calculated this indicator as the difference between the number of live embryos and the number of corpora lutea of pregnancy in the ovaries of female white rats at both stages of pregnancy. 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, we recorded the positive dynamics of reducing the influence of cadmium chloride under the influence of copper succinate by 52,17% and 32%, respectively **(fig. 1).**

A comparison of indicators of the effect of heavy metals on the body, namely cadmium chloride, shows its pronounced embryotoxic effect at a dose of 2.0 mg/kg on the processes of embryogenesis, which is a reliable increase in the total TEM compared to the control group, and positive changes were recorded in the group of combined introduction of cadmium chloride with copper succinates.

Table – Indicators of combustion of blood samples in the norm and in the experiment under the influence of cadmium chloride and copper succinate, on the 13th and 19th days of embryogenesis, (mcg/g)

Group	Metals	Period of pregnancy	Study samples
			Blood (mcg/g)
Control	Cd	13	0,026±0,003
		19	0,038±0,007
	Cu	13	0,880±0,083
		19	1,042±0,311
CdCl2	Cd	13	0,079±0,023
		19	0,116±0,005
	Cu	13	1,236±0,229
		19	1,180±0,160
CdCl2+Cu	Cd	13	0,068±0,002
		19	0,079±0,012
	Cu	13	1,490±0,316
		19	3,436±0,824

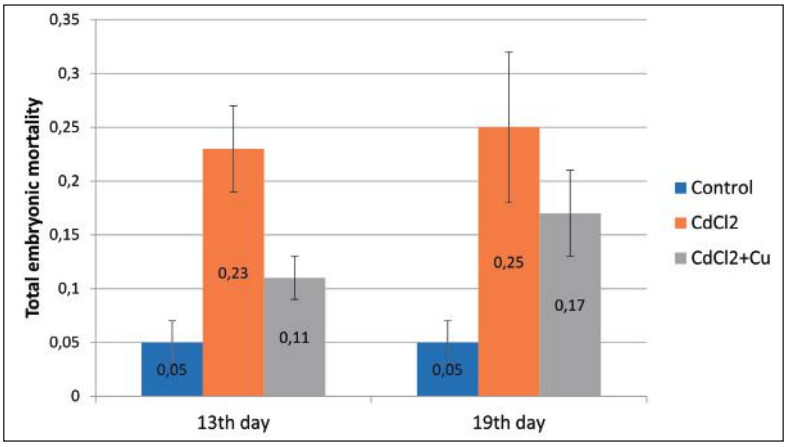

Figure 1 – The average rate of total embryonic mortality in experimental groups on the 13th and 19th day of embryogenesis.

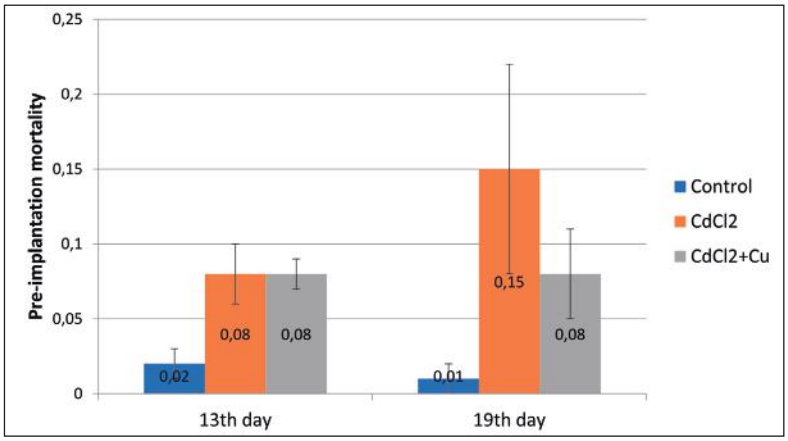

Figure 2 – Average rate of pre-implantation mortality in experimental groups on the 13th and 19th days of embryogenesis.

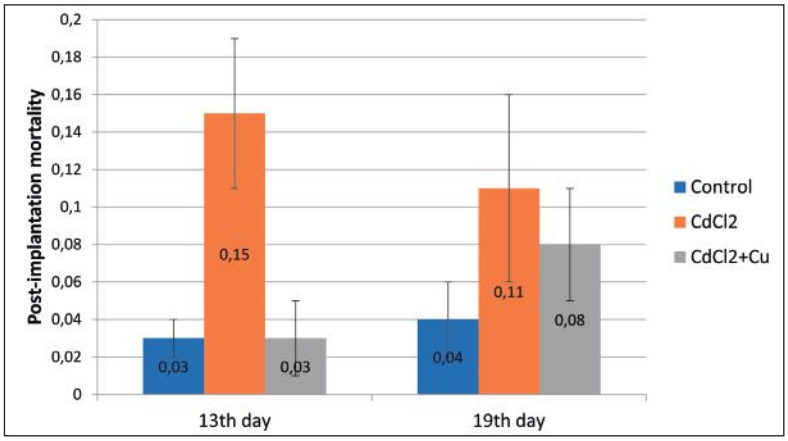

Figure 3 – The average rate of post-implantation mortality in experimental groups on the 13th and 19th days of embryogenesis.

Pre-implantation mortality (PIM) when exposed to cadmium on the 13th day of pregnancy increased 4,0 times and remained unchanged with the introduction of copper succinates, compared to the control group, and increased 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. 2).**

Post-implantation mortality, in the calculations of which the index of resorption of embryos after implantation was taken into account, significantly 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, in comparison with the group with independent administration of cadmium chloride. On the 19th day of pregnancy, there was 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 **(fig. 3).** 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.

When analyzing the results of the experiment, it is possible to notice an increase in the blood parameters of rats in the cadmium chloride group, when exposed to metals on the 13th and 19th days, respectively, and suppression of the effect indicators when the combined administration of cadmium chloride with copper succinates. In the control group, on the 13th day, a 3,0 times increase was noted under the action of the active agent, namely cadmium. Under the influence of copper, there is already 0,09 times decrease in the indicators of the influence of heavy metals. In the cadmium chloride groups, on the 19th day, a 3,0 times increase in indicators was recorded, compared to the control, and a 0,7 times decrease in indicators under the influence of copper. In the group of cadmium chloride and the active agent cuprum, on the 13th day, an increase in indicators was noted by 1,4 times compared to the control group, and when cuprum was administered to the group with cadmium chloride, the effect increased by 1,2 times. On the 19th day of embryogenesis, the effect of cadmium on the control group increased by 1,1 times, and when combined with copper succinates, the indicators increased by 2,9 times **(table).**

Conclusions.

The analysis of the obtained data indicates a pronounced embryotoxic effect of cadmium chloride on the processes of embryogenesis, which is 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 cadmium accumulation and conduct further histological studies of the small intestine in the organs of adult rats and embryos, after mediated exposure to cadmium and in combination of cadmium chloride with copper succinates, which will help to highlight changes at the tissue level and provide an opportunity to explain embryonic mortality rate.

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ЕКСПЕРИМЕНТАЛЬНЕ ДОСЛІДЖЕННЯ СУКЦИНАТУ МІДІ НА ЕМБРІОЛЕТАЛЬНІСТЬ ХЛОРИДУ КАДМІЮ У БІЛИХ ЩУРІВ

Тимчук К. М., Крижановський Д. Г., Трушенко О. С., Шевченко І. Ф., Жержова Т. А., Давиденко І. В., Коновалова О. С.

Резюме. В останні роки, у зв'язку з інтенсивним розвитком промисловості, в навколишньому середовищі спостерігається збільшення вмісту важких металів, що пагубно впливає на організм людини, тварин і рослин. В організм людини та тварин Cd потрапляє в основному такими шляхами: через шлунково-кишковий (аліментарний), органи дихання (інгаляційний) та поверхню тіла (транскутанний), звідки відбувається абсорбція цього елемента у кров, та, маючи високі кумулятивні властивості, до 20% накопичується в тонкій кишці. Кадмій (Cd) – найбільш поширений забруднювача довкілля та має токсичну дію в малих концентраціях, має гострий і хронічний вплив на здоров'я. Він накопичується протягом усього життя. Головну роль в плані природного біологічного бар'єру грає епітелій кишківника, який відображає здатність організму протистояти дії різних екзотоксикантів, в тому числі кадмію.

Метою дослідження 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 досліджували естральний цикл самиць методом піхвових мазків. На стадії еструсу та проеструсу парували з інтактними самцями за схемою 2:1. Розчини вводили через зонд, внутрішньошлунково, щоденно (раз на добу) з першого дня вагітності: I група – контроль, II група – введення розчину кадмій хлориду в дозі 2,0 мг/кг – група ізольованого введення кадмію, та III – група комбінованого введення хлориду кадмію, у дозі 2,0 мг/кг, разом з сукцинатом міді у дозі 0,1мг/кг. Розчин хлориду кадмію був іонної форми, а розчин сукцинату міді – наноаквахелатної. На 13-й і 19-й день вагітності самиць проводили оперативний забій.

Аналіз отриманих даних свідчить про виражений ембріотоксичний вплив хлориду кадмію на процеси ембріогенезу, що є достовірним підвищенням показників загальної ембріональної смертності, доімплантаційної і постімплантаційної смертності порівняно з контрольною групою, на всіх досліджуваних термінах ембріогенезу.

Ключові слова: кадмій, купрум, ембріогенез, ембріони, тонка кишка.

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

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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.

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