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CHANGE OF INDICATORS OF RAT EMBRYOTOXICITY UNDER ISOLATED INJECTION OF CADMIUM COMPOUNDS AND COMBINED WITH CERIUM

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Annotation. *In the scientific researches the general toxicity was determined, the peculiarities of metabolism were studied, the degree of carcinogenic, teratogenic, gonadotoxic, embryotoxic and mutagenic influence on the body of cadmium was established, but the scientific information concerning the influence on the general course of embryogenesis is extremely insufficient.*

Key words: *cadmium chloride, cadmium citrate, cerium citrate, embryogenesis, embryotoxicity.*

This study is a fragment of the interdepartmental planned scientific topic "Biological basis of morphogenesis of organs and tissues under the influence of nanometals in experiment" (state registration number 0115U004879), which was performed at the State Institution "Dnipropetrovsk Medical Academy of the Ministry of Health of Ukraine".

Modern functioning of the economy is accompanied by an increase in technogenic load.

Heavy metals are priority pollutants of atmospheric air, water of reservoirs and soils on a global and regional scale.

Due to their high migratory capacity, tendency to bioaccumulation and polytropicity, metals pose a risk to humans not only through direct action but also through a negative impact on the environmental health of the environment [1].

According to the literary data in the body of children is determined by the excess of biologically acceptable levels of a number of toxic metals, among which a significant place is the accumulation of cadmium [1], attributed to the second class of danger [2].

Cadmium and its compounds are widely used for the manufacture of nuclear reactor cores, chemical power sources, paints, colored glass; as a plastic stabilizer; in electroplating and in the automotive industry.

Very small amounts of cadmium are present in the body of any person. It is entering our body from the air and soil, that actively polluted by this metal and its compounds through human activity: tobacco smoke (tobacco stores cadmium well), food of plant origin (mushrooms, sunflower seeds, cereals, wheat, nuts), polluted air (combustion products of coal, diesel, galvanic, glass, cement production) [1,3].

In a case of excess admission of cadmium to the body, it adversely affects the liver, kidneys, central nervous system, reproductive organs, and in conditions of chronic exposure exhibits mainly nephrotoxic, immunotoxic and osteotoxic effects [1,4,5,6].

Like most heavy metals, cadmium has a high cumulative capacity: its half-life is 10-35 years.

Cadmium is deposited mainly in the kidneys (30-60%) and the liver (20-25%) [7,8].

Its action is related to the synthesis of the metallothionein protein in the body, which binds and transports cadmium ions [9].

Excess cadmium impairs the absorption and metabolism of a number of microelements: zinc, copper, selenium, iron [6,10,11].

A characteristic feature of heavy metals, including cadmium, after entering the body is their uneven distribution between cells and tissues and the ability to form a depot in the body, and secreted through the urinary tract, the mucous membranes of the digestive canal and various glands cause pathological changes in them [11,12].

The actual problem is the identification of substances or compounds that have the ability to reduce the adverse effects of heavy metals on humans and animals.

Nanotechnology has opened up new and promising areas in modern biology and medicine.

A promising area is the use of microelements in the form of carboxylates of food acids, especially in the form of citrates, which is a natural protective system against many toxicants.

Cerium nanoparticles have antihypoxic and antioxidant activity, that are essential during pregnancy and lactation, growth, development and normal functioning of the body.

Organic and complexing compounds of cerium. exhibit immunomodulatory, antitumor, antiviral, neuro-, cardio-, hepatoprotective, detoxifying, membrane-protecting effect, capable of increasing the life span of micro- and macro-organisms, affecting meiotic maturation of oocytes and follicles in the ovaries of aging mice [12,13,14].

The purpose of the article. The aim of this study was to determine the effect of low cadmium chloride and cadmium citrate on isolated administration and in combination with cerium citrate on the overall course of rats' embryogenesis.

As a biological test-object there were used mature female Wistar rats, at the beginning of the experiment exposed to research of estrous cycle by examining vaginal smears, at the stage of estrus matched with intact males by the scheme 2:1. The first day of pregnancy was determined by the presence of spermatozoons in vaginal smears, animals were divided into groups and administration of the test substances started: solutions of cadmium chloride, cadmium citrate and cerium citrate,, obtained by means of aquanotechnology (manufacturer – Research Institute of Nanobiotechnologies and resource of Ukraine, the city of Kyiv).

Materials and methods. The experimental part of the work was performed on 80 white pregnant female Wistar rats, which were divided into 5 groups of 16 animals in each: Group 1 (E № 1) - animals that were administered a solution of cadmium chloride at a dose of 1.0 mg / kg; Group 2 (E № 2) - animals that were administered a solution of cadmium citrate at a dose of 1.0 mg / kg; Group 3 (E № 3) - animals that were administered a solution of cadmium chloride at a dose of 1.0 mg / kg and a solution of

cerium citrate at a dose of 1.3 mg / kg; Group 4 (E № 4) - animals that were administered a solution of cadmium citrate at a dose of 1.0 mg / kg and a solution of cerium citrate at a dose of 1.3 mg / kg body weight of the animal, group 5 was control.

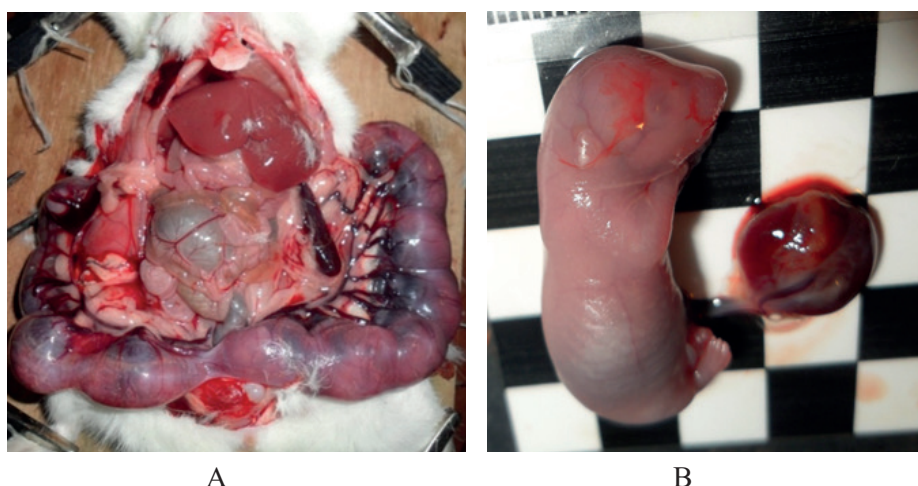
The solutions of the test substances were administered to the females intragastrically through the probe once a day, at the same time throughout pregnancy.

During the administration of solutions, the status and behavior of females, the dynamics of body weight, rectal temperature, and duration of pregnancy were registered.

In each group, the females were divided into 2 subgroups of 8 animals in each depending on the pregnancy period that was studied.

On the 13th and 20th day of pregnancy, an operative slaughter was performed.

At autopsy, the rats were removed from the uterus, tested for the live-dead test, weighed, determined the fetal development to be normal, performed macroscopic examination of the embryos to detect external abnormalities, photographed and fixated in 10% formalin for further histological examination. (Fig. 1)



A
B
Fig. 1 - Photograph of a two-sided uterus with the results of a pregnant female of a control (A) rat during surgery on the 20th day of pregnancy and (B) fetal placenta removed from the uterus.

In the ovaries were determined the number of yellow bodies of pregnancy, weight and size. (Fig. 2)

Investigation of the morphofunctional state of ovaries and their remodeling with defining changes of general course of rats' embryogenesis in terms of enteral injection of isolated cadmium chloride and cadmium citrate isolated and in combination with cerium citrate was conducted by means of: modeling, anatomical preparation, macroscopic examination, histological morphometric one, cytological and bio-statistical methods.

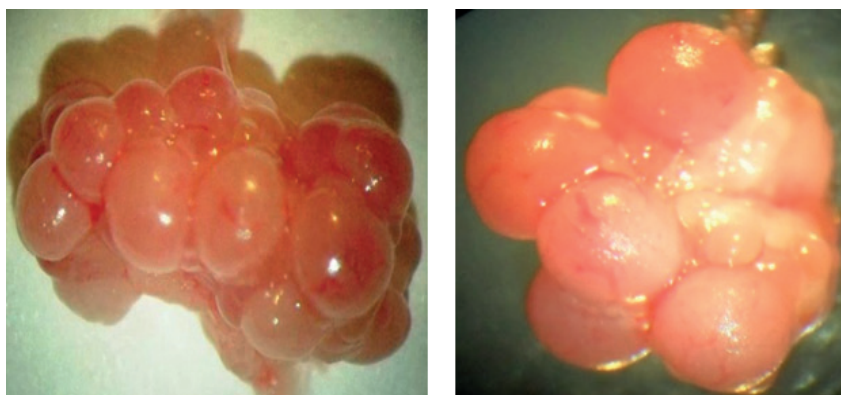


Fig. 2 - Ovaries of female rats of control (A) and experimental group # 1 (B) of the 20th gestation day. Reduction of the number of yellow bodies of pregnancy in study group # 1 can be observed with the isolated action of cadmium chloride.

Results and discussions. In the analysis of indicators of the number of yellow bodies per 1 female, it was found that on the 13th day of gestation there was an unreliable change of this indicator as follows compared with the control group: EN₂ (+ 4.9%) = EN₃ (+4.9%) > EN₄ (+ 1.9%) > EN₁ (- 9.7%). On the 20th day of prenatal development, indicators of the number of yellow bodies were arranged as follows in the order of decrease relative to the control group: EN₃ (+ 27,7%) > EN₄ (+ 9,9%) > EN₂ (+ 2,5%) > EN₁ (- 3.0%).

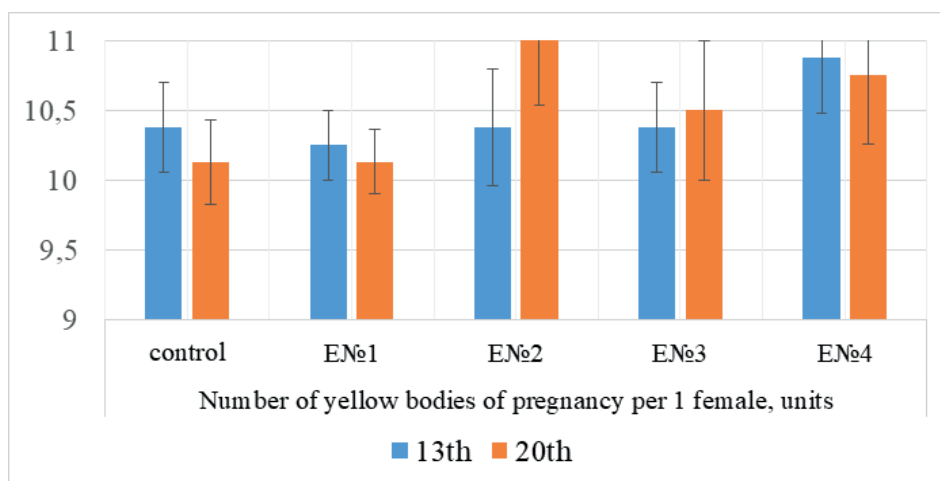


Fig. 3 - The number of yellow bodies in the ovaries of control and test rats

Indicators of embryotoxicity are generally accepted criteria: preimplantation (preimplantation, PMU) and postimplantation embryonic mortality (PEU), total embryonic mortality (TEM), morphological (anatomical) malformations, as well as the

overall delay in the development of fruits, which were calculated according to well-known formulas

The obtained results were processed by the method of variational statistics, their reliability was assessed using the Student's t test (t). The obtained data were considered to be significant at $p < 0.05$.

Analysis of the results of the experimental study revealed a negative effect of cadmium compounds on the embryotoxicity indicators and the number of alive fetuses in the females at the 13th and the 20th days of pregnancy ((Table 1, Fig. 4).

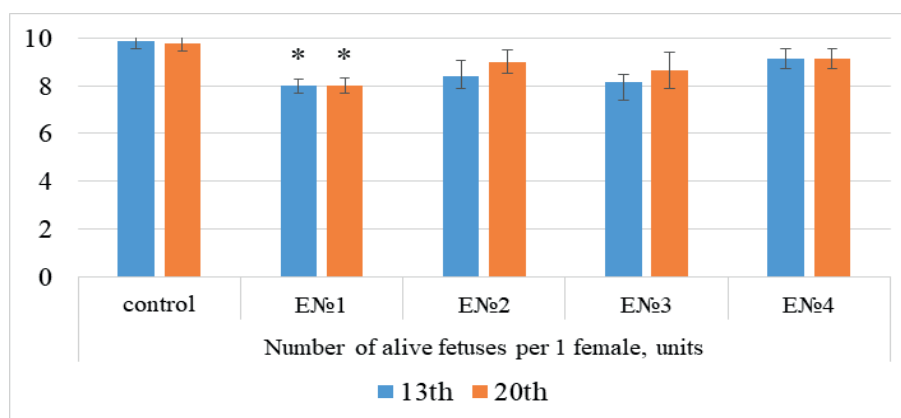


Fig. 4– Number of alive fetuses per 1 female, units

Table 1

Embryotoxicity indicators of control and experimental groups on 13th and 20th days of embryogenesis, (M ± m)

Indicator	Day	control	Study groups			
			ENo1	ENo2	ENo3	ENo4
Total embryonic mortality, % (TEM)	13	6,02 ± 1,71	26,83 ± 2,25***	17,86 ± 3,61**	20,48 ± 2,62***	17,24 ± 3,49*
	20	4,94 ± 1,81	20,24 ± 4,77**	15,60 ± 3,24**	18,22 ± 4,73*	14,82 ± 2,72**
Preimplant (preimplantation) mortality, unit (PMU)	13	0,02 ± 0,01	0,20 ± 0,03***	0,12 ± 0,03	0,13 ± 0,02**	0,12 ± 0,04*
	20	0,02 ± 0,01	0,14 ± 0,04*	0,11 ± 0,03*	0,12 ± 0,04*	0,10 ± 0,02**
Post-implantation mortality, units (PEU)	13	0,04 ± 0,02	0,09 ± 0,38	0,07 ± 0,31	0,08 ± 0,44	0,05 ± 0,33
	20	0,03 ± 0,38	0,07 ± 0,25	0,05 ± 0,29	0,07 ± 0,30	0,05 ± 0,32

Note. * - $p < 0.05$, ** - $p < 0.01$; *** - $p < 0.001$ compare to the control group

The number of alive fetuses per 1 female on the 13th day of pregnancy under the influence of cadmium chloride (EN₁) decreased by 23.1% ($p < 0.001$), and on the 20th day by 13.0% ($p > 0, 05$) compared to the control group. (Fig. 4).

At the same time, indicators of TEM, PMU, and PEU increased relative to the control group on the day 13: TEM 4.5 times ($p < 0.001$), PEU 10 times ($p < 0.001$), PIS 2.3 times ($p > 0, 05$) and on the 20th day of fruit development we observed increase of TEM indicators by 4.1 times ($p < 0.01$), PMU by 7.0 times ($p < 0.05$), PEU by 2.3 times ($p > 0.05$) compared to the control group ($p < 0.05$).

In the experimental group EN₂, isolated effects of cadmium chloride on the 13th day of embryogenesis relative to the control group, the indices of the number of living fruits per female decreased by 11.5% ($p > 0.05$), while the TEM indexes increased by 3.0 times ($p < 0.01$), PMU 6 times ($p > 0.05$), PEU 1.8 times ($p > 0.05$).

On the 20th day of fetuses development, there was a decrease in the number of alive fetuses per female by 4.0% ($p > 0.05$), but the TEM indexes increased 3.2 times ($p < 0.01$), the PMU in 5, 5 times ($p < 0.05$), PEU 1.7 times ($p > 0.05$) compared to the control group ($p < 0.05$).

Under the combined influence of cadmium chloride with cerium citrate (EN₃), the number of living fetuses per female decreased on the 13th (by 15.4% ($p > 0.05$)) and by the 20th day of embryogenesis (by 10.4% ($p > 0.05$)) compared to the control group.

Also, mortality rates increased by the 13th: TEM 3.4 times ($p < 0.001$), PMU 6.5 times ($p < 0.01$), PEU 2.0 times ($p > 0.05$), and by the 20th day of prenatal development: TEM 3.7 times ($p < 0.05$), PMU 6.0 times ($p < 0.05$), PEU 2.3 times ($p > 0, 05$) compared to the control group.

During analysis of the indexes of embryotoxicity in the experimental group No. 4 combined introduction of cadmium citrate with cerium citrate compared with the control group found a decrease in the number of alive fetuses per female on the 13th day (by 7.7% ($p > 0,05$)) and 20th day of embryogenesis (5.2% ($p > 0.05$)).

On the contrary, embryo-mortality rates increased as at the 13th day of gestation: TEM 2.9 times ($p < 0.05$), PMU 6.0 times ($p < 0.05$), PEU 1.3 times ($p > 0.05$) and on the 20th day of prenatal development: TEM 3.0 times ($p < 0.01$), PMU 5.0 times ($p < 0.01$), PEU 1.7 times ($p > 0.05$) compared to the control group.

Conclusions. The analysis of the obtained results indicates a pronounced embryotoxic effect of cadmium compounds on the processes of embryogenesis, which is a significant increase in total embryonic mortality compared with the control group in both embryogenesis timeframes, especially in the experimental groups of isolated administration of cadmium compounds: chloride EN₁ - 13th day (+ 345,7%) and 20th day (+ 309,7%) and citrate EN₃ (13th day (+ 309.7%) and the 20th day (+268.8%)), especially due to an increase in pre-implantation mortality in EN₁ (10.0 times on the 13th day and 7.0 times on the 20- and day 3) and in EN₃ (6.5 times on the 13th day and 6.0 times on the 20th day).

At the same time, the number of alive fetuses per 1 female in the isolated cadmium chloride (EN₁) and cadmium citrate (EN₃) groups decreased by 23.1% at the 13th

day of embryogenesis and by 15.4% at the 20th day (EN₁) and 13.0% and 10.4% respectively (EN₃) compared to the control group.

More pronounced embryotoxic effect was found in the cadmium chloride isolated action group.

At the same time, the group of cadmium chloride combined with cerium citrate showed improvements in all indicators of embryonic development relative to the cadmium chloride isolated effect group.

Thus, PMU indices were painted by 23.7%, by the 13th day - by 10.0%, by the 20th day, by 35% and by 14.3%

Similar changes in the studied parameters occurred in the group of combined effects of cadmium citrate with cerium citrate, indicating the modifying effect of cerium citrate on the toxicity of cadmium compounds.

In our opinion, it is promising to study the effect of cadmium compounds with metal citrate on organogenesis at histological level.

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