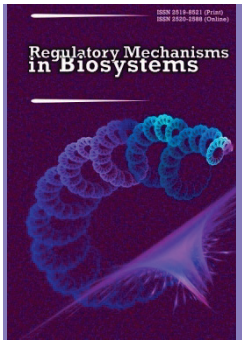

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Toxicity of cadmium salts on indicators of embryogenesis of rats

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Cadmium is a toxic heavy metal which is considered a dangerous environmental pollutant and has a detrimental effect on the organs of the reproductive system, the period of implantation and the development of embryos. The experiment presented in this article established the effect of cadmium salts (chloride and citrate) on the general progress of embryogenesis. For this purpose, 60 rats were randomly divided into three groups: control, experimental group with cadmium chloride exposure and experimental group with cadmium citrate exposure. Cadmium chloride solvent, cadmium citrate solvent at a dose of 1.0 mg/kg and distilled intragastric water were injected from the first to the thirteenth (first subgroup) and from the first to the twentieth days of embryogenesis (second subgroup). When cadmium chloride was injected, total embryonic (by 4.24 and 3.67 times), pre-implantation (by 6.50 and 14.03 times) and post-implantation mortality (by 3.07 and 2.49 times) increased with a reduction of the number of surviving fetuses by 24.0% and 25.9% compared with the control group on the 13th and 20th days of embryogenesis respectively. At the same time, during exposure to cadmium citrate, indicators of total embryonic mortality increased by 4.02 and 3.52 times, pre-implantation mortality by 6.04 and 13.03 times, and post-implantation mortality by 3.09 and 2.26 times, and indicators of the number of live fetuses decreased by 18.3% and 22.2% in relation to the control group. When determining the accumulation of cadmium in embryos on the 20th day of gestation, polyelement analysis of biological materials using the atomic emission method with electric arc atomization revealed a 15.83-fold increase in cadmium chloride and 9.00 times in cadmium citrate relative to the control group. Embryolethality rates increased in animals of both experimental groups while the number of live fetuses per female decreased, which indicated an obvious embryotoxic effect of cadmium compounds. It is would be useful to conduct histological studies, which will help detect changes at the tissue level and possibly explain the level of embryonic mortality.

Keywords: cadmium chloride; cadmium citrate; embryotoxicity of cadmium; total embryonic mortality; pre-implantation mortality.

Introduction

In connection with the growing pollution of the environment as a result of anthropogenic influence, the relevance of studying the effects of toxic substances on pregnant women and the developing fetus is increasing in medical practice (Johnston et al., 2014; Röllin et al., 2015; Jeong et al., 2017; Liang et al., 2021). The third place after poisoning by pesticides and nitrates is occupied by poisoning by heavy metals (cadmium, lead, mercury and others) – which are the main anthropogenic pollutants of the environment (Wai et al., 2017; Kozak & Brygadyrenko, 2018; Gull et al., 2018; Gutyi et al., 2019; Gutyj et al., 2019; Bashchenko et al., 2020; Genchi et al., 2021). The main natural source of cadmium entering the atmosphere (10–15%) is volcanic and human activity. Anthropogenic sources of cadmium pollution of air, soil, and water include local emissions associated with industrial complexes that produce or use Cd, diffuse scattering over the ground by sources of varying degrees of power, ranging from thermal power plants and motors to mineral fertilizers, cigarette smoke, as well as waste dumps containing this metal and production facilities for their utilization (Rehman et al., 2018).

Cadmium is used in the production of galvanic cells, in the manufacture of ceramics, in electrogalvanization and as a pigment in paints and plastics, while cadmium-nickel alloys are used in the production of accumulators and batteries, fertilizers, cigarettes, X-ray screens, kinescopes (Parizi et al., 2021). Cadmium can be obtained as a by-product during the refining of copper, lead and zinc, in which context mankind has polluted the environment with cadmium during mining of these metals for several centuries. Cadmium easily enters the environment with wastewater, contaminated fertilizers and atmospheric air, easily accumulates and is transmitted through food chains, entering the human body (Trakhtenberg, 2018). A significant amount of cadmium enters the body with food and water through the gastrointestinal tract and respiratory tract with air, as well as during smoking (Trouiller-Gerfaux et al., 2019, Repic et al., 2020). Even small doses of cadmium are dangerous, which over a long time accumulate to toxic levels, since cadmium has a long half-life (10–30 years) and the use of contaminated products for a long time can lead to one or another form of cadmium intoxication: damage to the respiratory organs (bronchitis, tracheitis, pneumonia, pulmonary edema, hemoptysis, etc.), gastrointestinal tract (gastritis, duodenitis, dyspeptic disorders in the form of nausea and vomiting, diarrhea with an admixture of blood), cardiovascular diseases, anemia associated with impaired iron absorption in the intestines, fragility of the skeleton, suppression of the immune system, kidney dysfunction (protein, glucose, and hyperaminoaciduria), fatty degeneration of the liver (Zhang et al., 2019). A fatal outcome is possible. From a prognostic point of view, pneumonia, pulmonary edema and nephropathy with signs of irreversible damage to the renal tubules are considered the most unfavourable due to the toxic effect of Cd. In most cases, the accumulation of cadmium in the soft tissues of organs does not affect their activity until the metal content reaches a critical level (Genchi et al., 2020; Lv et al., 2021; Qing et al., 2021).

Cadmium is strongly accumulated in cases of insufficiency of zinc, iron and selenium and aggravates the deficiency of these trace elements, causing dyselementoses (Ishitobi & Watanabe, 2005; Wang et al., 2016; Nefiodova et al., 2017; Skalny et al., 2019), which have recently been associated with an increase in the number of infertile marriages, especially among the population living in ecologically unfavourable areas (Oberlys et al., 2008).

The mechanism of the toxic effect of cadmium, as a polytropic toxin, is associated with its ability to inhibit numerous enzymes, blocking their

sulfhydryl, carboxyl, and amine groups. At a high concentration of Cd, their irreversible inhibition may occur, although the body has a significant reserve of protection (Oberlys et al., 2008). According to the chemical properties surrounding zinc, it is able to replace them in biochemical reactions, which means that it acts as a pseudo-inducer or pseudo-inhibitor of zinc-containing enzymes (Khopta et al., 2017; Skalny et al., 2019).

Heavy metals, and in particular cadmium, can lead to hormonal disorders in women, prevention of ovulation and fertilization, spontaneous abortions, early and late toxicosis of pregnant women, development of abnormalities in children (Jeong et al., 2017; Zhu et al., 2018; Liang et al., 2021). Also, cadmium (Cd) is an embryotoxic, genotoxic and teratogenic metal for various species of animals. When present in the blood of the mother, it can cause disruption of the placental function and the transport of nutrients to the fetus due to the creation of reactive oxygen species (ROS) and reactive nitrogen species (RNS), which cause oxidative stress by reacting with macromolecules and damaging them (Thompson & Bannigan, 2008; Kippler et al., 2012; Palmieri et al., 2019).

However, despite the variety of scientific publications devoted to the study of the influence of cadmium compounds on the postnatal development of humans and animals, the influence of this metal on embryogenesis has not been sufficiently studied. The aim of the study was to test the truthfulness of the hypothesis that suggests that the injection of cadmium salts to pregnant female rats even in low doses will lead to an increase in embryolethality, a decrease in the number of fetuses and the intrauterine survival rate. To achieve our goal it was necessary to observe the changes in these indicators and the accumulation of cadmium in the tissues of the embryos under conditions of intragastric injection of cadmium salts to female rats during the first half and the whole period of pregnancy.

Materials and methods

All procedures with animals at all stages of the research were carried out in compliance with the generally recognized bioethical principles of the "three Rs", as well as the provisions of the Helsinki Declaration of the World Medical Association (2000), the Council of Europe Convention on Human Rights and Biomedicine (1997), the relevant provisions of the WHO, the International Council of Medical Scientific Societies, International Code of Medical Ethics (1983), "General Ethical Principles of Experiments on Animals", approved by the First National Congress on Bioethics (Kyiv, 2001) according to the provisions of the "European Convention for the Protection of Vertebrate Animals, which are used in experiments and other educational purposes" (Strasbourg, March 18, 1986), "Regulations on the Committee on Ethics (Bioethics)", 2012. It was established by the Bioethics Commission of the Dnipropetrovsk State Medical University (protocol No. 1 of February 10, 2020) that the conducted scientific studies of ovaries and fetuses of experimental animals met the ethical requirements in concordance with the order of the Ministry of Health of Ukraine No. 231 dated November 1, 2005.

The rats were kept in the vivarium of the Dnipro State Medical University under natural lighting, with free access to water and food, feeding was performed in accordance with the norms of the Institute of Nutrition of the Academy of Medical Sciences of Ukraine, that are intended for this species of animals. The experiment was conducted in a ventilated room at an air temperature of 20–25 °C and a relative humidity of 50–65%, and animals were kept in standard plastic cages with no more than 3–4 individuals in each. Taking into account the circadian biorhythms of the regulation and functioning of the female reproductive system, and the associated fluctuations in the intensity of metabolic processes in body tissues, all manipulations (injection of distilled water, isolated cadmium chloride and cadmium citrate, removal of animals from the experiment) were carried out at the same time of the day – from 11 a.m. to 12 p.m. Vaginal swabs were taken at a fixed time of day (9:00 a.m.).

Before the start of the experiment, all rats were examined, weighed; their age, motor activity, skin condition were taken. Sick animals were not included in the experiment.

The rats of the control and experimental groups were kept in identical conditions, which is necessary for the creation of a structural group, and the material taken for research was studied in parallel. At the preparatory stage before carrying out the experiment 60 female rats with an initial weight of 150–180 g, aged 2.5–3.0 months were examined for the estrous cycle by the method of vaginal smears to obtain females with a fixed gestation period.

To solve the tasks, females with a stable rhythm of the estrous cycle in the stages of proestrus and estrus (when fertilization is possible) were mated with intact males according to the 2:1 scheme. The first day of pregnancy was determined in normally cycling females by the presence of spermatozoa in vaginal smears.

The main task of the experimental simulation of the effect of solutions of cadmium compounds (chloride and citrate) at a dose of 1.0 mg/kg was to obtain the necessary data for conducting a comparative analysis. In order to bring the experiment as close as possible to natural conditions, we chose the oral route of administration in accordance with methodological recommendations for the study of embryotoxicity of metals – using an intragastric probe, as due to technogenic pollution of the environment and toxicity for living organisms, cadmium and its compounds can accumulate in the body of humans and animals. Cadmium-containing compounds can lead to multi-organ damage in a woman's body even at low concentrations, especially it can lead to damage to the reproductive system, affect the quality of gametes, fertilization and the course of pregnancy, can penetrate the placental barrier and affect the development of the fetus according to the literature and the results of our own research.

Low doses of metals, which reflect their real ratio in the daily rations of women, including pregnant women in industrial regions, were chosen for the research. The dose of cadmium-containing compounds equates to 1/100 of the LD_{50} for cadmium (Fedorenko, 2019).

Modeling of the effect of cadmium salts on changes in rat embryogenesis was carried out according to the following plan. The selection of female rats for the control and experimental groups was carried out in a random order with the formation of groups of animals homogeneous in average weight. All rats were divided into three groups of 20 animals each:

– group of cadmium chloride exposure – animals that were injected with an isolated solution of cadmium chloride dose of 1.0 mg/kg;

– group of cadmium citrate exposure – animals injected with a solution of cadmium citrate dose of 1.0 mg/kg;

– control group – animals that were injected with distilled water.

In each group were 20 pregnant females (n = 20), of which 10 were injected with an solution of cadmium salt from 1st to 13th day of gestation, while the other 10 were injected with an solution of cadmium salt from 1st to 20th day of gestation.

The number of repetitions in the experiment and the control was enough to conduct a valid statistical analysis of the results (10 repetitions).

Female rats were exposed to chemical factors from the 1st to the 12th or 19th day of pregnancy, respectively, surgical slaughter was performed on the 13th or 20th day of pregnancy. Adhering to the humane principles of animal treatment, they were removed from the experiment by means of overdose with ether anesthesia, and operations were performed to remove fetuses from the uterus for further research. To carry out macroscopic, morphological and morphometric studies during the operation of pregnant females, the internal organs, uterus, ovaries, correspondence of the number of fetuses in each part of the bicornuate uterus were carefully examined and the data were recorded in the protocol.

The embryotoxic effect of the studied substances was evaluated according to the following indicators: fertility index, total embryonic mortality (TEM), pre-implantation mortality (PIM), post-implantation mortality (PIM), number of fetuses per one female, survival index.

In addition, we determined the gradient of cadmium accumulation in the tissues of embryos of experimental rats with the isolated introduction of cadmium salts (chloride and citrate) using polyelement analysis of biological materials by the atomic emission method with electric arc atomization. Measurements of the samples were performed at the State Enterprise "Ukrainian Research Institute of Transport Medicine of the Ministry of Health of Ukraine (Odesa) in accordance with the agreement on scientific and technical cooperation of the "DMA" Medical Center of the Ministry of Health of Ukraine (2018). Preparation of samples and measurement of metal content were carried out according to the state standards of Ukraine (No. 30823-2002). Quantitative measurement of metal content in samples was carried out on an Emas-200 CCD atomic emission spect-

rometer (verified on 30.11.2017, verification certificate 4706-FG). The current strength in the arc in all cases was 15A, the size of the optical gap of the device when measuring cadmium and zinc was equal to 0.4. Quantitative determination of cadmium in the analyzed objects was carried out at a wavelength of 228.802 nm, zinc at 213.856 nm.

During our experiments, we analyzed the accumulation of cadmium as a marker of cadmium intoxication and the level of the trace element zinc, as an element that has pronounced bioantagonistic properties in relation to cadmium and, according to the literature, its balance is primarily disturbed in case of cadmium intoxication (Pykhteeva et al., 2013; Shafran et al., 2014; Pykhteeva & Bolshoi, 2015).

The term of embryogenesis – the 20th day and it was not chosen by chance: it is precisely this term that demonstrates the result of long-term exposure to cadmium intoxication.

The process of measuring the level of accumulation of metals took into account the peculiarities of preparing samples for research. According to the state standards of Ukraine, the samples are not subject to fixation, so some of the 20-day-old embryos was frozen to measure the metal content and compare the levels of cadmium accumulation in the embryos of the experimental groups compared to the control group. Statistical processing of the results was calculated using the software product Statistica 6.1 (StatSoft Inc., Tulsa). The results were presented as the mean ± standard error of mean (x ± SE), n – number of experiments. For the analysis and interpretation of the obtained results, before the application of statistical criteria, the hypothesis about the normal distribution of random variables was tested (according to the Kolmogorov-Smirnov and Shapiro-Wilk criteria). Data were analyzed using Kruskal-Wallis nonparametric one-way analysis of variance followed by the Mann-Whitney U-test. P-values lower than 0.05 were considered statistically significant.

Results

During the operation on the female rats of the experimental groups, the fetuses were removed from the uterus together with the placenta, without disturbing the umbilical cord, they were examined externally for the presence of visually noticeable developmental anomalies, the colour of the integuments, and the appropriateness of the stage of normal development was determined according to generally accepted criteria: the formation of eyelids, auricles, laying of vibrissae, limb development, etc., massometric indicators were taken (Fig. 1, 2).

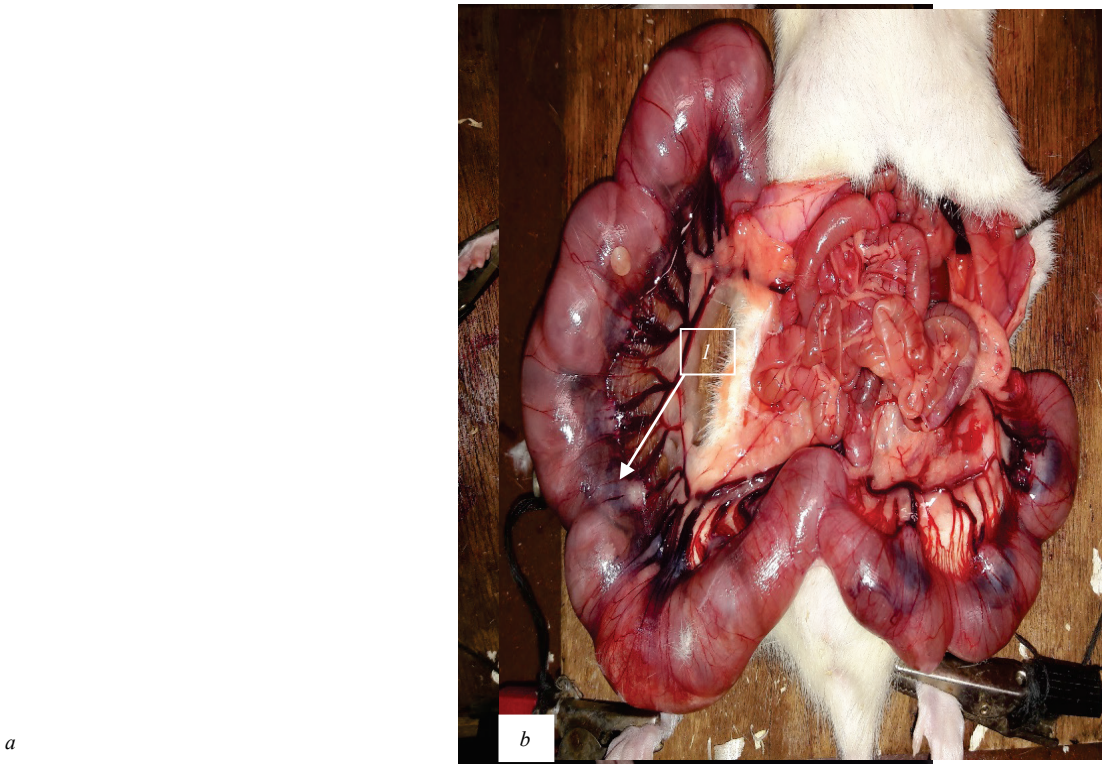

Fig. 1. Pregnant female rats of the control group on the 13th day (*a*) and 20th (*b*) day of the experiment during surgery: *1* – places of implantation are clearly visible

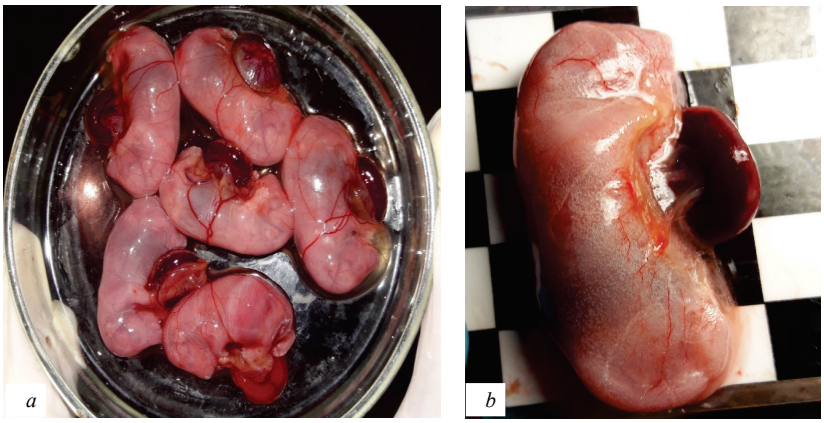

Fig. 2. Fetuses removed from the uterus with placenta on the 13th day of the experiment (*a*) and embryos on the 20th day (*b*) of the control group during surgery, the photo of the embryo was taken on a calibration surface for size comparison (length of the square wall = 10 mm)

All fetuses of the control group in our experimental studies met the standard criteria for the development of rat fetuses and were used as a standard for comparing the studied stages of development of rats in the experimental groups.

During the operation on female rats who were injected solutions of cadmium salts during pregnancy, asymmetry of the location of the fetuses (in one corner of the uterus) was observed: when exposed to 3.0% cadmium chloride, and when exposed to 2.3% cadmium citrate, and resorption of fetuses at both stages of development (Fig. 3, Fig. 4). When studying the embryotoxicity of cadmium chloride and cadmium citrate on the progress of embryogenesis, the extracted fetuses were studied under a MBS-2 binocular magnifier and photographed under the same magnifier at different magnifications.

We did not detect the formation of external mutilations, which means that the tested compounds do not have a teratogenic effect in the indicated doses and method of administration.

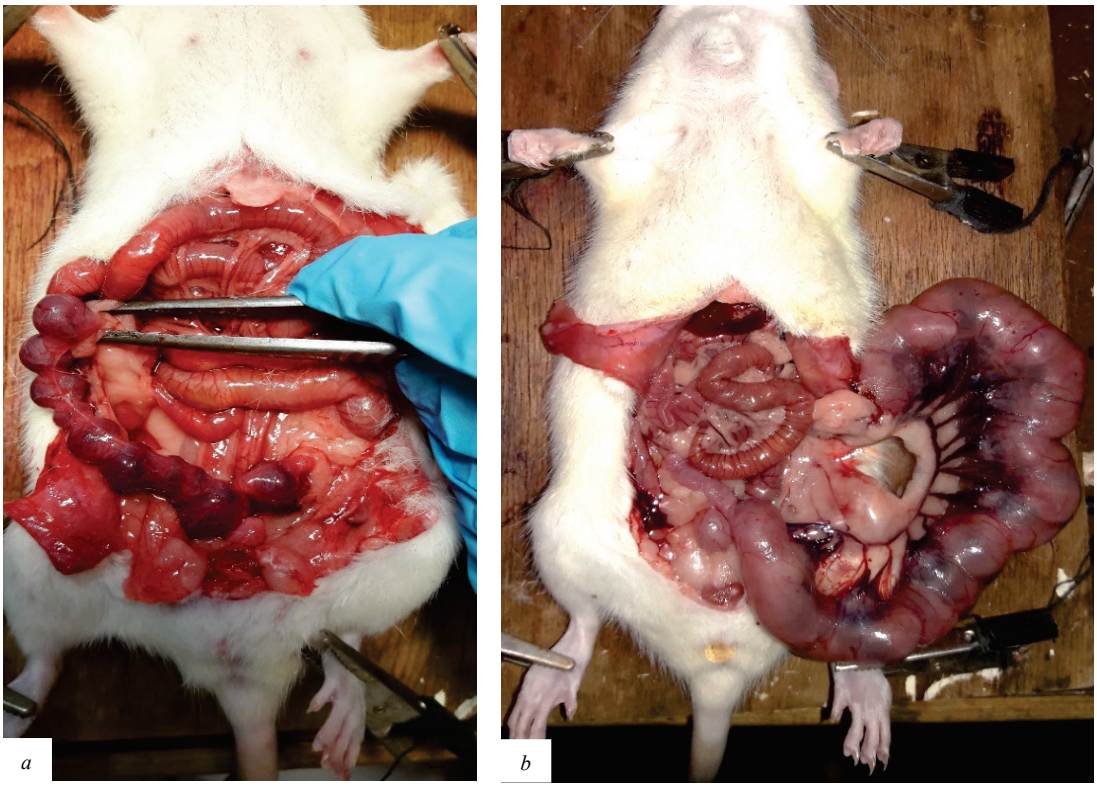

Fig. 3. Pregnant female rats of the group exposed to cadmium chloride (*a*) on the 13th day of the experiment and cadmium citrate (*b*) on the 20th day of the experiment during dissection, the location of the fetuses in one corner of the uterus is clearly visible

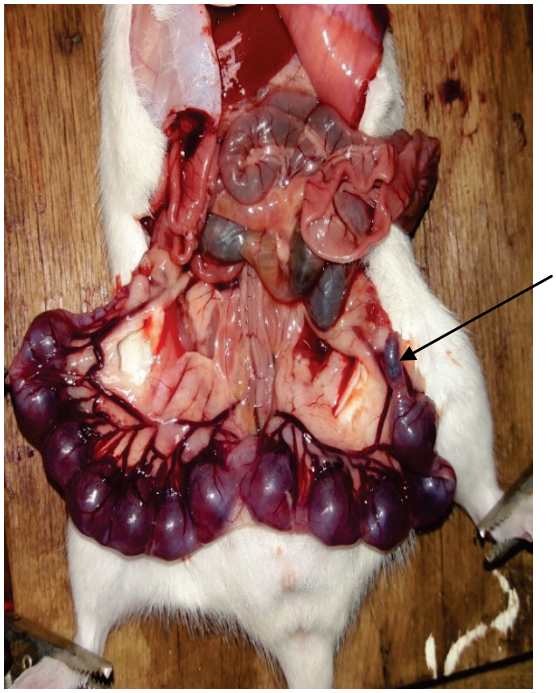

Fig. 4. Pregnant female rat of the group exposed to cadmium chloride on the 13th day of the experiment during dissection, the arrow indicates resorption of the fetus

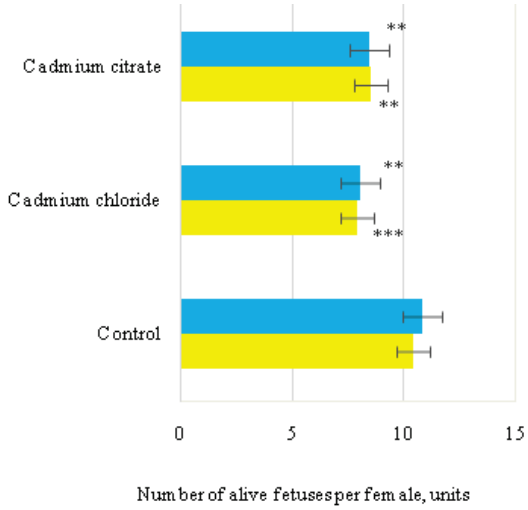

Fig. 5. Average indicators of the number of live fetuses per female: blue colour – the 13th day of embryogenesis, yellow colour – the 20th day of embryogenesis (x ± SE, n = 10 in each subgroup, units), the symbol (**) indicates statistical difference from control ($P < 0.01$), symbol (***) indicates statistical difference from control ($P < 0.01$) by Mann-Whitney U-test, group of cadmium chloride exposure and group of cadmium citrate exposure on the 13th day are compared with control group on 13th day, group of cadmium chloride exposure and group of cadmium citrate exposure on the 20th day of embryonic development are compared with the control group on 20th day

Analysis of the experimental results revealed a negative effect of cadmium chloride on embryogenesis indicators both on the 13th and 20th days of pregnancy (Fig. 5). The calculation of the average values in the experimental groups showed that the indicators of the number of live fetuses per female on the 13th day of pregnancy in the group exposed to cadmium chloride decreased by 24.0%, and in the exposure group with cadmium citrate by 18.3%, and on the 20th day: by 25.9% (cadmium chloride) and 22.2% (cadmium citrate) respectively in relation to the control group (Fig. 5). In the group exposed to cadmium citrate, the indicator of the number of live fetuses per female increased unreliably by 7.6% on the 13th day, and by 1.3% on the 20th day of embryogenesis compared to the group exposed to cadmium chloride. The conducted studies determined that in both experimental groups of isolated injection of cadmium salts, the indicators of accumulation of cadmium level in embryos on the 20th day of gestation significantly increased by 15.83 times (cadmium chloride) and 9.00 times (cadmium citrate) relative to the control group (Fig. 6a). At the same time, the accumulation of zinc in experimental groups of isolated administration of cadmium salts differed: in the group exposed to cadmium chloride, the level of zinc significantly increased by 1.32 times, and under the influence of cadmium citrate it decreased by 0.70 times compared to the control group (Fig. 6b).

According to the results of this study, the lowest average values of the number of yellow bodies of pregnancy per 1 female were observed in the group of isolated exposure to cadmium chloride, both on the 13th day (10.00 ± 0.27) and on the 20th day of embryonic development (10.30 ± 0.27) respectively, but had no significant difference (Fig. 7).

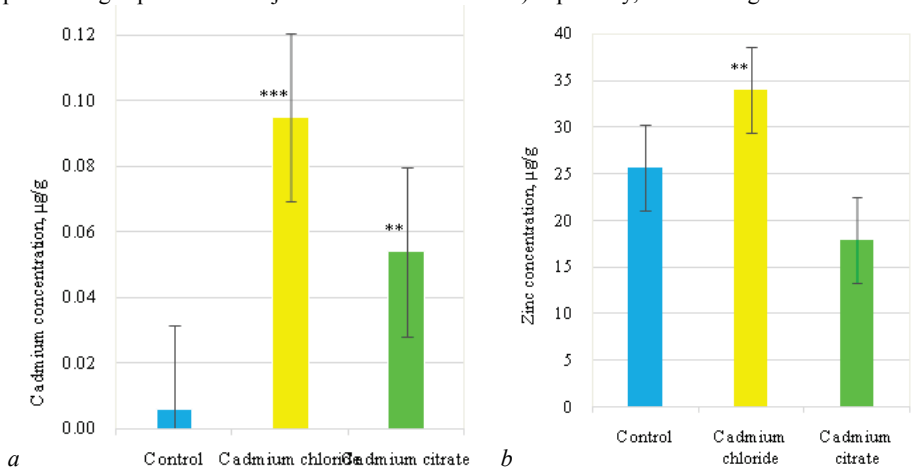

Fig. 6. Indicators of the level of accumulation of cadmium (*a*) and zinc (*b*): bue colour – rats of the control group, yellow colour – rats that received a solution of cadmium chloride, green colour – rats who were injected with cadmium citrate solution (x ± SE, n = 10 in each subgroup, µg/g); the symbol (**) indicates statistical difference from control (P < 0.01), symbol (***) indicates statistical difference from control (P < 0.01) by Mann-Whitney U-test, group of cadmium chloride exposure and group of cadmium citrate exposure on the 20th day of embryonic development are compared with the control group on 20th day

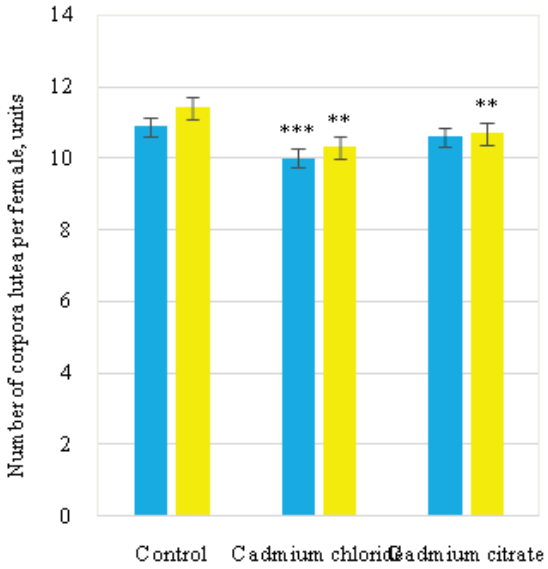

Fig. 7. The average number of corpora lutea per female: see Fig. 5

The analysis of the obtained results indicates an obvious embryotoxic effect of cadmium chloride and cadmium citrate in doses of 1.0 mg/kg on the processes of embryogenesis, which is evidenced by a significant increase in total embryonic mortality (TEM) compared to the control group at both studied periods of pregnancy (Fig. 8). Thus, by the 13th day of embryogenesis, this indicator in the group exposed to isolated cadmium chloride had increased by 4.24 times and under the influence of cadmium citrate by 4.02 times, and on day 20 day of embryonic development was higher by 3.67 times and 3.52 times, respectively, compared to the control group (Fig. 8).

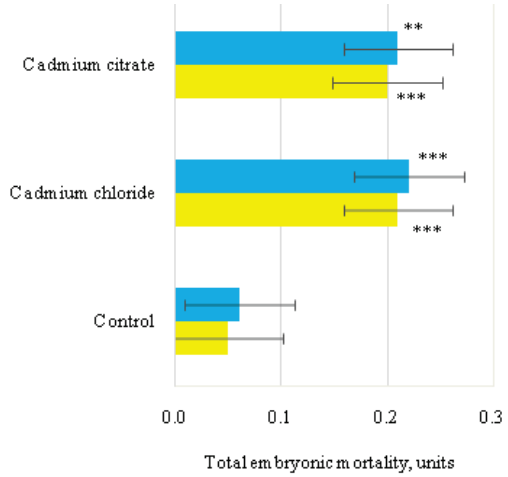

Fig. 8. Average indicators of total embryonic mortality: see Fig. 5

Such results are explained by a reduction in both pre-implantation and post-implantation mortality in these groups (Fig. 9). In the cadmium chloride exposure group, by the 13th day of embryogenesis, the pre-implantation mortality rate had increased by 6.50 times, and the post-implantation mortality rate had increased by 3.07 times, and on the 20th day of embryonic development the pre-implantation mortality score was 14.03 times higher, and the post-implantation score was 2.49 times less reliable than the control group. At the same time, in the group exposed to cadmium citrate on the 13th day of embryogenesis, the pre-implantation mortality rate was shown to have increased by 6.04 times, and the post-implantation mortality rate to have increased by 3.09 times, and on the 20th day of embryonic development, the pre-implantation mortality indicator was 13.03 times higher, and the the post-implantation mortality indicator was 2.26 times higher than in the control group.

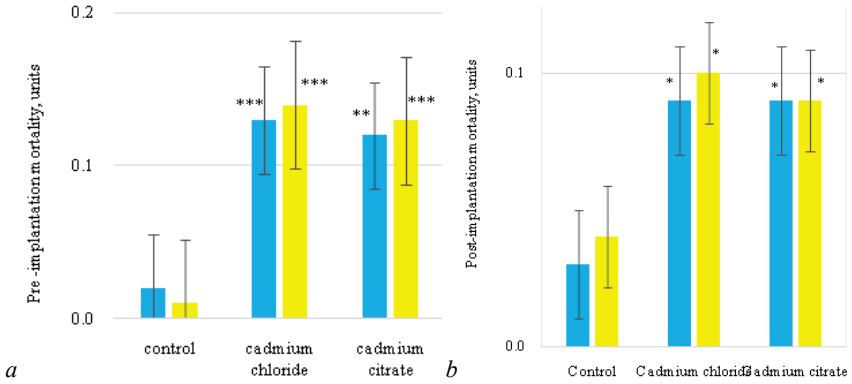

Fig. 9. Average indicators of pre-implantation mortality (*a*) and post-implantation mortality (*b*): see Fig. 5

The intrauterine survival rate was the highest in the control group both on the 13th day (95.4%) and on the 20th day of embryogenesis (94.4%). In the experimental groups, this indicator decreased on both studied terms of embryogenesis compared to the control group on the 13th day: by 17.1% (cadmium chloride exposure group, P = 0.0002) and by 16.3% (cadmium citrate exposure group, P = 0.005), and on the 20th day: by 17.9% (cadmium chloride exposure group, P = 0.0004) and by 16.7% (cadmium citrate exposure group, P = 0.0004).

Thus, the analysis of the received results indicates an obvious embryotoxic effect of cadmium salts (chloride and citrate) at a dose of 1.0 mg/kg 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 in both studied groups during embryogenesis.

Discussion

Salts of heavy metals, the amount of which is constantly increasing in the ecosystem, attract the special attention of researchers. Recently, there has been an expansion of the scope of use of various cadmium compounds and a significant increase in the anthropogenic contribution to their environmental pollution, which has led to excesses over the maximum permissible amount of cadmium in many countries and areas in food, water and air.

As cadmium compounds are poorly excreted from the body and accumulate for a long time in the blood, kidneys and liver, they have a negative effect on the body, both during embryogenesis and during the postnatal period. It was experimentally established that exposure to low doses of cadmium in early childhood led to a decrease in the coefficient of mental development of children (Kippler et al., 2012; Chatterjee & Kortenkamp, 2022). As for adults, cadmium disrupts DNA repair mechanisms and affects certain phases of the cell cycle and apoptosis (Vandegehuchte & Janssen, 2011; Severson et al., 2012).

Recent studies indicate that cadmium is able to induce epigenetic changes in mammalian cells, which play an important role in the development of various cancers and chronic diseases (Patkin & Sofronov, 2012). At the same time, changes in the embryonic period affect cellular plasticity, thereby influencing susceptibility to cardiovascular diseases, type 2 diabetes, obesity and other chronic diseases in adults, including the reproductive system (Weaver et al., 2005; Waterland et al., 2006; Anway et al., 2008). Thus, changes related to the violation of the blood-testicular barrier and oxidative stress were detected in the testes of adult mammals (Siu et al., 2009). In *ex vivo* experiments, it was established that the number of spermatogonia and meiotic cells decreases over time with chronic use of low doses of Cd, which is explained by a violation of the structure and function of proteins involved in chromosome conjugation or meiotic recombination (Geoffroy-Siraudin et al., 2012), and $CdCl_2$ leads to disruption of the formation of sperm from spermatogonia as a result of abnormal restructuring of the spermatogenic epithelium caused by the effect of Cd on the connection of adhesive membrane proteins with actin (Xiao et al., 2013). The onset of necrosis is noted at high doses of cadmium. Exposure to cadmium at puberty markedly reduces the weight of the testes, prostate, and seminal vesicles in adult mice, which is accompanied by a significant decrease of testosterone levels in blood serum and testes (Ji et al., 2010). Also, exposure to low, ecologically relevant doses of Cd affects male reproductive capacity and depends on genetic polymorphism (Wirth et al., 2010). In addition, cadmium negatively affects the female reproductive system, reducing female fertility and oocyte maturation, reducing the number of ovulated oocytes, and disrupting the rate of meiotic maturation of oocytes both *in vivo* and *in vitro* and further embryonic development (Zhu et al., 2018; Cheng et al., 2019). The concentration of Cd in the ovaries increases with age, disrupting the processes of oocyte development, which leads to the impossibility of ovulation. Depending on the dose, Cd either enhances or inhibits progesterone biosynthesis, affecting ovarian and reproductive morphology (Verma et al., 2011).

During pregnancy, toxicants, which include cadmium and its compounds, often have a negative effect on the processes of embryogenesis: they can cause spontaneous abortion, due to the slowing down of trophoblast development, placental necrosis, inhibition of steroid biosynthesis and disruption of the supply of necessary metals to the placenta, which contributes to the delay of implantation and possible early pregnancy loss, intrauterine fetal death or birth of weakened offspring (Sabra et al., 2017; Geng & Wang, 2019; Xiong et al., 2020). As was shown, cadmium accumulates in embryos starting from the four-cell stage, and in high doses inhibits development up to the blastocyst stage and can further lead to degeneration and decompression of the blastocyst, which is accompanied by apoptosis and disruption of cell adhesion, and in embryos, Cd dose-dependently causes damage to DNA, cell cycle arrest and oxidative stress (Beyersmann & Hartwig, 2008; Qiu et al., 2013). Potential gender differences in the toxic-kinetics and toxic-dynamics of cadmium were established in the experiment, which were manifested in the inverse relationships between prenatal exposure to Cd and anthropometry only in female newborns, but this requires additional research (Kippler et al., 2012; Röllin et al., 2015).

The embryotoxic effect of cadmium, as in our experiment (Shatornaya et al., 2020), occurred when animals were exposed to the toxicant during the majority of the pregnancy period (from the 1st to the 16th day) in the experiment of Russian researchers. A comparative analysis of the massometric indicators of fetal organs and extraembryonic organs when cadmium sulfate was administered in different periods of embryogenesis showed that the early postimplantation period is the most sensitive to the influence of cadmium (Zalavina et al., 2018; Hudson et al., 2019).

With a large number of works devoted to the study of the effect of heavy metals on the body, the mechanisms of adaptation to chronic intoxication with low, permissible doses of heavy metals and the possibility of forming resistance to higher doses are poorly researched.

Conclusions

Our results confirmed our hypothesis and showed that intragastric injection of cadmium chloride and cadmium citrate to Wistar rats (the dose of 1.0 mg/kg in terms of metal) has an embryolethal effect. This is manifested in a significant increase in mortality rates both on the 13th and on the 20th day of embryogenesis. Thus, as a result of the injection of cadmium chloride, the total embryonic mortality increased by 4.24 and 3.67 times, pre-implantation mortality by 6.50 and 14.03 times, and post-

implantation mortality by 3.07 and 2.49 times with a decrease in the number of live fetuses by 24.0% and 25.9% compared to the control group. At the same time, under the influence of cadmium citrate, indicators of total embryonic mortality increased by 4.00 and 3.50 times, pre-implantation mortality by 6.04 and 13.03 times, post-implantation mortality by 3.09 and 2.26 times, indicators of the number of live fetuses decreased by 18.3% and 22.2% relative to the control group. The revealed indicators of embryonic losses testify to the ability of pregnant females under the influence of adverse factors to maintain the abilities of the functional system "mother-fetus" as a whole, albeit with a selective loss of its individual elements. In both experimental groups of the injection of cadmium salts, indicators of accumulation of cadmium level in embryos on the 20th day of gestation increased by 15.83 times (exposure to cadmium chloride) and 9.00 times (exposure to cadmium citrate), and the level of zinc in the group exposed to cadmium chloride increased by 1.32 times, and under the action of cadmium citrate it decreased by 0.70 times compared to the control group. It would be promising to carry out histological studies, which would help to reveal changes at the tissue level and, possibly, explain the level of embryonic mortality.

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