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ЕЖЕМЕСЯЧНЫЙ НАУЧНЫЙ ЖУРНАЛ

Медицинские новости Грузии

საქართველოს სამედიცინო სიახლენი

GEORGIAN MEDICAL NEWS

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GMN: Georgian Medical News is peer-reviewed, published monthly journal committed to promoting the science and art of medicine and the betterment of public health, published by the GMN Editorial Board since 1994. GMN carries original scientific articles on medicine, biology and pharmacy, which are of experimental, theoretical and practical character; publishes original research, reviews, commentaries, editorials, essays, medical news, and correspondence in English and Russian.

GMN is indexed in MEDLINE, SCOPUS, PubMed and VINITI Russian Academy of Sciences. The full text content is available through EBSCO databases.

GMN: Медицинские новости Грузии - ежемесячный рецензируемый научный журнал, издаётся Редакционной коллегией с 1994 года на русском и английском языках в целях поддержки медицинской науки и улучшения здравоохранения. В журнале публикуются оригинальные научные статьи в области медицины, биологии и фармации, статьи обзорного характера, научные сообщения, новости медицины и здравоохранения. Журнал индексируется в MEDLINE, отражён в базе данных SCOPUS, PubMed и ВИНИТИ РАН. Полнотекстовые статьи журнала доступны через БД EBSCO.

GMN: Georgian Medical News – საქართველოს სამედიცინო სიახლენი – არის ყოველთვიური სამეცნიერო სამედიცინო რეცენზირებადი ჟურნალი, გამოიცემა 1994 წლიდან, წარმოადგენს სარედაქციო კოლეგიისა და აშშ-ის მეცნიერების, განათლების, ინდუსტრიის, ხელოვნებისა და ბუნებისმეტყველების საერთაშორისო აკადემიის ერთობლივ გამოცემას. GMN-ში რუსულ და ინგლისურ ენებზე ქვეყნდება ექსპერიმენტული, თეორიული და პრაქტიკული ხასიათის ორიგინალური სამეცნიერო სტატიები მედიცინის, ბიოლოგიისა და ფარმაციის სფეროში, მიმოხილვითი ხასიათის სტატიები.

ჟურნალი ინდექსირებულია MEDLINE-ის საერთაშორისო სისტემაში, ასახულია SCOPUS-ის, PubMed-ის და ВИНИТИ РАН-ის მონაცემთა ბაზებში. სტატიების სრული ტექსტი ხელმისაწვდომია EBSCO-ს მონაცემთა ბაზებიდან.

WEBSITE

www.geomednews.com

К СВЕДЕНИЮ АВТОРОВ!

При направлении статьи в редакцию необходимо соблюдать следующие правила:

1. Статья должна быть представлена в двух экземплярах, на русском или английском языках, напечатанная через **полтора интервала на одной стороне стандартного листа с шириной левого поля в три сантиметра**. Используемый компьютерный шрифт для текста на русском и английском языках - **Times New Roman** (**Кириллица**), для текста на грузинском языке следует использовать **AcadNusx.** Размер шрифта - **12**. К рукописи, напечатанной на компьютере, должен быть приложен CD со статьей.

2. Размер статьи должен быть не менее десяти и не более двадцати страниц машинописи, включая указатель литературы и резюме на английском, русском и грузинском языках.

3. В статье должны быть освещены актуальность данного материала, методы и результаты исследования и их обсуждение.

При представлении в печать научных экспериментальных работ авторы должны указывать вид и количество экспериментальных животных, применявшиеся методы обезболивания и усыпления (в ходе острых опытов).

4. К статье должны быть приложены краткое (на полстраницы) резюме на английском, русском и грузинском языках (включающее следующие разделы: цель исследования, материал и методы, результаты и заключение) и список ключевых слов (key words).

5. Таблицы необходимо представлять в печатной форме. Фотокопии не принимаются. **Все цифровые, итоговые и процентные данные в таблицах должны соответствовать таковым в тексте статьи**. Таблицы и графики должны быть озаглавлены.

6. Фотографии должны быть контрастными, фотокопии с рентгенограмм - в позитивном изображении. Рисунки, чертежи и диаграммы следует озаглавить, пронумеровать и вставить в соответствующее место текста **в tiff формате**.

В подписях к микрофотографиям следует указывать степень увеличения через окуляр или объектив и метод окраски или импрегнации срезов.

7. Фамилии отечественных авторов приводятся в оригинальной транскрипции.

8. При оформлении и направлении статей в журнал МНГ просим авторов соблюдать правила, изложенные в «Единых требованиях к рукописям, представляемым в биомедицинские журналы», принятых Международным комитетом редакторов медицинских журналов - http://www.spinesurgery.ru/files/publish.pdf и http://www.nlm.nih.gov/bsd/uniform_requirements.html
В конце каждой оригинальной статьи приводится библиографический список. В список литературы включаются все материалы, на которые имеются ссылки в тексте. Список составляется в алфавитном порядке и нумеруется. Литературный источник приводится на языке оригинала. В списке литературы сначала приводятся работы, написанные знаками грузинского алфавита, затем кириллицей и латиницей. Ссылки на цитируемые работы в тексте статьи даются в квадратных скобках в виде номера, соответствующего номеру данной работы в списке литературы. Большинство цитированных источников должны быть за последние 5-7 лет.

9. Для получения права на публикацию статья должна иметь от руководителя работы или учреждения визу и сопроводительное отношение, написанные или напечатанные на бланке и заверенные подписью и печатью.

10. В конце статьи должны быть подписи всех авторов, полностью приведены их фамилии, имена и отчества, указаны служебный и домашний номера телефонов и адреса или иные координаты. Количество авторов (соавторов) не должно превышать пяти человек.

11. Редакция оставляет за собой право сокращать и исправлять статьи. Корректура авторам не высылается, вся работа и сверка проводится по авторскому оригиналу.

12. Недопустимо направление в редакцию работ, представленных к печати в иных издательствах или опубликованных в других изданиях.

При нарушении указанных правил статьи не рассматриваются.

REQUIREMENTS

Please note, materials submitted to the Editorial Office Staff are supposed to meet the following requirements:

1. Articles must be provided with a double copy, in English or Russian languages and typed or compu-ter-printed on a single side of standard typing paper, with the left margin of **3** centimeters width, and **1.5** spacing between the lines, typeface - **Times New Roman** (**Cyrillic**), print size - **12** (referring to Georgian and Russian materials). With computer-printed texts please enclose a CD carrying the same file titled with Latin symbols.

2. Size of the article, including index and resume in English, Russian and Georgian languages must be at least 10 pages and not exceed the limit of 20 pages of typed or computer-printed text.

3. Submitted material must include a coverage of a topical subject, research methods, results, and review.

Authors of the scientific-research works must indicate the number of experimental biological species drawn in, list the employed methods of anesthetization and soporific means used during acute tests.

4. Articles must have a short (half page) abstract in English, Russian and Georgian (including the following sections: aim of study, material and methods, results and conclusions) and a list of key words.

5. Tables must be presented in an original typed or computer-printed form, instead of a photocopied version. **Numbers, totals, percentile data on the tables must coincide with those in the texts of the articles.** Tables and graphs must be headed.

6. Photographs are required to be contrasted and must be submitted with doubles. Please number each photograph with a pencil on its back, indicate author's name, title of the article (short version), and mark out its top and bottom parts. Drawings must be accurate, drafts and diagrams drawn in Indian ink (or black ink). Photocopies of the X-ray photographs must be presented in a positive image in **tiff format**.

Accurately numbered subtitles for each illustration must be listed on a separate sheet of paper. In the subtitles for the microphotographs please indicate the ocular and objective lens magnification power, method of coloring or impregnation of the microscopic sections (preparations).

7. Please indicate last names, first and middle initials of the native authors, present names and initials of the foreign authors in the transcription of the original language, enclose in parenthesis corresponding number under which the author is listed in the reference materials.

8. Please follow guidance offered to authors by The International Committee of Medical Journal Editors guidance in its Uniform Requirements for Manuscripts Submitted to Biomedical Journals publication available online at: http://www.nlm.nih.gov/bsd/uniform_requirements.html
http://www.icmje.org/urm_full.pdf
In GMN style for each work cited in the text, a bibliographic reference is given, and this is located at the end of the article under the title "References". All references cited in the text must be listed. The list of references should be arranged alphabetically and then numbered. References are numbered in the text [numbers in square brackets] and in the reference list and numbers are repeated throughout the text as needed. The bibliographic description is given in the language of publication (citations in Georgian script are followed by Cyrillic and Latin).

9. To obtain the rights of publication articles must be accompanied by a visa from the project instructor or the establishment, where the work has been performed, and a reference letter, both written or typed on a special signed form, certified by a stamp or a seal.

10. Articles must be signed by all of the authors at the end, and they must be provided with a list of full names, office and home phone numbers and addresses or other non-office locations where the authors could be reached. The number of the authors (co-authors) must not exceed the limit of 5 people.

11. Editorial Staff reserves the rights to cut down in size and correct the articles. Proof-sheets are not sent out to the authors. The entire editorial and collation work is performed according to the author's original text.

12. Sending in the works that have already been assigned to the press by other Editorial Staffs or have been printed by other publishers is not permissible.

Articles that Fail to Meet the Aforementioned Requirements are not Assigned to be Reviewed.

ავტორთა საყურადღებოდ!

რედაქციაში სტატიის წარმოდგენისას საჭიროა დავიცვათ შემდეგი წესები:

1. სტატია უნდა წარმოადგინოთ 2 ცალად, რუსულ ან ინგლისურ ენებზე,დაბეჭდილი სტანდარტული ფურცლის 1 გვერდზე, 3 სმ სიგანის მარცხენა ველისა და სტრიქონებს შორის 1,5 ინტერვალის დაცვით. გამოყენებული კომპიუტერული შრიფტი რუსულ და ინგლისურენოვან ტექსტებში - **Times New Roman (Кириллица)**, ხოლო ქართულენოვან ტექსტში საჭიროა გამოვიყენოთ **AcadNusx.** შრიფტის ზომა – 12. სტატიას თან უნდა ახლდეს CD სტატიით.

2. სტატიის მოცულობა არ უნდა შეადგენდეს 10 გვერდზე ნაკლებს და 20 გვერდზე მეტს ლიტერატურის სიის და რეზიუმეების (ინგლისურ, რუსულ და ქართულ ენებზე) ჩათვლით.

3. სტატიაში საჭიროა გაშუქდეს: საკითხის აქტუალობა; კვლევის მიზანი; საკვლევი მასალა და გამოყენებული მეთოდები; მიღებული შედეგები და მათი განსჯა. ექსპერიმენტული ხასიათის სტატიების წარმოდგენისას ავტორებმა უნდა მიუთითონ საექსპერიმენტო ცხოველების სახეობა და რაოდენობა; გაუტკივარებისა და დაძინების მეთოდები (მწვავე ცდების პირობებში).

4. სტატიას თან უნდა ახლდეს რეზიუმე ინგლისურ, რუსულ და ქართულ ენებზე არანაკლებ ნახევარი გვერდის მოცულობისა (სათაურის, ავტორების, დაწესებულების მითითებით და უნდა შეიცავდეს შემდეგ განყოფილებებს: მიზანი, მასალა და მეთოდები, შედეგები და დასკვნები; ტექსტუალური ნაწილი არ უნდა იყოს 15 სტრიქონზე ნაკლები) და საკვანძო სიტყვების ჩამონათვალი (key words).

5. ცხრილები საჭიროა წარმოადგინოთ ნაბეჭდი სახით. ყველა ციფრული, შემაჯამებელი და პროცენტული მონაცემები უნდა შეესაბამებოდეს ტექსტში მოყვანილს.

6. ფოტოსურათები უნდა იყოს კონტრასტული; სურათები, ნახაზები, დიაგრამები - დასათაურებული, დანომრილი და სათანადო ადგილას ჩასმული. რენტგენოგრამების ფოტოასლები წარმოადგინეთ პოზიტიური გამოსახულებით **tiff** ფორმატში. მიკროფოტოსურათების წარწერებში საჭიროა მიუთითოთ ოკულარის ან ობიექტივის საშუალებით გადიდების ხარისხი, ანათალების შეღებვის ან იმპრეგნაციის მეთოდი და აღნიშნოთ სურათის ზედა და ქვედა ნაწილები.

7. სამამულო ავტორების გვარები სტატიაში აღინიშნება ინიციალების თანდართვით, უცხოურისა – უცხოური ტრანსკრიპციით.

8. სტატიას თან უნდა ახლდეს ავტორის მიერ გამოყენებული სამამულო და უცხოური შრომების ბიბლიოგრაფიული სია (ბოლო 5-8 წლის სიღრმით). ანბანური წყობით წარმოდგენილ ბიბლიოგრაფიულ სიაში მიუთითეთ ჯერ სამამულო, შემდეგ უცხოელი ავტორები (გვარი, ინიციალები, სტატიის სათაური, ჟურნალის დასახელება, გამოცემის ადგილი, წელი, ჟურნალის №, პირველი და ბოლო გვერდები). მონოგრაფიის შემთხვევაში მიუთითეთ გამოცემის წელი, ადგილი და გვერდების საერთო რაოდენობა. ტექსტში კვადრატულ ფჩხილებში უნდა მიუთითოთ ავტორის შესაბამისი N ლიტერატურის სიის მიხედვით. მიზანშეწონილია, რომ ციტირებული წყაროების უმეტესი ნაწილი იყოს 5-6 წლის სიღრმის.

9. სტატიას თან უნდა ახლდეს: ა) დაწესებულების ან სამეცნიერო ხელმძღვანელის წარდგინება, დამოწმებული ხელმოწერითა და ბეჭდით; ბ) დარგის სპეციალისტის დამოწმებული რეცენზია, რომელშიც მითითებული იქნება საკითხის აქტუალობა, მასალის საკმაობა, მეთოდის სანდოობა, შედეგების სამეცნიერო-პრაქტიკული მნიშვნელობა.

10. სტატიის ბოლოს საჭიროა ყველა ავტორის ხელმოწერა, რომელთა რაოდენობა არ უნდა აღემატებოდეს 5-ს.

11. რედაქცია იტოვებს უფლებას შეასწოროს სტატია. ტექსტზე მუშაობა და შეჯერება ხდება საავტორო ორიგინალის მიხედვით.

12. დაუშვებელია რედაქციაში ისეთი სტატიის წარდგენა, რომელიც დასაბეჭდად წარდგენილი იყო სხვა რედაქციაში ან გამოქვეყნებული იყო სხვა გამოცემებში.

აღნიშნული წესების დარღვევის შემთხვევაში სტატიები არ განიხილება.

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CHRONIC EFFECTS OF CADMIUM CHLORIDE ON RAT EMBRYOGENESIS

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Abstract.

The purpose of this study was to analyze the effects of cadmium toxicity on rat embryogenesis when exposed to other heavy metal citrates. Despite the variety of scientific publications discussing the influence of cadmium on mammalian postnatal development, the effect of this metal on embryogenesis has not yet been sufficiently studied. In this experimental study, cadmium chloride was administered to experimental pregnant female Wistar rats at a daily dose of 1.0 mg/kg. Rats were allocated at random into groups receiving either cadmium chloride alone or additional zinc citrate, cerium citrate, or nanocomposite (based on iodine, sulfur, and selenium citrate). The control group received distilled water at an equivalent volume. In each group, operational intervention occurred at the 13th and 20th day of gestation to assess numbers of live fetuses, corpora lutea, pre-implantation losses, post-implantation losses, and total implantation losses. When cadmium chloride alone was administered, a pronounced embryotoxic effect was observed, manifested as a significant decrease in the number of live fetuses. Experimental groups which received cadmium chloride with zinc citrate, cerium citrate, or nanocomposite had an increased number of live fetuses and corpora lutea, as well as a decreased number of implantation losses, compared to the group which only received cadmium chloride. Each combination of cerium, zinc, and selenium nanocomposite citrates demonstrated a compensatory effect on all measures of embryogenesis impacted by cadmium embryotoxicity. Thus, administration of the citrates of cerium, zinc, and selenium nanocomposite reduces cadmium embryotoxicity and its accumulation in the body.

Key words. Cadmium chloride, cerium citrate, zinc citrate, embryogenesis, toxicity, antagonism.

Introduction.

The effects of heavy metals on human health and the environment remain some of the most relevant problems of modern science. Industrialization has caused severe air, water, and soil pollution, which have devastating effects on the health, survival, and activities of humans.

Among the most dangerous toxins of prolonged action are salts of heavy metals. Cadmium is an accumulative heavy metal with potential toxicity to the excretory, musculoskeletal, digestive, cardiovascular, and reproductive systems. When cadmium enters the human body, it is excreted very slowly and, therefore, accumulates in organs and tissues, disrupting metabolic processes and physiologic functions. The peculiarities of the harmful effects of cadmium include a rapid absorption by the body and a biologic half-life of 10 to 30 years. Cadmium has mutagenic and carcinogenic properties, and it tends to accumulate in the liver, kidneys, spleen, brain, and reproductive organs [1-3]. This metal causes *itai-itai* disease, manifested by bone softening, calcification, kidney pyelonephritis, and disruption of iron and calcium metabolism [4-7]. Despite the variety of scientific publications on the influence of cadmium on postnatal development in humans and animals, the effect of this metal on embryogenesis has not been sufficiently studied [8-10].

Cadmium affects the accumulation and distribution of other chemical elements. It has been experimentally proven that cadmium is a biological antagonist of zinc; therefore, its toxicity, absorption, and distribution in the body may be influenced by the content of zinc [11]. In this study, we aim to analyze the effects of cadmium toxicity on developing rat fetuses in the womb, when exposed to different metals and factors which interact with cadmium. The goal of this original article is to provide insight into the effects of cadmium toxicity on embryogenesis and to assess potential factors which may serve as additional avenues for safe treatment options.

Materials and Methods.

A total of 100 adult pregnant female Wistar laboratory rats and 853 rat fetuses were analyzed in the study. The animals were kept in the vivarium of Dnipro State Medical University under natural light, with free access to food and water. Animal procedures at all stages of the study were performed in compliance with generally accepted bioethical principles of the Declaration of Helsinki of the World Medical Association.

Selection of female rats to the control and experimental groups was carried out in a random order. All animals were divided into five groups, and each group consisted of 20 rats. In Experimental Group 1 (EG1), animals received an isolated solution of cadmium chloride at a daily dose of 1.0 mg/kg. In Experimental Group 2 (EG2), animals received cadmium chloride solution at a daily dose of 1.0 mg/kg in combination with cerium citrate solution at a concentration of 1.3 μg/kg. In Experimental Group 3 (EG3), animals received cadmium chloride solution at a daily dose of 1.0 mg/kg in combination with zinc citrate solution at a concentration of 1.5 μg/kg. In Experimental Group 4 (EG4), animals received cadmium chloride solution at a daily dose of 1.0 mg/kg in combination with nanocomposite (iodine, sulfur, and selenium citrate solution) at a concentration of 2.0 μg/kg . In the control group, animals received distilled water in an equivalent volume.

Rats in each experimental group were further divided into two subgroups. Ten females received operational intervention at a gestational period of 13 days, and the remaining ten females received operational intervention at a gestational period of 20 days. Pregnancy in Wistar Rats lasts around 21-23 days, and the

majority of internal organ growth occurs between 17- and 21-days gestational age. Changes seen on 20th day of embryogenesis thus demonstrate the result of the long-term effects of cadmium intoxication [12,13]. Internal organs and fetuses were examined and analyzed.

Embryological analysis included counting the number of corpora lutea, implants, live embryos, and dead embryos. Based on these data, pre-implantation losses, post-implantation losses, and total implantation losses were calculated (Appendix A).

Determination of cadmium and zinc metabolism in tissues of embryos was performed by atomic emission spectroscopy. The samples were measured at the Ukrainian Research Institute of Transport Medicine. The atomic emission spectrometer Emas-200 CCD was used for quantitative measurement of metal content in the samples. The direct current utilized was 15 amperages. Quantification of cadmium and zinc in the examined objects was carried out at wavelengths of 228.802 nm and 213.856 nm respectively. Analyses of accumulation of cadmium as a marker of cadmium intoxication and the level of zinc as a marker of cadmium antagonism were conducted.

The statistical analyses of the results were performed using the Statistica 12 software program. Results are presented as mean ± S.E.M. The data were analyzed using the Kruskal-Wallis test and the Mann-Whitney U-test. All statistical significance levels were set at alpha of 0.05.

Results.

Experimental study results revealed a negative effect of cadmium chloride on embryogenesis. In female rats that received an isolated cadmium chloride solution during pregnancy, fetal asymmetry and fetal resorption were noted (Figure 1).

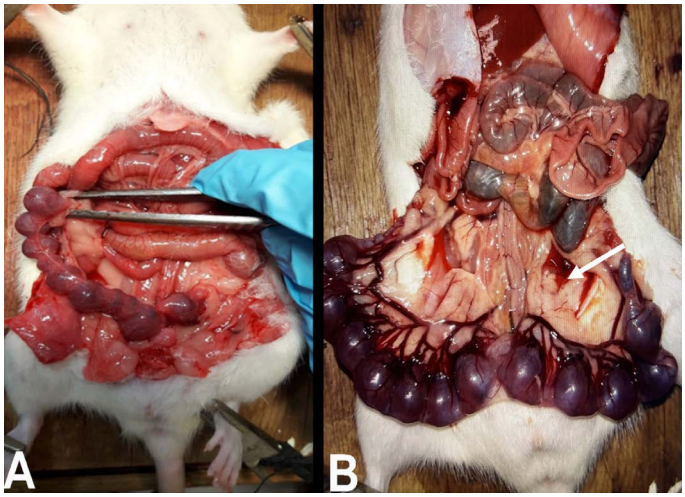

Figure 1. *(A) asymmetry of the fetal position in one corner of the uterus and fetal resorption, indicated by the arrow (B).*

At 13 days gestation, the mean number of live fetuses per female in EG1 decreased by 24.04% (p=0.0002) compared to the control group. A similar pattern was observed in the groups exposed to combination of cadmium chloride with metal citrates. Compared to the control, at 13 days gestation, the mean number of live fetuses per female decreased by 7.70% (p=0.11) in EG2, 4.80% (p=0.13) in EG3, and 11.54% (p=0.005) in EG4.

On the 20th day of gestation, the mean number of live fetuses per female decreased by 25.92% (p=0.003) in EG1, 14.81% (p=0.03) in EG2, 6.48% (p=0.36) in EG3, and 5.56% (p=0.38) in EG4, compared to the control group. Therefore, when cadmium chloride was administered with metal citrates, there was an increase in live fetuses compared to cadmium chloride alone (Figure 2).

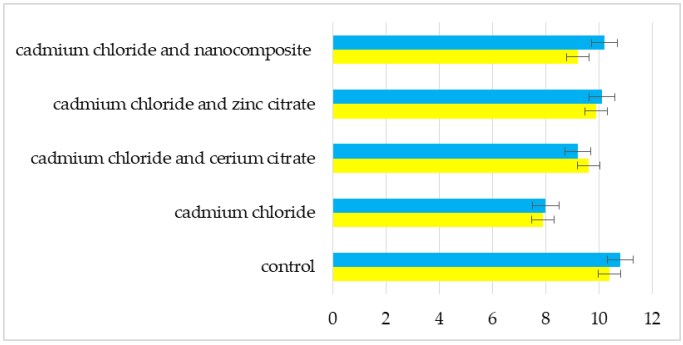

Figure 2. *Mean ± S.E.M of live fetuses per female, on the 13th day (yellow) and 20th day (blue) of embryonic development in the control and experimental groups.*

Our research shows that cadmium chloride administration without metal citrates increased the cadmium levels by 15.8 times (p=0.0002) and increased zinc levels by 1.3 times (p=0.0002) in the embryos at 20 days gestation, compared to the control group. In the experimental groups of cadmium chloride in combination with metal citrates, cadmium levels increased 12.3 times (p=0.0002) in EG2, 12.3 times (p=0.0002) in EG3, and 14.0 times (p=0.0002) in EG4, compared to the control group (Figure 3).

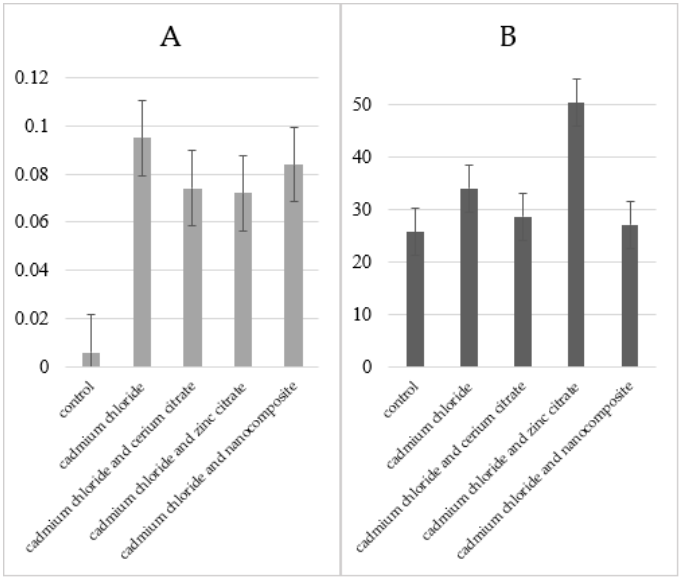

Figure 3. *Mean ± S.E.M. of cadmium (A) and zinc (B) accumulation levels in rat embryos of experimental groups on the 20th day of embryogenesis.*

The lowest mean number of corpora lutea per one female was observed with administration of cadmium chloride alone, both

on the 13th day (10.00±0.27) and the 20th day (10.30±0.27) of embryonic development respectively, but the results were not statistically significant (Figure 4).

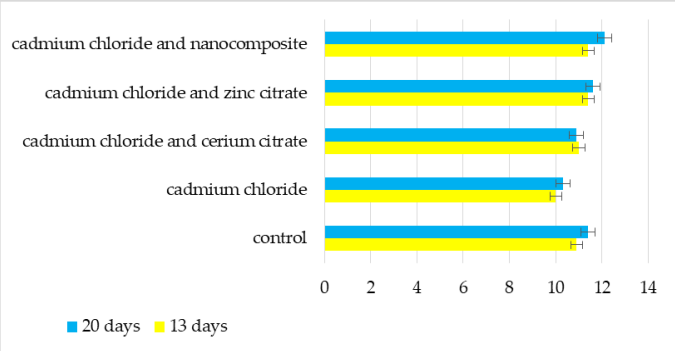

Figure 4. *Mean ± S.E.M. of corpora lutea per a female, on the 13th and 20th days of embryonic development in the control and experimental groups.*

The results of this study indicate a pronounced embryotoxic effect of cadmium chloride at a dose of 1.0 mg/kg/day, which led to a significant increase in total implantation losses compared to the control group in both studied gestational periods. By the 13th day of embryogenesis, total implantation losses increased 4.2-fold (p=0.0003) in EG1 compared to the control. In the groups of combined exposure to cadmium chloride and metal citrates, total implantation losses also increased compared to the control. At 13 days gestation, total implantation losses increased 2.6-fold in EG2 (p=0.02), 2.6-fold in EG3 (p=0.02), and 3.8-fold in EG4 (p=0.003), compared to the control group. On the 20th day of embryogenesis, total implantation losses increased 3.7-fold in EG1 (p = 0.0004), 2.5-fold in EG2 (p=0.004), 2.3-fold in EG3 (p=0.03), and 2.7-fold in EG4 (p=0.06), compared to the control. When cadmium chloride was administered with metal citrates, less total implantation losses were observed compared to cadmium chloride administered alone, at both day 13 and day 20 of embryogenesis (Figure 5).

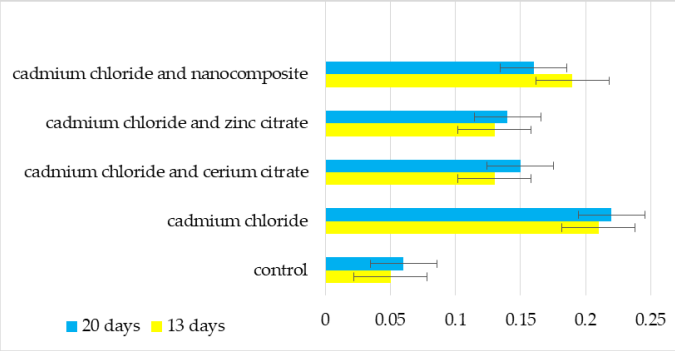

Figure 5. *Mean ± S.E.M. of total implantation losses on the 13th day (yellow) and the 20th day (blue) of embryonic development in the control and experimental groups.*

In rats exposed to cadmium chloride alone, by 13 days gestation, pre-implantation losses increased 6.5-fold (p=0.0002), and post-implantation losses increased 3.0-fold (p>0.05) compared to the control. By 20 days gestational age, in the cadmium chloride exposure group, pre-implantation losses increased 14.0-fold (p=0.0002), and post-implantation losses increased 2.5-fold (p>0.05) compared to the control group. At 13 days of embryogenesis, EG2 had a 3.00% decrease (p>0.05), EG3 had a 30.78% decrease (p=0.01), and EG4 had a 30.77% increase (p>0.05) in pre-implantation losses compared to cadmium chloride alone. Post-implantation losses at 13 days gestation decreased 2.3-fold (p>0.05) in EG2, decreased by 66.67% (p>0.05) in EG3, and decreased by 77.78% (p>0.05) in EG4, compared to cadmium chloride alone. By 20 days of embryogenesis, EG2 had a 10-fold decrease (p = 0.05), EG3 had a 42.86% decrease (p=0.13), and EG4 had a 21.43% (p=0.03) decrease in pre-implantation losses compared to cadmium chloride alone. Post-implantation losses at 20 days gestation decreased 1.3-fold in EG2, decreased by 50.00% (p>0.05) in EG3, and decreased by 60.00% (p=0.04) in EG4, compared to cadmium chloride alone (Figure 6 and Figure 7).

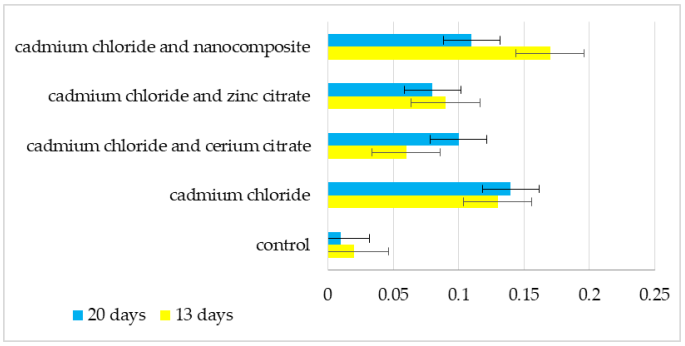

Figure 6. *Mean ± S.E.M. of pre-implantation loss on the 13th day (yellow) and the 20th day (blue) of embryonic development in the control and experimental groups.*

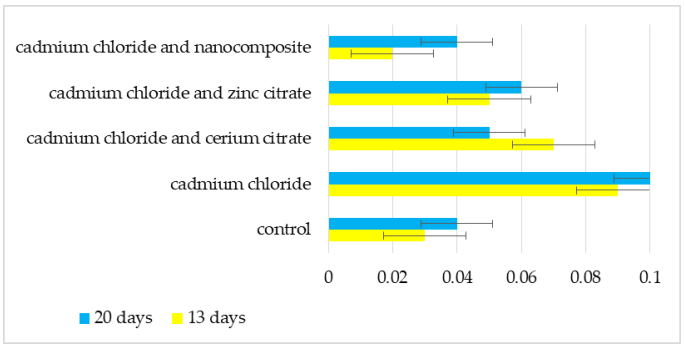

Figure 7. *Mean ± S.E.M. of post-implantation loss, on the 13th day (yellow) and the 20th day (blue) of embryonic development in the control and experimental groups.*

Discussion.

Cadmium is one of the most toxic heavy metals. Due to bioaccumulation, its potential toxicity can have negative effects on humans and animals. All cadmium compounds are poisonous, and the accumulation of this metal in different organs depends on

the way it enters the body, orally or by inhalation. Cadmium can form biological complexes with metallothioneins and disrupt reduction and oxidation processes. One of the primary biologic responses to cadmium is the activation of free-radical lipid peroxidation processes, the end products of which are aldehydes and ketones, which both have toxic effects. As a result, the activity of membrane-bound enzymes, ion transport, and cell membrane permeability are impaired. Hypoxia develops, blood oxygen transport is disrupted, and hemoglobin levels decrease. Thus, cadmium can cause imbalance of enzyme systems in the cell, which leads to changes in cell homeostasis. In the event of acute intoxication, the lungs, liver, kidneys, and reproductive organs are primarily affected. Cadmium compounds negatively impact female fertility, oocyte maturation, pregnancy, and formation of the fetus [14-18]. In rats, the sensitivity of the "mother-fetus" system to the toxicant varies and is related to the duration of exposure.

It should be noted that cadmium chloride intake during the first days of pregnancy in rats leads to an increase in fetal death due to pre-implantation losses, as shown in previous literature [19]. These findings support the idea of the presence of defense mechanisms that act against destabilizing factors introduced prior to implantation, as it is energetically advantageous for rats to abort the fetuses in the initial period of pregnancy rather than in later periods of gestation. In addition, the results obtained during our research suggest a pronounced embryotoxic effect of cadmium chloride at a dose of 1.0 mg/kg/day, which is illustrated by a significant increase in the pre-implantation losses, post-implantation losses, and total implantation losses, as well as a decrease in implants and number of live fetuses per female, compared to the control group in both studied gestational terms.

The mechanism of cadmium toxicity is believed to be a result of interactions with multiple biological and oxidative processes, such as through enhancement of the production of oxidative free radicals upon inhibition of manganese-superoxide dismutase and copper/zinc-dismutase [20]. Although cadmium interacts with many heavy metals including calcium, copper, iron, selenium, and zinc, the relationship between cadmium and zinc is more commonly noted due to their similarities as well as nutritional deficiencies in zinc. In our research, when combined with the citrates of zinc, cerium, and selenium-based nanocomposite, the toxic effects of cadmium chloride were decreased, as visualized by a reversal of these parameters, indicating a potential antagonistic mechanism. When zinc citrate was added to cadmium chloride, a resultant increased number of live fetuses and decreased number of total implantation losses were visualized. The addition of either cerium citrate or nanocomposite boasted similar effects as zinc citrate, indicating a similar mechanism against cadmium. Differences among these groups were not significant enough to discuss.

Previous literature has discussed tobacco smoking as the most common cause of cadmium exposure in humans, although other sources may be through contaminated food and water as well as through certain manufacturing industries in developing countries. However, during pregnancy, cadmium absorption from dietary sources increases, and nutritional deficiencies in other heavy metals, notably iron and zinc, exacerbate its toxic profile [21]. Smoking and increased dietary cadmium intake in pregnant mothers are associated with increased cadmium levels in the placenta, which correlate with increased miscarriage and fetal death [21,22]. It has been shown in previous research that zinc and magnesium are two important trace minerals that can treat cadmium toxicity and reverse damage. In medical practice, common chelating agents such as EDTA, penicillamine, dimercaprol, and others have been used in attempts to treat cadmium toxicity, but adverse effects and minimal human research has led to insufficient evidence to justify any chelator as the best form of treatment at this point in time [23]. Currently, the best-known intervention for pregnant patients with cadmium exposure is through prevention of exposures, which requires community-wide and environmental interventions. Moreover, there is little knowledge on the ability to reduce cadmium absorption after smoking or other forms of inhalation [24].

Before the onset of our study, there was minimal known research describing the effects of metal citrates on the recovery of fetal and placental cadmium toxicity. Our research indicates that in rats exposed to cadmium levels indicative of toxic effects, the addition of zinc citrate, cerium citrate, and selenium-based nanocomposite can lead to salvageable effects on the developing fetus. Assuming direct extension of these results to the human population, administration of these metal citrates or nanocomposites in pregnant patients with dietary cadmium toxicity could lead to decreased chance of miscarriages and fetal cadmium exposure.

Recent literature has shown that selenium nanocomposites are exciting forthcoming modalities in the field of toxicology that have been shown to have protective roles in drug toxicities, cancer, infections, neurodegenerative disorders, and diabetes [25]. Cerium nanocomposites are also notable mainly for their robust antioxidizing profile, while currently claiming minimal toxicity [26]. There is much to learn through further research on how metal citrates and nanocomposites may influence biological effects of heavy metal toxicities and how they may be implemented in future medical practice.

Conclusion.

As a result of this study, intragastric administration of cadmium chloride at a dose of 1.0 mg/kg/day to Wistar rats throughout the first 13 days of gestation was found to lead to a significantly decreased number of live fetuses and a significantly increased amount of pre-implantation, post-implantation, and total implantation losses. Through 20 days gestation, the results also showed a significant decrease in number of live fetuses and a significant increase in pre-implantation, post-implantation, and total implantation losses in rats exposed to cadmium chloride alone. Exposure to cadmium chloride combined with metal citrates improved all the studied parameters compared to the isolated exposure to cadmium chloride. Thus, each combination of cerium, zinc, and nanocomposite citrates demonstrated a compensatory effect on all measures of embryogenesis impacted by cadmium embryotoxicity. According to the results of this experiment, administration of the citrates of cerium, zinc, and nanocomposite reduces cadmium embryotoxicity and its accumulation in the body.

Appendix A

Formulas:

Pre-implantation losses (%) = ((No. of corpora lutea – No. of implants)/No. of corpora lutea) × 100.

Post-implantation losses (%) = ((No. of implants – No. of live embryos)/No. of implants) × 100.

Total implantation losses (%) = ((No. of corpora lutea – No. of live embryos)/No. of corpora lutea) × 100. [27].

Author Contributions.

Conceptualization, K.I. and S.V.; methodology, K.I. and S.V.; formal analysis, K.I. and S.V.; investigation, K.I. and S.V.; data curation, K.I. and S.V.; writing—original draft preparation, K.I., S.V., M.K., and K.S.; writing—review and editing, M.K., A.W., and K.S.; visualization, A.W. All authors have read and agreed to the published version of the manuscript.

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None

Conflict of interest statement.

The author declares no conflict of interest.

Ethical approval.

The present study conforms to the ethical standards and guidelines of the journal.

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Institutional Review Board Statement.

The animal study protocol was approved by the Bioethics Commission of the Dnipro State Medical University (protocol No. 1 dated February 10, 2020) and established that the conducted scientific research on the ovaries and fetuses of experimental animals meets the ethical requirements in accordance with the order of the Ministry of Health of Ukraine No. 231 dated November 1, 2005.

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