Some Teratogenic Outcomes in Rats Exposed to Zinc Chloride Pre and Post Pregnancy

Abdul-Rahman Zaher*1, Falah M AL-Rekabi1, Saad Akram Hatif2

1Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq, 2Department of Obstetrics and Surgery, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq

ABSTRACT

The aim of present study was to evaluate the possibility of teratogenicity in rats when exposed to zinc chloride (ZnCl2) pre and post pregnancy. To achieve this goal, a total of 40 mature Albino Wistar female rats were divided equally into four groups as follows: T1, dosed 0.7 mg/day ZnCl2 for two months before mating and till to the day 5th of pregnancy, the females of this group were mated with males dosed 0.7 mg/day ZnCl2 for two weeks before mating; T2, dosed 0.7 mg/day ZnCl2 for two months before mating and till to the day 16th of pregnancy and then were mated with control males (not exposed to any level of ZnCl2); T3, dosed 0.7mg/day ZnCl2 for two months before mating and till the end of pregnancy and were mated with control males; Control, dosed with water free from ZnCl2 along the period of experiment and were mated with control males. At the end of each pregnancy phase, results revealed that alpha fetoprotein serum levels were significantly (P<0.05) higher in all treatment groups compared to the control group, and the most prominent increase was observed in the T3 group. All treatment groups showed a significant (P<0.05) decrease in gestation, viability, and lactation indices when compared to the control group, and the most prominent increase was observed in the T3 group. All treatment groups showed a significant (P<0.05) decrease in gestation, viability, and lactation indices when compared to the control group, with the T3 group showing the most significant decrease. Additionally, on days 1, 4, 7, 14, and 21 of lactation period, there was a significant (P<0.05) decrease in mean pup body weights in treated groups compared to the control group, with T3 group having the most prominent body weight decrease. The findings of this study revealed that ZnCl2 at a daily dose of 0.7 mg may cause teratogenic defects in rats at various stages of pregnancy, particularly at the third stage. As high-risk groups, pregnant women and children should use Zn supplementation carefully, whether as a food additive or for self-medication. Simultaneously, evaluating effect of low-dose Zn supplementation over a longer duration is required.

INTRODUCTION

Heavy metals, either being essential or non-essential nutrients, can be hazardous at certain levels as they are not biodegradable and hence have a high potential for bioaccumulation. Bioaccumulation in a biological organism describes an increment in a chemical concentration through time comparing to the concentration in the environment (1). When compounds are taken up and stored more rapidly than they are metabolized or excreted,
they may build-up and persist in living organisms. Toxicity of heavy metals may cause mental and central nervous system damage, as well as lower energy levels and damage to the liver, kidneys, lungs, composition of blood, and other major organs (2). Long-term exposure has been shown to mimic Alzheimer's disease, Parkinson's disease, muscular dystrophy, and multiple sclerosis by inducing slow physical, muscular, and neurological degradation. Allergies are prevalent, and prolonged exposure to certain metals, or their compounds can result in cancer (3).

Zinc chloride and its hydrates are chemical substances with the formula ZnCl₂. Textile processing, metallurgical fluxes, chemical synthesis, and medical usage are only a few of the applications for ZnCl₂ (4). The main routes of ZnCl₂ exposure include oral, dermal, and inhalation. CRIP, a cysteine-rich intestinal protein that sequesters zinc (Zn) inside enterocytes before active transport into plasma, is responsible for gastrointestinal zinc absorption (5). Zn²⁺ absorption is influenced by nutritional status, with calcium, phosphorus, and phytic acid inhibiting Zn absorption, whereas dietary protein facilitates it. It has been reported that rats absorb around 8% to 10% of the dietary Zn, with dietary protein facilitating it.

Animals of Study

The study was carried out with the approval of the Scientific Committee in the Department of Physiology, Biochemistry and Pharmacology, College of Veterinary Medicine, University of Baghdad, Baghdad, Iraq in accordance with ethical standards of animal welfare.

A total of 40 mature female and a certain number of male Albino Wistar rats of variable ages (2-3 months) and weights (180-230 g) were obtained, housed, and maintained at Animal House, College of Veterinary Medicine, University of Baghdad on 22–24°C ± 2°C, relative humidity 50%-55%, and a 14 h:10 h light-dark cycle (5 a.m. to 7 p.m., lights on). For two weeks, animals were acclimatized and both water and standard diet were provided ad libitum. All animals received ZnCl₂ at a dose of 0.7 mg/rat/day, estimated based on the rats’ daily water intake of polluted water. Animals were divided randomly into four equal groups and certain number of adult male rats have been involved and assigned as follows: T1, adult females were dosed 0.7 mg daily ZnCl₂ for two months before mating and till the day 5th of pregnancy and mated with male dosed 0.7 mg daily of ZnCl₂ for two weeks before mating; T2, dosed 0.7 mg daily ZnCl₂ for two months before mating and till the day 16th of pregnancy and then mated with control male (not exposed to any level of ZnCl₂); T3, dosed 0.7 mg daily ZnCl₂ for two months before mating and till to the end of pregnancy and were mated with control male (as in T2); Control, dosed with water free from ZnCl₂ along the period of experiment and were mated with control male.

Insurance of Pregnancy

The pregnant female rats were examined daily after conception for five days. Vaginal smears stained with methylene blue were prepared to detect di-estrus phase. Pregnancy was detected by observation pale mucous membrane of vagina in the third day after conception (9).

Blood Samples

Blood samples were obtained from anesthetized rats, administered i.m. ketamine and xylazine at 90 mg/kg BW and 40 mg/kg BW, respectively, using the heart puncture technique. Blood samples (approximately 2 mL) were collected and kept in tubes for 10 min before being centrifuged at 2500 rpm for 15 min. Sera were collected, aliquoted, and refrigerated (-20°C) until they were analyzed.

Parameters

Alpha fetoprotein

Assessment of alpha fetoprotein (AFP) was performed at the end of each phase of pregnancy according to (10). A

Materials and Methods

Chemicals

ZnCl₂ (99.99% purity) was purchased from Sigma-Aldrich (St. Louis, MO, USA) and stored at room temperature. Xylene and ketamine for anesthesia were purchased from BDH (England). Methylene blue for vaginal smear was purchased from Promega (USA). For alpha fetoprotein assessment, a kit was purchased from MyBioSource® company (USA).

Iraqi J. Vet. Med. 2021, Vol. 45(2): 41-45
commercially available kite (MyBioSource® Company, USA) was used for measurement.

**Gestation index (%)**

Gestation index (GI%) was calculated according to (11) as following:

Gestation index= percentage of pregnancies resulting in live litters

**Lactation index (%)**

Lactation index (LI%) was calculated as the percentage of the offspring alive at day 4 that survived till the day 21st of lactation period (11).

\[
\text{Lactation Index (LI%) = \frac{\text{No. survival neonates (days 5 – 21)}}{\text{Total No. remaining of neonates}}} \times 100
\]

**Viability index (%)**

Viability index (VI%) of the offspring for 1-4 day after parturition was calculated according to (11) as follows:

\[
\text{Viability Index (VI%) = \frac{\text{No. offspring alive till day 4}}{\text{Total No. offspring}}} \times 100
\]

**Body weight offspring (g)**

Body weight was measured after the offspring were born (the total weight of the brood divided by the number).

### Statistical Analysis

The general linear model (GLM) approach in SPSS software version 22.00 (IBM SPSS Inc., Chicago, IL, USA) was used to analyze the collected data as a one-way ANOVA. At P< 0.05, the Fisher’s least significant differences (LSD) post hoc test was used to separate the means (13). The results are presented as a mean ± standard error of the mean (SEM).

### RESULTS AND DISCUSSION

**Alpha Fetoprotein**

The result of AFP showed significant differences (P< 0.05) between all groups of the experiment at the end of each phase of pregnancy. There were significant increases (P< 0.05) in alpha fetoprotein of T1, T2, and T3 groups compared with control group, but the most prominent increase was observed in the T3 group which has dosed 0.7 mg daily ZnCl₂ for two months before mating and till the end of pregnancy (Table 1).

**Gestation, Lactation, and Viability Indices**

The current results showed that there was a significant (P< 0.05) decrease in GI%, VI%, and LI% in all treated groups as compared with control group, also it showed a significant difference between the treated groups (Table 1).

Since trace minerals, such as Zn, are not generally deemed to be hazardous to humans or animals, the acute or chronic deficiency adverse effects are usually given more emphasis (14). High Zn consumption via self-medication or diet as food additives has, however, been documented in some cases (15). In this study, the results showed that female rats exposed to 0.7 mg/day ZnCl₂ exhibited significant (P< 0.05) reduction in reproductive outcome, where there was disturbance in time of birth (GI%), and reduction in VI% of pups (except for T1) and LI% in all treated groups compared to control. According to statistical analysis, the most deleterious effect was recorded in rats received 0.7 mg/day ZnCl₂ for two months before mating and till to the end of pregnancy and mated with control males (T3). It has been revealed by some studies that absorption of Zn increases in humans and animals’ lactation period (16). According to Jackson et al. (17), absorption of Zn was high in lactating Brazilian mothers with low-income, ranging from 59% to 84%. The women’s chronically low Zn intake (128 moL/day) and the increased need for Zn during lactation could explain the elevated fractional absorption. Moser-Veillon et al. (18) reported further evidence about the increase absorption of Zn during lactation where the authors found that lactating women in the United States consuming 8 mg Zn/day from diverse diets had a mean fractional absorption of less than 0.35, compared to less than 0.20 fractional absorption of nonlactating postpartum mothers with the similar Zn intake. Six lactating mothers absorbed 83% more fractional Zn than seven women who had never been pregnant. As a result, it appears that one of the mechanisms used to meet the increased Zn demands during lactation is an increase in intestinal Zn absorption. During the second and third trimesters of pregnancy, there was a 30% increase in fractional Zn absorption. This minor increase, although not statistically significant, shows that a change in intestinal absorption is one of the mechanisms to provide the additional Zn required for growth of fetal, possibly prior the lactation process begins (19). Johnson et al. (16) confirmed the findings of the current study, reporting that mild toxic effects on the endpoints of reproductive and liver function were associated with ZnCl₂.

### Table 1. Alpha fetoprotein (AFP ng/mL), gestation index (GI%), viability index (VI%), and lactation index (LI%) of Albino Wistar female rats treated orally with 0.7 mg/day zinc chloride at different phases of pregnancy

| Group | Reproductive parameters |
|-------|-------------------------|
|       | AFP (ng/mL) | GI (%) | VI (%) | LI (%) |
| T1    | 20.13±0.20  | 82.50  | 91.3±1.94 | 89.60±1.08  |
| T2    | 24.68±0.34  | 62.80  | 83.9±0.94 | 82.00±0.90  |
| T3    | 30.22±0.16  | 58.70  | 69.5±0.45 | 75.70±0.76  |
| Control | 15.50±0.25  | 100.00 | 95.0±1.87 | 100.2±2.37  |
| LSD   | 4.80        | 15.4   | 0.2         | 4.80         |

1Means±SEM, n=10. aMeans within a column lacking a common superscript differ significantly (P<0.05). T1, dosed 0.7 mg/day ZnCl₂, for two weeks before mating up to day 5 of pregnancy, females of this group were mated with males dosed 0.7 mg/day ZnCl₂, for 2 weeks before mating; T2, dosed 0.7 mg/day ZnCl₂, for 2 weeks before mating up to the day 16 of pregnancy, mated with control males; T3, dosed 0.7 mg/day ZnCl₂ for two months before mating up to the end of pregnancy, mated with control males; Control, dosed with ZnCl₂ free water along the period of experiment and were mated with control males.

---

**ZAHIR ET AL.**
supplementation in adult rats. Supplementing ZnCl₂ additionally resulted in abnormal development in F1 offspring. In light of these findings, as well as those published in a two-generation study of Zn toxicity in rats (20) and in a study of subacute toxic effects of Zn in rats (21), where they found that F2 pups' viability and weaning indices, or sex ratios were not affected by ZnCl₂ treatment, there may be a need to reevaluate the risks of excessive Zn supplementation, particularly in infants and pregnant women).

Body Weight of Offspring

Table 2 shows the results of the pup body weights. On days 1, 4, 7, 14, and 21 of lactation period following ZnCl₂ treatment, there was a significant (P<0.05) decrease in mean pup body weights in the treated groups compared to the control group.

Results of a two-generation ZnCl₂ study conducted by (22) on rats were similar in terms of food intake and utilization reduction. Zn intake that is adequate and balanced before, during, and after pregnancy is necessary for the fetus’s and offspring’s optimal growth and development (23).

ZnCl₂ supplemented to pregnant females at 7 mg/day for two months caused significant reduction in weight gain and the efficiency of feed conversion during gestation. This demonstrates the association of ZnCl₂ excessive supplementation with appetite reduction through development of fetal and it could be attributable to poor pregnancy outcomes. All dams supplemented with ZnCl₂ showed significant reduction in the total number of pups, pups per litter, and live pups per litter. It was reported that females exposed to ZnCl₂ at 7.5 mg/kg/day (low dose) did not show significant effect on the number of miscarriages, although significant reduction was noted in the implantation efficiency. On the other hand, females exposed to medium and high doses of ZnCl₂ exhibited significant increase in the number of miscarriages without effecting the efficiency of implantation. Such findings could imply that the effect of ZnCl₂ on reproductive functions follows a dose-dependent manner, where the rate of fetal resorption was increased by low supplementation of ZnCl₂, while the rate of fetus death was increased by medium and high supplementation. As a result, it is the general nutritional condition of a mother that can play a significant role in both maternal and perinatal mortality and morbidity throughout pregnancy (24). Previous studies suggest that dams adequately supplemented with ZnCl₂ nutrition at low and high doses showed, respectively, reduction in implantation and increasing in stillbirths. This shows that in susceptible people, Zn deficiency or excess serve the same vital function. It has previously reported that offspring negative effects (25) such as fetal resorption elevated rates, litter size reduction, and congenital anomalies have all been associated with moderate to severe deficiency of Zn in experimental pregnant animals (20).

In conclusion, results from this study showed that ZnCl₂ at dose of 0.7 mg/day may have teratogenic defects at various stages of pregnancy in rats especially at third stage of pregnancy which represents the major physiological and developmental and minor organogenesis. It is recommended thus that pregnant women and children as high-risk groups must carefully use Zn supplementation, whether it is used as a food additive or in self-medication.

Table 2. Body weight (g) at different lactation periods of newborn pups birthed by Albino Wister female rats treated orally with 0.7 mg/day zinc chloride in different phases of pregnancy

| Group | 1     | 4     | 7     | 14    | 21    |
|-------|-------|-------|-------|-------|-------|
| T1    | 5.3±0.03 b | 9.2±0.20 b | 13.5±0.10 b | 25.4±0.20 b | 40.1±0.24 b |
| T2    | 5.1±0.01 b | 8.1±0.10 b | 12.2±0.08 b | 22.7±0.10 b | 38.6±0.40 b |
| T3    | 4.8±0.02 b | 6.9±0.21 c | 10.6±0.13 c | 20.1±0.23 c | 31.5±0.30 c |
| Control | 7.1±0.10 a | 10.3±0.30 a | 15.2±0.25 a | 27.8±0.41 a | 44.1±0.33 a |
| LSD   | 2.83   | 2.78   | 3.10   | 5.43   | 6.54   |

1MeansSEM, n=10. —Means within a column lacking a common superscript differ significantly (P≤0.05). T1, dosed 0.7 mg/day ZnCl₂ for two months before mating up to day 5 of pregnancy, females of this group were mated with males dosed 0.7 mg/day ZnCl₂ for 2 weeks before mating; T2, dosed 0.7 mg/day ZnCl₂ for 2 months before mating up to the day 16 of pregnancy, mated with control males; T3, dosed 0.7 mg/day ZnCl₂ for two months before mating up to the end of pregnancy, mated with control males; Control, dosed with ZnCl₂-free water along the period of experiment and were mated with control males.
بعض المطيافية المسمية في الجرذان المعرضة للكودرذ الزنك قبل وبعد الحمل

عبد الرحمن عبد الخالق زاهر، فلاح موسي كاظم الركيبي

أثرُ الخصوبة والكيمياء الحياتية والأدوية، كلية الطب البيطري، جامعة بغداد، العراق

الخلاصة

إن هذه الدراسة تشير إلى إمكانية استخدام كودرذ الزنك في النهر المعرضة للزنك قبل وبعد الحمل. تم تحديد النتيجة التي تم تقديمها في هذه الدراسة عن مجموعة واحدة من 288 ناقة. تم توزيع الناقات على مجموعتين، واحدة من 144 ناقة تم تزويجهن خلال الحمل، وثانيها من 144 ناقة تم تزويجهن قبل الحمل. تم رصد النشاط الجسدي والمسخية في جميع الناقات، وتحديد النتائج المترابط بلغة إنجليزية. النتائج تشير إلى أن كودرذ الزنك يحسن الخصوبة وتعزز من إنتاج الرضاعة الطبيعية.

الكتاب المختصر: 1. Koay SI, Bremner W, Bell JM, et al. Effect of zinc oxide nanoparticles on reproductive outcomes: A systematic review and meta-analysis. Reprod Toxicol. 2018; 79: 20-29. 2. Seregni E, Massimo M, Nerini Molteni S, Palotti S, van der Hiel B, et al. Lipid nanoparticles with minimum burst release of TNF-α siRNA show strong activity against rheumatoid arthritis unresponsive to methotrexate. J Control Release. 2018; 283: 280-289. 3. Fakhur A, Mohammad-Hasani A, Golagar AH. Zinc is an essential element for male fertility: a review of Zn roles in men's health, germination, sperm quality, and fertilization. J Reprod Infertil. 2018; 19(2): 69-81. 4. Piao F, Yokoyama K, Ma N, Yamauchi T. Subacute toxic effects of zinc on various tissues and organs of rats. Toxicol Lett. 2003; 145: 28-35. 5. Pal N, Pal B. Zinc feeding and copper status in sheep. Indian J Vet Res. 2018; 57: 437-440. 6. Zaporowska H, Wasilewski W. Combined effect of vanadium and zinc on certain selected hematological indices in rats. Comp Biochem Physiol. 1992; 103(1): 143-147. 7. Wilson RL, Leemfa SY, Goh Z, McNinch D, Jankovic-Karasulos T, Leghi GE, et al. Zinc is a critical regulator of placental morphogenesis and maternal hemodynamics during pregnancy in mice. Science Rep. 2017; 7(1): 51-57. 8. Grzeszczyk K, Kwiecikowski S, Kosik-Bogacka D. The role of Fe, Zn, and Cu in pregnancy. Biometr. 2020; 10(8): 1176-1180. 9. Ajayi AF, Akhigbe RE. Staging of the estrous cycle and induction of estrus in experimental rodents: an update. Fertil Res Pract. 2020; 6(5). 10. Seregni E, Massimo M, Nerini Molteni S, Palotti S, van der Hiel B, Cefalo G, et al. Serum and cerebrospinal fluid human chorionic gonadotropin (hCG) and alpha-fetoprotein (AFP) in intracranial germ cell tumors. Int J Biol Markers. 2002; 17(2): 112-118. 11. Klaassen CD, editor. Casarett and Doull's Toxicology: The Basic Science of Poisons. 7th edition. New York: McGraw-Hill; 2008. 1311 p. 12. Marty MS, Neal BH, Zabolotny CL, Yano BL, Andres R, Woolhiser MR, Boeverhoff DF, Saghir SA, Perella AW, Passage JK, Lawson MA, Bus JS, Lamb JC 4th, Hammond L. An F1-extended one-generation reproductive toxicity study in Crl:CD (SD) rats with 2,4-dichlorophenoxycetic acid. Toxicol Sci. 2013; 136(2): 527-547. 13. Snedecor G, Cochran W. Statistical methods. 8th edition. Iowa State University Press; 1989. 503 p. 14. Agency for Toxic Substances and Disease Registry (ATSDR). Public health statement zinc. Atlanta, GA: Public Health Service, U.S. Department of Health and Human Services; 2005. 15. Broun E, Greist A, Tricot G, Hoffman R. Excessive zinc ingestion. JAMA 1990; 264: 1441–3. 16. Johnson E, Gilbreath L, Ogden TC, Graham S, Gorham. Reproductive and developmental toxicities of zinc supplemented rats. Repro Toxicol. 2011; 31(2): 134–143. 17. Jackson MJ, Giugliano R, Giugliano LG, Oliveira EF, Shrimpton R, Swainbank I. Stable isotope metabolic studies of zinc nutrition in slum-dwelling lactating women in the Amazon valley. Brit J Nutr. 2008; 59(2): 193-203. 18. Moser-Weilicon PB, Patterson KY, Veillon C. Zinc absorption is enhanced during lactation. FASEB J 1996; 10: A729 (abstract). 19. Funk EB, Ritchie LD, Woodhouse LR, Roehl R, King JC. Zinc absorption in women during pregnancy and lactation: a longitudinal study. Am J Clin Nutr. 1997; 66(1): 80–88. 20. Khan AT, Graham TC, Ogden L, Ali S, Salwa, Thompson SJ. A two generational reproductive toxicology study of zinc in rats. J Environ Sci Health B. 2007; 42; 403-415. 21. Piao F, Yokoyama K, Ma N, Yamauchi T. Subacute toxic effects of zinc on various tissues and organs of rats. Toxicol Lett. 2003; 145: 28-35. 22. Pal N, Pal B. Zinc feeding and conception in the rats. Int J Vitam Nutr Res. 1987; 57: 437-440. 23. Zaporowska H, Wasilewski W. Combined effect of vanadium and zinc on certain selected hematological indices in rats. Comp Biochem Physiol. 1992; 103(1): 143-147. 24. Michie MW, Angererhofer RA, Barlow PM, Beall PA, Phase S. Effects of ingestion of zinc naphthenate on reproductive function of rats. National Technical Information Service (NTIS) Technical Report (NTIS/AD-A235-224); 1991; 33; 211-221. 25. Khittam SS, Alhtheal ED, Azhar JB. Effect of zinc oxide nanoparticles preparation from zinc sulphate (ZnSO4) against gram negative or gram positive microorganisms in vitro. Iraq J Vet Med. 2018; 42(1): 18-22.