IRAQI JOURNAL OF EMBRYOS AND INFERTILITY RESEARCHES (IJEIR)
Nisreen et al., Vol. 14, Issue 2, Pp. 17-38 (2024) Original Research
DOI: http://doi.org/10.28969/IJEIR.v14.i2.r2.24 E-ISSN: 2616-6984 P-ISSN: 2218-0265

The protective influence of Zinc Sulphate on some histological changes in Male mice embryos belonging to the mother injected with lead acetate.

Nisreen Flayyih[1], Ban Th. Saeed[1], Rana A. Al-Saadi[1], Zahraa Alzaidi[1]

[1]High Institute of Infertility Diagnosis and Assisted Reproductive Technologies, Al Nahrain University, Baghdad, Iraq.

nisreen.alseadi@st.nahrainuniv.edu.iq

ABSTRACT

Lead is a heavy metal toxicant, present widely in the environment and workplaces. Zinc has been shown to reduce the toxicity of lead. The present experimental animal study was designed to observe the changes in the testes due to excessive use of lead. This study aims to detect the effect of antioxidants (zinc) on the toxicity of high doses of lead on the reproductive organs of male mice embryos compared to the control group as a model for human representative. Thirty mature female mice were used (Swiss-Webster), as a mammalian model. They were divided into three groups, 10 mice/group after labelling them. They were randomly assigned into three experimental groups, and these embryos were processed for histological observations.

The recorded results showed a severe disruption in the organization of testicular structure with primordial germ cells severely degenerated with the absence of basal lamina and peritubular myoid cells of the treated group (G1) compared with the control group at day 14 and day 17. The embryo testes of (G2) at day 14 showed irregular testes cords with prominent degenerative changes compared to that of the control group (G3).

It was concluded from these results that zinc sulphate plays a significant role in the improvement of histological damage of the testes belonging to mothers injected with lead acetate and protects the reproductive organs from injury during lead toxicity.

Received: 07-Oct- 2024
Accepted: 30-Oct-2024
Published: 08-Dec-2024

How to cite:

Nisreen Flayyih, Ban Th. Saeed, Rana A. Al-Saadi , Zahraa Alzaidi; The protective influence of Zinc Sulphate on some histological changes in Male mice embryos belonging to the mother injected with lead acetate; Iraqi Journal of Embryos and Infertility Researches (IJEIR), (2024); 14(2): 17-38.
Doi: http://doi.org/10.28969/IJEIR.v14.i2.r2.24

KEYWORD

Lead, Zinc, Teratogenicity, Testes development.

1. Introduction

Exposure to chemical material during the early stages of pregnancy is of particular concern. Both unborn and newborn babies are thought to be more susceptible to chemical exposure because these periods represent the most complex and sensitive in terms of body development (Altshuler, N., 2003 [1]). Chemical toxicants in the environment, poverty, and little or no access to health care are all factors contributing to life-threatening pediatric diseases; children are uniquely vulnerable to chemical toxicants because of their disproportionately heavy exposures and their inherent biological growth and development (Suk WA et al., 2003[2]) Lead is the oldest cumulative toxic metal that seriously contaminates the environment. Because of its malleability, resistance to corrosion, and low melting point, humans have used lead since prehistoric times to fabricate statues, jewelry, water pipes, and drinking vessels (RÍsovÁ V, 2019[3]).

Lead is present in batteries, leaded gasoline, paints, water pipes, insecticides, and some cosmetics. Air, water, soil, food, and consumer products are the major routes of human exposure to lead (M. M. El-Tohamy and W. S. El-Nattat, 2010 [4]). May be exposed by breathing, eating, or drinking the substance or by skin contact (Abadin H et al., 2007[5]).

The toxic effect of lead on reproduction is pervasive, affecting basically all aspects of the reproductive system (Moniem A. et al., 2010 [6]). One mechanism by which lead exerts its adverse effect is by inducing oxidative stress (OS). Oxidative stress represents an imbalance between reactive oxygen species (ROS) production and a biological system's antioxidant.

Defence mechanism (Singh RP et al., 2010[7]). Because of a high content of polyunsaturated membrane lipids, reproductive tissue becomes one of the targets for OS (Mishra M et al.,2004 [8]).

Heavy metals like lead and cadmium are well-known to cross the placenta and accumulate in fetal tissues. The study of the hypothalamic-pituitary-gonadal axis in animals exposed to the metal is of great interest since the levels of lead in air, water, soil, and foods have increased several-fold in many parts of the world as a result of emissions from industrial activities (Sebahat T et al., 2005[9]). Prenatal exposure to lead poses a health threat, particularly to the developing brain. Fetal exposure to lead correlates with reduced birth weight and birth size (Gundacker C, Hengstschläger M, 2010[10]). High lead blood levels during pregnancy increase the risk of miscarriage and can make the baby be born early or at a low birth weight. Even low lead levels in a child can cause behavior and learning problems. Lead toxicity includes damage to soft tissues, mainly the liver, kidney, and the reproductive system; depression of hematopoiesis) Greenpeace, 2005 [11]).

Reactive oxygen species (ROS) are the free radicals that result from O2 metabolism. They have short half-lives, but their high production harms the cell (Alfadda A. A. and Sallam R. M et al.,2012[12]). Antioxidants are the main defense mechanism against oxidative stress induced by free radicals. In general, they are free radical scavengers that suppress the formation of ROS and /or oppose their actions (Venkatesh S et al., 2009 [13]). They can deal with ROS, but excessive amounts of ROS or impaired antioxidant defense mechanism oxidative stress will occur, which is harmful to the cell. These antioxidants include enzymes.

Such as catalase, glutathione peroxidase, and reductase (Kurpisz M et al., 1996[14]), besides non-enzymatic antioxidants such as vitamin C, zinc, vitamin E, and micronutrients (Henkel, R.R. et al.,2003 [15]). Zinc is second to iron as the most abundant trace element in the body (Vish wanath, 1998 [16]). High doses of metals such as zinc, copper, and iron can become toxic. Klauder et al. reported that low dietary copper and iron enhanced lead absorption in rats (Klauder DS et al. 1979[17]). It has been observed that the gastrointestinal absorption of lead increases in the presence of ascorbic acid (Conrad ME, Barton JC, 1978[18]), whereas it decreases in the presence of iron and magnesium (Barltrop D, Khoo HE, 1976[19]). Lead and zinc interaction also have been observed at absorptive and enzymatic sites (Flora SJS et al., 1983[20]). Kagi and Vallee have shown that in the gastrointestinal tract, zinc and lead compete for similar binding sites on the metallothionein-like transport protein (Kagi JHR et al. 1961[21]). Zinc as a dietary supplement combined with ascorbic acid (Papaioannou RA et al., 1978[22]) and thiamine (Flora SJS et al., 1989[23]) has also been shown to reduce lead toxicity. As in most tissues, zinc ranks second to iron in concentration in the testis, bridging the gap between the macro- and micronutrients. Zinc is involved primarily in nucleic acid and protein metabolism and, hence, in cell replication's fundamental processes. In view of the prominent localization of zinc in the spermatozoa within the seminiferous tubules and the specific need for zinc in spermatogenesis, the present studies were designed to investigate the molecular basis of the interaction of zinc and lead in the rat testis. High zinc content has been noted in ocular tissues, seminal.

Vesicles, epididymes, and the prostate (Aitken, R.J. et al. 1978[24]). It has been shown that the majority of zinc present in seminal plasma comes from the prostate. It is an integral component of nearly 300 enzymes in different species. Zinc plays an important role in the physiology of spermatozoa. It may also have a role in sperm production and /or viability in preventing spermatozoa degradation and in sperm membrane stabilization (Corpas et al. 1995[25]).

2. Patients and Methods

The experiments were performed on 30 mature female Swiss-Webster mice; their ages ranged between 6-8 weeks with a body weight (B.wt) ranging between 28-30g; these females in the metestrus phase were left with mature, healthy males for mating (1male/2female). The occurrence of a vaginal plug was considered as the first day of pregnancy; the subsequent days were sequentially numbered. The pregnant female was removed into separate cages. 1g of the powdered lead acetate dissolved with 2500ml of normal saline. The solution obtained was well mixed, and then 12.5ml from this stock solution was withdrawn and mixed with 200ml of normal saline to get the concentration of 0.5mg/kg b. 0.1g of the powdered zinc dissolved with 250ml of normal saline. The solution obtained was well mixed, and then 1.25ml from this stock solution was withdrawn and mixed with 8ml of normal saline to get the concentration of 0.5mg/kg b. These animals were divided into three groups (10 mice/group) as follows:

1 .Experimental group (G1): Injection of lead acetate at a dose of 50 mg/kg body weight from the first day of gestation for 3 days.

2 .Experimental group (G2): This group was orally administrated with zinc sulphate (0.5mg/kg body weight/day from the first day for 3 weeks period of the experiment and Injection of lead acetate at a dose of 50

mg/kg body weight from the first day of gestation for 3 days.

3 .Control group (G3): This group was orally administered with distilled water during the 3-week period of the experiment.

When the females in G1, G2, and G3 reach day 14 of gestation, (5) females from each group were sacrificed. After the abdominal incision, a number of dead and live fetuses in each horn were recorded. Each fetus was washed and weighed for all groups to see the events that may occur during this period. The other (5) females in the experiment were sacrificed at day 17 to demonstrate the histological effect that occur at this period on testes. The

treated embryos were fixed in Bouins fixative for 24 hr., then dehydration, infiltration with paraffin, and embedded sections were stained with haematoxylin and eosin; the specimens were independently read and reviewed by two pathologists who were unaware of the drug and the dose.

3. Results

The histological sections of the male embryo belong to mothers from the control group (G3) show the linkage of the gonads to the mesonephros at the abdominal cavity; these gonads with the tunica albuginea at their periphery contained numerous, well-organized testicular cords in which primordial germ cells surrounded by peritubular myoid cells were separated by somatic Sertoli cells at the periphery, the interstitium contained steroidogenic Leydig cells precursors, pericyts were observed in close association with endothelial cells of normal gonadal capillaries at day 14 of pregnancy (Figure1).

At day 17 of gestation, the male embryo exhibits well-organized testicular cords with Sertoli cells surrounding the germ cells, peritubular myoid cells and extensive interstitial tissue, including Leydig cells that have round nuclei. The characteristic male-specific coelomic vessels, pericytes

around developing capillaries, were visible in the mesenchyme (Figure 2).

The histological sections of male embryos belong to mothers from (G1) that were Injected with lead acetate at a dose of 50 mg/kg body weight at days 14 and 17 show a severe disrupting in the organization of the testicular structure. Primordial germ cells severely degenerated with the absence of basal lamina and pritubule myoid cells. The characteristic male-specific coelomic vessels were reduced in mesenchyme with severe vaculation as compared to that of the control group figure [3, 4]. The histology of the male gonads of the

embryos belongs to mothers orally administrated with zinc sulphate (0.5mg/kg body weight/day during the 3 weeks period of the experiment and Injection of lead acetate at a dose of 50 mg/kg body weight from the first day of gestation for 3 days (G2), showed mild degenerative changes and necrosis of spermatogenic cells, no organized testicular cords, peritubular myoid cells or mesenchyme were seen. (Figure 5).

The histological sections of the testes of the male embryos at day 17 showed irregular testes cords with prominent degenerative changes compared to that of the control group (G3), the basal lamina was disrupted in some regions. The sections also showed disorganized and necrosis of testicular cords degenerative of primordial germ cells; numerous vacuoles were appeared throughout the structure of testicular cords. (Figure 6)

The results show the descending of the testes to its normal position in (G2) and control group (G3) at day 17, but it.

Remained adjacent to the kidneys (metanephron) at the upper part of the abdominal cavity at (G1) (Figure7: A, B.C).

The statistical analysis showed a highly significant decrease ($P< 0.01$) in the weights of fetuses belongs to mothers injected with lead acetate at a dose of 50 mg/kg body weight at day 14 in comparison with that of the control group, similar results revealed by

embryo belongs to mothers administered with zinc sulphate (0.5mg/kg body weight/day during the 3 weeks' period of the experiment and Injection with lead acetate at a dose of 50 mg/kg body weight at day 17 as shown in table (1). The statistical analysis showed a highly significant decrease ($P< 0.01$) in the number of fetuses belongs to mothers' injection with lead acetate at a dose of 50 mg/kg body weight (G1) in comparison with that of the control group, similar results revealed by embryo belongs to mothers administered with zinc sulphate

(0.5mg/kg body weight/day during the 3-week period of the experiment and Injection with lead acetate at a dose of 50 mg/kg body weight (G2) at day 17, as shown in Table (2).

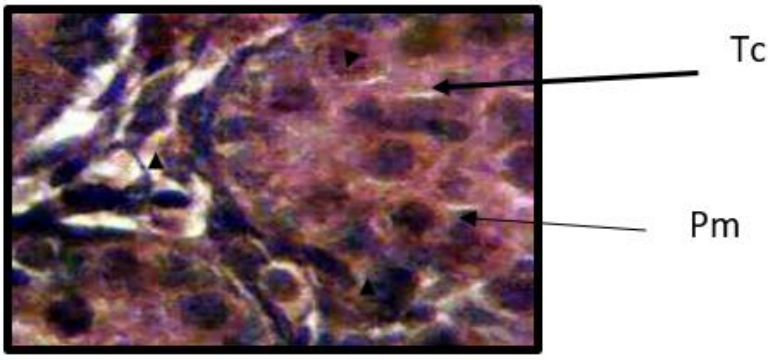

Figure (1): Testes section of male mice embryo aged 14 from the control group (G3). Note the numerous, well-organized testicular cords (Tc) in which primordial germ cells (Pm) (large cells with prominent nuclei) are separated by somatic Sertoli cells (Sc) (H&E, 40X).

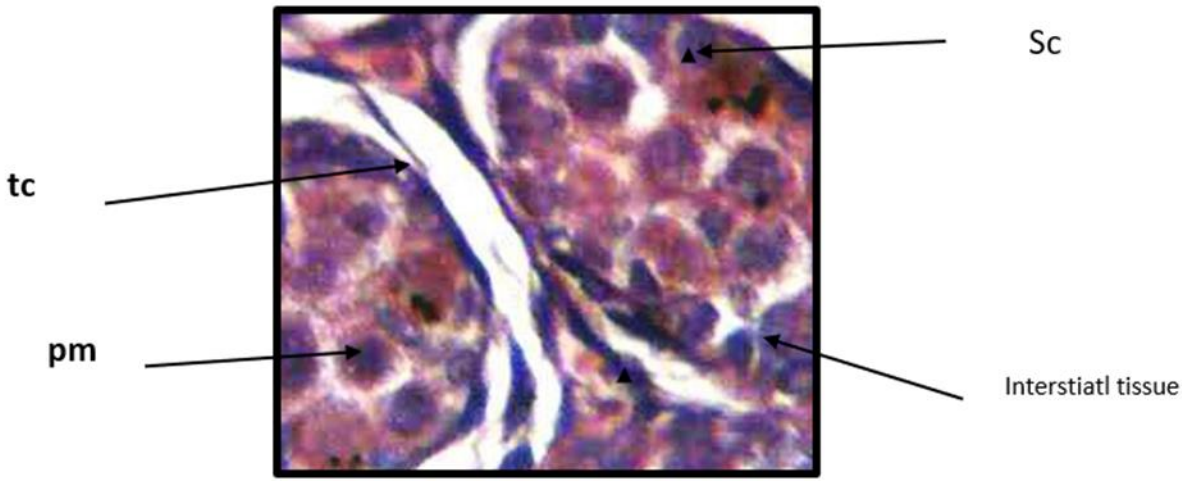

Figure (2): Testes section of male mouse embryo aged 17 of G3 (control group). Note the well-organized testicular cords (tc) filled with germ cells (g) and Sertoli cells (Sc) surrounding primordial germ cells (pm) with extensive interstitial tissue (H&E, 40X).

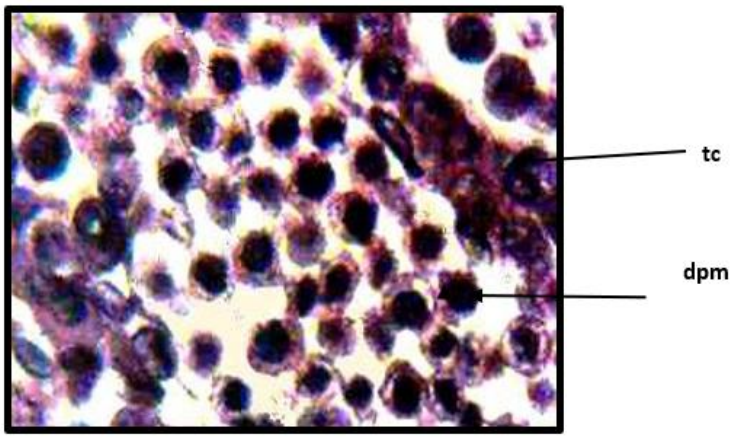

Figure (3): The test section of a male mouse embryo aged 14 (G1) belongs to the mother Injected with lead acetate at a dose of 50 mg/kg body weight. Note disorganized and lack of distinct testicular cords, severe degenerative of primordial germ cells (dpm), which are irregular in shape with less density (H&E 40)

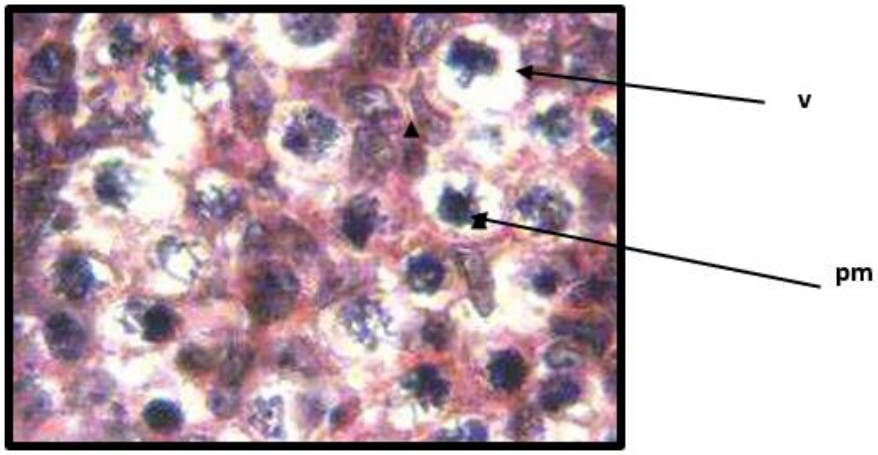

Figure (4): The test section of a male mouse embryo aged 17 (G1) belongs to the mother Injected with lead acetate at a dose of 50 mg/kg body weight. Note the severely affected primordial germ cells (pm) with typical vaculation (v) (H&E, 40X).

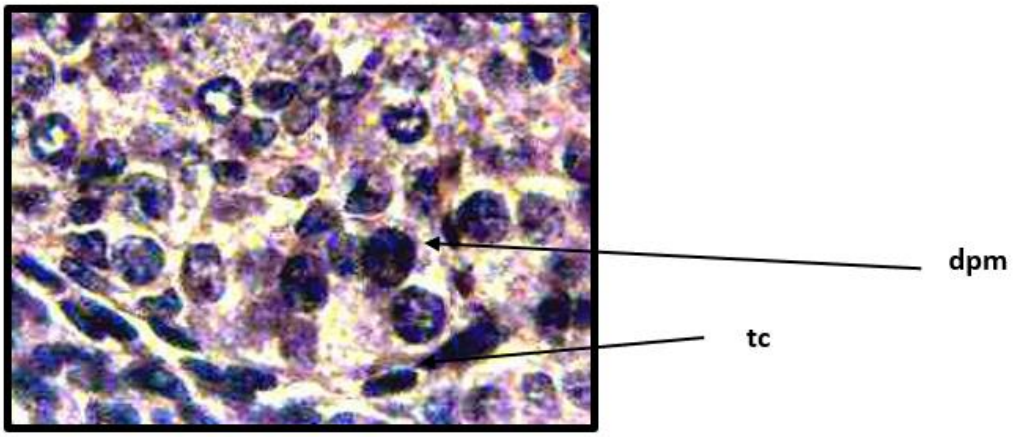

Figure (5): Testes section of a male mouse embryo aged 14 belongs to the mother orally administrated with zinc sulphate (0.5mg/kg body weight/day during the 3 weeks period of the experiment and Injection of lead acetate at a dose of 50 mg/kg body weight from the first day of gestation for 3 days (G2). Note disorganized and lack of distinct testicular cords (tc), degenerative seen in Primordial germ cells (dpm), which are irregular in shape with less density (H&E, 40X).

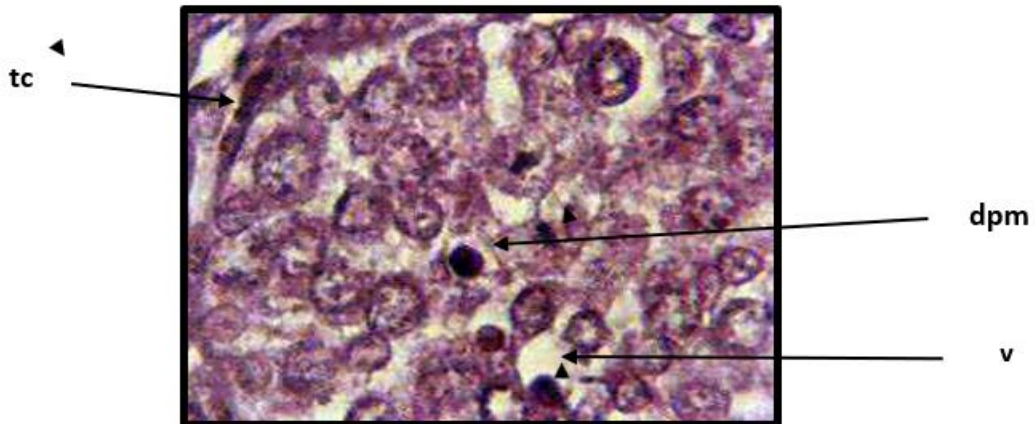

Figure (6): Testes section of a male mouse embryo aged 17 (G2) belongs to the mother orally administrated with zinc sulphate (0.5mg/kg body weight/day during the 3 weeks period of the experiment and Injection of lead acetate at a dose of 50 mg/kg body weight from the first day of gestation for 3 days. Note disorganized and necrosis of testicular cords (tc) degenerative of primordial germ cells (dpm). Numerous vacuoles (v) appeared throughout the structure of testicular cords (tc). (H&E, 40X).

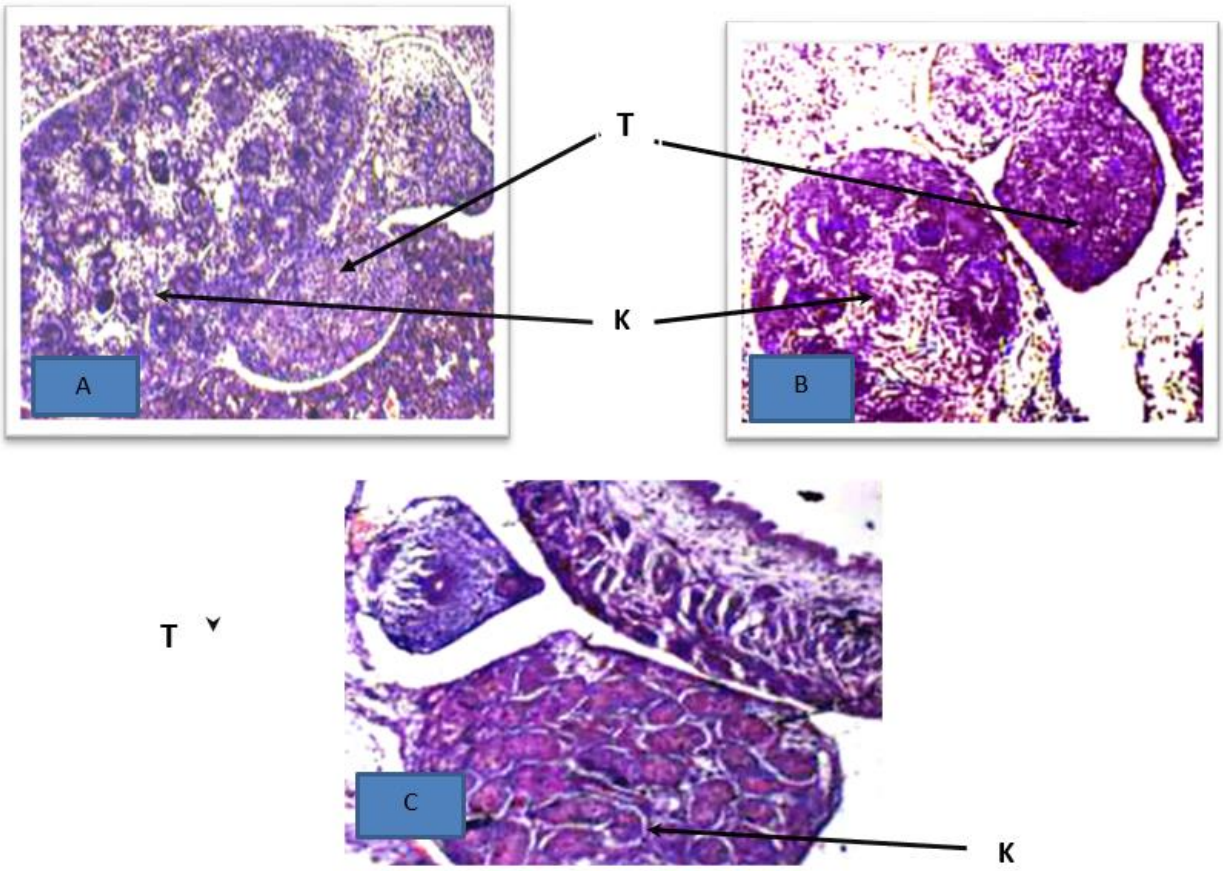

Figure (7): Longitudinal section of mouse embryo aged 17. A: showing the persistence of the testes adjacent (t) to the kidneys (k) (metanephron) in the abdominal cavity in the treated group (G1), B: showing the persistence of the testes adjacent (t) to the kidneys in the treated group (G2), C; showing the persistence of the testes adjacent (t) to the kidneys from the control group (G3), (H&E, 10X).

Table (1): Changes in weight of the embryos belonging to the mother Injected with lead acetate.

	groups	Mean± S.E.	P value			
			Among groups	Group 1 vs. control	Group 2 Vs. control	Group 1 vs. Group 2
Weigh of embryos on day 14	Group 1 (G1)	0.471±0.038	≤0.001**	≤0.001**	≤0.001**	0.029*
	Group 2 (G2)	0.584±0.03				
	Control (G3)	0.784±0.009				
Weight of embryos on day 17	Group 1(G1)	1.125±0.038	≤0.001**	≤0.001**	≤0.001**	≤0.001**
	Group 2(G2)	1.484±0.018				
	Control (G3)	1.696±0.032				

Values are mean ± standard error (SEM), (n=10 animals/group)

NS= no statistically significant difference

*=Statistically significant difference (P<0.05)

** =Highly statically significant difference (P< 0.01).

Table (2): Changes in the number of embryos belongs to the mother injection with lead.

	Group 1				Group 2				Control(G3)			
	No. of males		No. of Females		No. of males		No. of Females		No. of males		No. of Females	
	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent	No.	Percent
Total no. of embryos	44	100%	33	100%	56	100%	53	100%	68	100%	75	100%
Total male & female	77				109				143			
P value	0.833				0.771				0.007			

Values are mean ± standard error (SEM), (n=10 animals/group)

NS= no statistically significant difference *=Statistically significant difference, (P<0.05)

**=Highly statically significant difference (P< 0.01)

4. Discussion

In the present study, the injection of lead acetate at a dose of 50 mg/kg body weight from the first day of gestation for 3 days to pregnant female mice and sacrificed at 14, 17 revealed many negative effects, including numbers and weight of fetuses and the histology of the male testes. According to previous studies on rodents, the toxic dose of lead acetate was 100–200 mg kg (Shayne C, 2024 [26]); these studies agree with our results.

The results of lead in this study showed its ability to cross the placenta of pregnant female mice and reach the embryonic and fetal tissues; this is similar to the results of Homan and Brogan (Homan, C.S., and Brogan 1993[27]), which showed that lead can pass through the placenta of pregnant female mice, presumably by passive diffusion, and accumulate in embryos tissues over the period of gestation (McGivern, R.F et al., 1991[28]). Gonzale-Cossio et al. proved (Gonzale-Cossio, T. et al. 1997 [29]) that lead exposure during a period of pregnancy when structures related to the Hypothalamus Pituitary Gonadal (HPG) axis are undergoing rapid proliferation, the exposed animals at significant risk for reduced reproductive capacity in adulthood, and elicit toxic, pathological changes in the testes leading to atrophy of the organ.

Our study showed that lead exposure increased the risk of reduced fetal weight; these results agreed with that found by (IPCS) (IPCS, 1995[30]), who proved that infants born to mothers with prenatal occupational exposure had an increased risk of low birth weight. The mechanism by which the fetal body weight is reduced due to decreased fetal growth because lead has a wide range of biological effects depending on the level and duration of exposure. Effects at the subcellular level and overall function of the body have been noted and range from inhibition enzymes to the production of

marked morphological changes (Brent, R.L. et al., 2004[31]).

The timing and duration are critical; the level of response to a given dose may change dramatically depending on the stage of development at which a fetus is exposed (Danielson, B.R, et al. 1983 [32]). Furthermore, the dose of lead the fetus receives is determined by the amount of the substance transported across the placenta and the rate of metabolism and elimination of the substance (Odiette, W.O.,1999[33]).

The fetal weight reduction may also be attributed to the replacement of vital minerals such as calcium and potassium by lead, and binding with the red blood is believed to reduce the oxygen-carrying capacity of the cells, which makes red blood cells destroy more rapidly, thereby impairing the synthesis of haemoglobin in heamopeotic tissues) Guyton, A.C. and Hall, 2016 [34]).

In experimental group G1, G2 the significant decrease in fetuses' number may be related mainly to the high percentage of failure of implantation compared to that of the control group (G3); this result may be explained on the bases that implantation depend upon the presence of good amount of estrogen and progesterone (Heap, R.B.; et al., 1981[35]). Ovarian progesterone and estrogen are crucial for implantation in mice and rats (Bowman, P. and McLaren, A., 1970 [36]). There are a reduced number of embryos and a reduced number of cells per embryo in the absence of these hormones (Dey, S.K., 2003 [37]), and it has been found that estrogen plays a critical role in determining the window of uterine receptivity for embryo implantation. Moreover, a possible mechanism of action of lead as a cause of infertility might be due to its antiestrogenic activity (Tchernitchen, N.N,1998[38]).

Furthermore, a reduction in the number of offspring of laboratory animals and in the families of workers

occupationally exposure to lead might be due to impaired ovarian function as the impairment of normal maturation of this function (Pinon- Lataillaade, G.,1995,[39]).

The results of (G1), which was injected with lead acetate at a dose of 50 mg/kg body.

Weight from the first day of gestation for 3 days shows a severe disrupting in the organization of testicular structure, and primordial germ cells severely degenerated with the absence of basal lamina and pritubule myoid cells at day 14 and day 17 in comparison with G2 and control group, this may be due to receiving the whole dose by embryos at this period of gestation because the defense mechanism was not well developed leading to accumulation of lead in gonads which in turn causing more loose and disrupted structures of the testes and due to rapid growth at the period of this gestation (day 17), leading to more disruption and necrosis these results are in a good agreement with the findings of (Kostial, K. and Momcilovic, B. ,1974[40]) that cited the destruction of the lining epithelium of blood vessels, which lead to the prevalence of plasma and electrolytes and infiltration quantities of plasma to the interstitial tissues of the testes.

The reduction in testes recorded in (G1) are due to the accumulation of lead in the gonads and testicular tissues which. Affects the physiology of reproduction. (Silbergeld, E.K.,1983 [41]) , causing testicular damage and degenerative changes in the testicular tissues. (Moorman, W.J. et al., 1998 [42]) Leading to dysfunction of the Sertoli cells, which is responsible for germ cell proliferation and maturation (Moorman, W.J. et al.,1991[43]).

The present study showed the efficacy of zinc sulfate in preventing the toxic effects of lead acetate in mice embryos. Zinc has been shown to protect animals and cell cultures from the acute toxicity of heavy metals (Liu, D. Y., and Baker 1992, [44]) and zinc-containing.

Enzymes are known to be involved in the synthesis and/or degradation of carbohydrates, lipids, proteins, and nucleic acids (Robert, K. M et al. 1997[45]). Thus, it plays an important role in body metabolism which is reflected by an increase in body weight and reproductive organs weight.

In addition, zinc has been reported to have a membrane stabilizing.

Antioxidant activity and maintains sperm viability by inhibiting DNAase (Aitken, R.J, et al.,1987[46]). It is also an integral part of many metallo-enzymes, which is believed to stabilize membranes and protect them against free radical injury. Therefore, it appears to be a potent scavenger of excessive superoxide anion produced by defective spermatozoa. (Irvine, D.S, 1996,[47]).

Undescending of the testes to its normal position was observed in the experimental group (G2). It remained adjacent to the kidneys at the upper part of the abdominal cavity while it was.

Normally relocating at the base of the abdominal cavity as it appeared in the control group (G3) and G2 at day 17 and descent into the inguinal canal . Transfer of the male gonad from its site of origin at the urogenital ridge, as opposed to the kidney, into the scrotum is a critical event in male sexual differentiation (Bernstein, L.; et al.,1988 [48]) and since testicular descent is hormonally regulated in which the presence of testosterone induces regression of the cranial suspensory ligaments (CSL) (Hutson, J.M et al., 1994[49]), Furthermore the Leydig cells are an important target for the harmful action of lead which interferes with several steps in the testosterone biosynthetic pathway, leading to a reduction in plasma and intratesticular levels of testosterone (Thoreux-Manlay, A et al.,1995 [50]).

5. Conclusion:

It was concluded from these results that zinc sulphate plays a significant role in the improvement of histological

damage of the testes belonging to mothers injected with lead acetate and protected the reproductive organs from injury during lead toxicity.

Acknowledgement

We would like to acknowledge the High Institute of Infertility Diagnosis and Assisted Reproductive Technologies/ Al Nahrain University, Baghdad, Iraq.

Funding

This work received no funding .

Author Contribution

Nisreen Flayyih, Ban Th. Saeed, Rana A. Al-Saadi1, and Zahraa Alzaidi performed the study.

Conflict of Interest

The authors declare no conflict of interest .

Ethical Clearance

The Ethical Approval Committee approved the study.

Financial Disclosure

There is no financial disclosure.

References

[1] Altshuler, N. (2003). Children's environmental exposures. DCHP paper Series on Children's Health and the Environment. Washington, D.C. USA; US Environmental Protection Agency .

[2] Suk WA, Murray K, Avakian MD. Environmental hazards to children's health in the modern world. Mutat Res. 2003 Nov;544(2-3):235-42. Doi: 10.1016/j.mrrev.2003.06.007. PMID: 14644325.

[3] RÍsovÁ V. The pathway of lead through the mother's body to the child. Interdiscip Toxicol. 2019 Sep;12(1):1-6. doi: 10.2478/intox-2019-0001. Epub 2020 Feb 20. PMID: 32189981; PMCID: PMC7061448.

[4] M. M. El-Tohamy and W. S. El-Nattat, "Effect of Antioxidant on Lead-Induced Oxidative Damage and Reproductive Dysfunction in Male Rabbits," Journal of American Science, Vol. 6, No. 1, 2010, pp. 613-622.

[5] Abadin H, Ashizawa A, Stevens YW, Llados F, Diamond G, Sage G, Citra M, Quinones A, Bosch SJ, Swarts SG. Toxicological Profile for Lead. Atlanta (GA): Agency for Toxic Substances and Disease Registry (US); 2007 Aug. PMID: 24049859.

[6] Moniem A, Dkhil M, Al-Quraishy S: Protective role of flaxseed oil against lead acetate induced oxidative stress in testes of adult rats. Afr J Biotechnol 2010, 9(42):7216–7223 .

[7] Singh RP, Shashwat S, Suman K: Free Radicals and Oxidative Stress in Neurodegenerative Diseases: Relevance of Dietary Antioxidants. Journal of Indian Academy of Clinical Medicine JIACM 2004, 5(3):218–225.

[8] Mishra M, Acharya UR: Protective action of vitamins on the spermatogenesis in lead-treated Swiss mice. J Trace Elem Med Biol 2004, 18(2):173–178.

[9] Sebahat T, Bünyamin K, Günfer T, Gülten E and Osman G. Effects of cadmium and zinc on plasma levels of growth hormone, insulin-like growth factor I, and insulin-like growth factor-binding protein 311. Biological Trace Element Research, 2005; 108: 197-204.

[10] Gundacker C, Hengstschläger M. The role of the placenta in fetal exposure to heavy metals. Wien Med Wochenschr. 2012 May;162(9-10):201-6. doi: 10.1007/s10354-012-0074-3. PMID: 22717874.

[11] Greenpeace (2005). A Chinese child sits amongst a pile of wires and- waste, picture. Pp 53-82.

[12] Alfadda A. A. and Sallam R. M., Reactive oxygen species in health and disease, Journal of Biomedicine and Biotechnology. (2012) 2012, 14, 936486, https://doi.org/10.1155/2012/936486, 2-s2.0-84866133072.

[13] -Venkatesh S, Gurdeep Singh M, Prasad Gupta N, Kumar R, Deecaraman M, Dada R. Correlation of sperm morphology and oxidative stress in infertile men. Iran J Reprod Med. 2009;7:29–34.

[14] Kurpisz, M., Miesel, R., Sanocka, D. and Jendrzejczyk, P. Seminal plasma can be a predictive factor for male infertility. Hum. Reprod., 1996; 11: 1223–1226 .

[15] Henkel, R.R., Defosse, K. and Koyro, H.W. Estmate of oxygen consumption and intercellular zinc concentration of human spermatozoa in relation to motility: Asian, J.Anodrol., 2003; 5:3-8.

[16] Vish wanath, M. Inorganic elements in: Sardesai, Vish wanath, M., Introduction to clinical Nutrition. 2nd Ed. New York, 1998; 98-100.

[17] Klauder DS, Murthy L, Petering HG. Effect of dietary intake of lead acetate on copper metabolism in male rats. In: Hemphill DD, ed. Trace substances in environmental health. Columbia, MO: University of Missouri; 1973:131. 9. Six KM ,

[18] Conrad ME, Barton JC. Factors affecting the absorption and excretion of lead in the rat. Gastroenterology. 1978;74:731±40.

[19] Barltrop D, Khoo HE. The influence of dietary minerals and fat on the absorption of lead. Science Tot Environ. 1976;6:265±73 .

[20] Flora SJS, Jain VK, Behari JR, Tandon SK. Protective role of trace metals in lead intoxication. Toxicol Lett. 1982;13:51±6 .

[21] Kagi JHR, Vallee BL. Metallothionein, a cadmium and zinc-containing protein from the equine renal cortex. J Biol Chem. 1961; 236:2435±42.

[22] .Papaioannou RA, Sohler A, Pfeiffer CC. Reduction of blood lead levels in battery workers by zinc and vitamin C. J Orthomol Psychiat. 1978;7:1±13 .

[23] Flora SJS, Singh S, Tondon SK. Thiamine and zinc in prevention or therapy of lead intoxication. J Internat Med Res. 1989;17:68± 75.

[24] Aitken, R.J., Fisher, H.M., Fulton, N., Gomez, E., Knox, W., Lewis, B., and Irvine, S. Reactive oxygen species generation by human spermatozoa is induced by exogenous NADPH and inhibited by the flavor protein inhibitors diphenylene iodonium and quinacrine. Mol Reprod Dev.,1987; 47: 468 - 482.

[25] Corpas, I.; Gaspar, I.; Martinez, S.; Codesal, J.; and Candelas, S. (1995). Testicular alteration in rats due to gestational and early lactation administration of lead. Reprod. Toxicol. 9: 307-313.

[26] Shayne C. Gad, in Encyclopedia of Toxicology (Fourth Edition), 2024.

[27] Homan, C.S. and Brogan, G.X. (1993). Lead Toxicity. In Handbook of Medical Toxicology. Viccellio, P. (ed.). Little, Brown and Company, London. Pp: 271-273.

[28] McGivern, R.F; Sokol, R.Z. and Berman, N.G. (1991). Prenatal lead exposure in the rat during the third week of gestation; Long-term behavioral, physiological, and anatomical effects associated with reproduction. Toxicol. Appl. Pharmacol. 110: 206-215.

[29] Gonzale-Cossio, T.; Peterson, K.E.; Sanin, L.; Fishbein, S.E.; Palazuelos, E.; Aro, A.; Hernandez-Avila, M. and Hu, H. (1997). Decrease in birth weight in relation to maternal bone lead burden. Pediatrics. 100: 856-862 .

[30] (IPCS) (1995). International Panel of Chemical Safety. Inorganic lead- Environmental Health Criteria 165. International Programme on Chemical Safety. World Health Organization, Geneva, Switzerland .

[31] Brent, R.L. (2004). Environmental causes of human congenital malformations, the pediatrician's role in dealing with these complex clinical problems caused by a multiplicity of environmental and genetic factors. Pediatrics. 113(4 suppl). 957-68 .

[32] Danielson, B.R.; Dencker, L. and Lindgren, A. (1983). Transplacental movement of inorganic lead in early and late

gestation in the mouse. Arch. Toxicol. 54: 97-107 .

[33] Odiette, W.O. (1999). Environmental physiology of animals and pollution. Diversified Resources Ltd. Lagos. P 261.

[34] Guyton, A.C. and Hall, J.E. (2016). Textbook of Medical Physiology, Used, Elsevier Saunders, Philadelphia, Pennsylvania. Pp: 1017-1018 .

[35] Heap, R.B.; Flint, A.P.; Hartmann, P.E.; Gadsby, J.E.; Staples, L.D.; Ackland, N. and Hamon, M. (1981). Oestrogen production in early pregnancy. J. Endocrinol. 89 (Suppl). 77 -94 .

[36] Bowman, P. and McLaren, A. (1970). Cleavage rate of mouse embryos in vivo and in vitro. J. Embryol. Exp. Morphol. 24: 203-207 .

[37] Dey, S.K. (2003). Fertility study shows that increased estrogen shortens the window of implantation in mice. Vanderbilt University Medical Center, Clinton.colmenares@vanderbilt.edu.615: 322-4747 .

[38] Tchernitchen, N.N.; Villagra, A. and Tchernitchen, A.N. (1998). Antiestrogenic activity of lead. Environ. Toxicol. Water Qual. 13: 43-53 .

[39] Pinon- Lataillaade, G.; Thoreux-Manlay, A.; Coffigny, H.; Masse, R. and Soufir, J.C. (1995). Reproductive toxicity of chronic lead exposure in male and female mice. Hum. Experiment. Toxicol. 14: 872-878 .

[40] Kostial, K. and Momcilovic, B. (1974). Transport of lead 203 and Calcium 47 from mother to offspring. Arch. Environ. Health. 29: 28-30 .

[41] Silbergeld, E.K. (1983). Experimental studies of lead neurotoxicity. Biol. Reprod. 33: 722-728.

[42] Moorman, W.J.; Skaggs, S.R.; Clarck, J.C. and Simon, S.D. (1998). Male reproductive effects of lead, including species extrapolation for the rabbit model. Reprod. Toxicol. 12: 333-46 .

[43] McGivern, R.F; Sokol, R.Z. and Berman, N.G. (1991). Prenatal lead exposure in the rat during the third week of gestation; Long-term behavioral, physiological, and anatomical effects associated with reproduction. Toxicol. Appl. Pharmacol. 110: 206-215.

[44] Liu, D. Y. and Baker, H. W. G. Tests of human sperm function and fertilization in vitro. Fertil. Steril., 1992; 58:465–483 .

[45] Robert, K. M., Daryl, K.G., Peter, A. M., and Victor, W. K. Harpers Biochemistry.24th Ed.Appleton &Lange, 1997; Pp.155-521.

[46] Aitken, R.J., Fisher, H.M., Fulton, N., Gomez, E., Knox, W., Lewis, B., and Irvine, S. Reactive oxygen species generation by human spermatozoa is induced by exogenous NADPH and inhibited by the flavor protein

inhibitors diphenylene iodonium and quinacrine. Mol Reprod Dev., 1987; 47: 468.

[47] Irvine, D.S. Glutathione as a treatment of male infertility. Rev Reprod., 1996; 1: 6-12.

[48] Bernstein, L.; Pike, M.C.; Depue, R.H.; Ross, R.K.; Moore, J.W. and Henderson, B.E. (1988). Maternal hormone levels in early gestation of crypt orchid males: A case-control study. Br.J. Cancer. 58: 379-381 .

[49] Hutson, J.M.; Baker, M.; Terada, M.; Zhou, B. and Paxton, G. (1994). Hormonal control of testicular descent and the cause of cryptorchidisim. Reprod. Fertil. Dev. 6: 151-156 .

[50] Thoreux-Manlay, A.; Velezdelacalle, J.F.; Olivier, M.F.; Soufir, J.C.; Masse, R. and Pinon-Lataillade, G. (1995). Impairment of testicular endocrine function after lead intoxication in the adult rat. Toxico. 100; 101-109.