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Assessment of the protective effect of vitamin E on the quality of spermatogenesis and sperm parameters in rats exposed to lead

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ABSTRACT

Background and aims: Vitamins are a rich source of antioxidants that can neutralize the harmful effects of lead on spermatogenesis and sperm parameters. The aim of this study was to determine the effects of vitamin E on spermatogenesis and sperm parameters in lead-exposed rats.

Methods: This is an experimental study on 45 male Wistar that were allocated to 5 groups: distilled water gavage, intraperitoneal distilled water, vitamin E, lead, and lead+ vitamin E. All the rats were exposed to lead for 30 days. Then, the rats were sacrificed and sperm count, motility and morphology of sperm chromatin, and epididymitis and testis weight were investigated.

Results: The findings showed significant differences in weight gain, epididymitis weight, sperm death, the highest mobility of sperm among the groups ($P<0.05$). Vitamin E and control (gavage) rats experienced more weight than other groups. There was a significant difference in the highest mobility of sperm between peritoneal control group and other groups ($P<0.001$), and in lower than 50% mobility between lead group and other groups ($P<0.05$). There was a significant difference in sperm death between lead group, and the control groups and vitamin E group ($P<0.05$) with the least sperm death. Vitamin E group had a significantly lower sperm death than other groups. There was no significant difference in the testis weight, normal morphology, and normal DNA among the groups ($P>0.05$). Vitamin E had noticeable effects on gaining weight, epididymitis weight, and survived sperm, and a noticeable reducing effect on lead's harmful side effects on the sperm.

Conclusions: The results indicate the protective effects of vitamin E on spermatogenesis quality and some sperm parameters in rats exposed to lead.

Keywords: Lead, VitaminE, Spermatogenesis.

Original article

INTRODUCTION

Todays, heavy metal poisoning is a main bioenvironmental problem that has been caused mainly by metals extraction and melting. The vital organs are continuously

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exposed to several toxic metals in polluted environments.[1]

Lead is a heavy metal which has been used by human beings since ancient times and is still being used. Lead may enter into the body and consequently the vital organs of human beings. Because of having toxic effects, this metal, even in small amounts, can cause several side effects on biological processes.[2-6] The side effects of lead contamination on semen are caused by activating one of the pathways of producing activated reactive oxygen species (ROS). The human sperm has a specific sensitivity to oxidative damage, because it contains high levels of non-saturated fatty acids with multiple bipolar covalence and can produce ROS, superoxide anion, and hydrogen peroxide.[7] Free radicals have a main role in incidence of several diseases, such as diabetes, atherosclerosis, cardiovascular disease, neurologic disorder, cancer and the side effects due to drugs and toxic compounds.[8-14] The toxic effects of the lead on several organs have been assessed and found to be caused by increased lipid peroxidation and finally disturbed functions of different organs of the body such as reproductive system.[15-18] The non-saturated fatty acids are the main elements of sperm cell wall. Because of low antioxidant potential, sperm cells are predisposed to oxidative damage.[15,19] Lead can induce several side effects on sperm parameters and reduces fertility by damaging sperm cell wall through oxidative pathway.[19,20] There are different criteria to assess the reproductive system function, including use of specific biological criteria in exposure to contaminants to assess sperm function including its concentration, morphology, and mobility. Vitamin E is a strong non-enzymatic antioxidant that can limit lipid per oxidation, prevent free radical action, and finally protect the cell wall against damage.[21] In male reproductive system, vitamin E has been reported to prevent the side effects of free radicals on testis and sperm.[22-24] Therefore, the aim of this study was to determine the effects of vitamin E on spermatogenesis and sperm parameters (mobility, number, morphology) in lead-exposed male rats. In this study, we are going to assess the specific effects of vitamin E in lead-exposed rats to find an approach to prevent lead harmful effects on fertility criteria using a different method and dosage of vitamin E that have never been used in previous studies.

METHOD

This animal experimental study was conducted to investigate the protective effects of vitamin E on sperm parameters and spermatogenesis quality in rats exposed to lead. The study protocol was approved at Applied Pharmaceutical Research Center, Tabriz University of Medical Sciences in 2014 (with ethic code number 741 in August 2013) and researchers considered the national law on the care and use of laboratory animals.

According to the Declaration of Helsinki in animal study should be paid more attention to environmental protection and welfare of laboratory animals.[25]

Accordingly, in this study, it was tried to be used minimum sample to run and correct conclusion is obtained. Also, during the study, as well as before and after the study, it was tried to create the right conditions for feeding and life samples. Moreover, all phases of the work and living conditions were conducted under the supervision of a veterinarian consultant.

In the present study, sample size was determined regarding to previous study by GolshanIranpour and et al.[26] Moreover, sample size was estimated by STATA software that α=0.05, ß=80%, Sd_1=9, Sd_2=1, M_1=67, and M_2=56 were considered.

A total of 45 male Wistar rats, 8-10 weeks old ranging in 180±20 gr, were

purchased from Tehran Pasteur Institute. To accommodate the animals, they were treated in the animal house of Pharmaceutical Research Center, Tabriz University of Medical Sciences for 1 week under 12 hours of light and 12 hours of darkness at 25°C and humidity of 40-70% based on the principles of Laboratory Animal Care. Then they were weighed; numbered and allocated randomly in 5 groups (in each group 9 rat) with the similar average weights using the SPSS 13 Inc, Chicago software

Interventions were carried out 3 days in a week for one month as following groups: Control group (C1): 0.5 ml of distilled water (double distilled and filtered) by gavage; Intraperitoneal control group (C2): 0.5 ml of distilled water intraperitoneally; Lead acetate (L): 10 mg/kg/body weight (bw) intraperitoneally; Vitamin E: 100 mg/kg/bw by gavage; Lead acetate + Vitamin E (L+E) as above dose and route.

24 hours after the end of intervention, all the animals were weighed, with consideration of ethic criteria anesthetized with ether and sacrificed. Their right reproductive organs consist of epididymis and testis were removed and weighed by a digital scale with 0.001 accuracy (Mettler-Toledo B303-S Co, Swiss) and dissolved in phosphate-buffered saline (PBS). The digital scale was calibrated before each measurement. Subsequently, sperm samples were collected from distal part of epididym and examined based on sperm count, motility, and morphology.[27] These sperms were released in the medium provided by GibcoHam's F10 Company. The medium osmolarity and pH were set at 285 mmol and 7.2-7.4 respectively. Sperm mortality was examined by placing a drop of the medium on the slide using an optical microscope with a magnification of × 40.

Another smear were fixed on the slides by Carnoy's solution and stained with acridine orange for the detection of sperms with normal DNA based on Tejada.[28]

The percentage of sperms with normal (green colour) and denaturated DNA (red colour) was reported.[29]

A drop of medium containing-sperm was placed on a 20 × 20 mm glass slide and then a cover slip was placed on. To assess the sperm condition, the prepared slides were observed under an optical microscope (Olympus bx 40, Japan) with the object lens of × 40 and the numbers of motile and non-motile sperms were counted (200 spermatozoa per slide) and their ratio (sperm motility) was determined.

Regarding to the vital sperms' membrane permeability, the numbers of live and dead sperms were counted. For eosin-nigrosin staining, a drop of sperm-containing medium was placed on the glass slides and fixed with alcohol (more than 96%). Eosin cannot penetrate in the membrane of living cells. Therefore, viable cells remained uncolored and the dead sperms were seen in pink color. At least 300 spermatozoa were evaluated per slide and the percentage of live sperm cells was calculated.[30]

In order to increase the accuracy of observation, one observer did different laboratory tests. All stages of experiment were blind and after the intervention, laboratory expert was unaware about the study groups.

To determine normality of data, descriptive indices (skewness, kurtosis) were used. All the data in the study have been reported as IQR ± median and frequency. The normality assumptions of data were checked using one sample Kurtosis and Skewness tests (K-S) and for normal and non-normal data parametric and non-parametric test were used respectively. The data were analyzed by Kruskal Wallis, Wilcoxon, Mann-Whitney U-test, and Chi square, tests using SPSS Inc, Chicago

software. The P<0.05 is considered as significant difference in this study.

RESULTS

The findings of this study showed a significant differences of increasing weight among the five study groups (P=0.04). Vitamin E and control (gavage) groups have more increasing of the weight than other groups. In analyzing between two groups, the findings showed a significant difference of increasing weight between control (gavage) group with peritoneal control group, lead group and Vitamin E group (P=0.02, P=0.03, P=0.008) (Table 1).

Table 1: The abundance of increasing weight among the study groups

Group	Before intervention weight(gr)	After intervention weight(gr)	Weight increasing(gr)
Peritoneal control	215.00 (162,265)	237.50 (195.00,262.75)	13.00 (-4/75,45.00)
Gavage control	170.50 (146.25,274.25)	213.00 (196.25, 312.25)	42.00 (37.00, 51.50)
Lead	205.00 (167.50,245.00)	230.00 (171.50,278.50)	17.00 (4.00, 38.00)
Vitamin E+ Lead	207.00 (179.50,249.00)	240/00 (188.00,259.50)	-8.00 (-17.00,87.00)
Vitamin E	202.00 (193.00, 275.00)	245/00 (227/00,270/00)	22.00 (0.50, 39.50)
(P1)	0.82	0.94	0.04

The findings showed no significant differences of testis weight among the groups (P=0.48), and showed a significant difference of epididymis weight among the groups (P=0.006), that Vitamin E and peritoneal control groups had more increase of epididymis weight than other groups. In analyzing between 2 groups using Mann-Whitney test, the findings showed a significant difference between control (peritoneal) group with lead group (P=0.017), lead+ Vitamin E group (P=0.004), and lead + Vitamin E group with gavage and Vitamin E groups (P=0.03, P=0.008) (Table 2).

Table 2: The abundance of testis and epididymis weight increasing among the study groups

Group	Testis weight (gr)	Epididymis weight (gr)
Control(peritoneal)	1.22 (1.17, 1.41)	0.53 (0.45, 0.59)
Control(gavage)	1.37 (1.21, 1.48)	0.41 (0.23, 0.62)
Lead	1.28 (1.13, 1.49)	0.32 (0.21, 0.47)
Lead+ Vitamin E	1.25 (1.19, 1.54)	0.21 (0.18, 0.28)
Vitamin E	1.46 (1.22 , 1.58)	0.46 (0.40, 0.52)
P	0.48	0.006

Significance between 2 variable based on coroscalvalis test was mentioned P<0.05.

The findings showed a significant difference of sperm mobility>50% among the 5 groups (P<0.001), that this range in control groups was higher than intervention groups. In between 2 groups analyzing, the findings showed a significant difference between control (peritoneal) group with other study groups (P=0.009, P=0.009, P=0.000, P=0.002) (Table 3).

Table 3: Spermatography in study groups

Variable \ Groups	Control (peritoneal)	Control gavage	Lead	Lead + Vitamin E	Vitamin E	P
	N (%)	N (%)	N (%)	N (%)	N (%)	
Sperm mobility>50%	9(100)	7(77.6)	3(33.2)	1(11.11)	5(55.5)	P<0.001

The findings showed no significant difference of normal sperm morphology percentage and normal DNA percentage (P=0.87, P=0.82) (Table 4).

Table 4: Abundance of surviving potential, abnormal morphology, alteration in sperm DNA in study groups

Group	Death sperm percentage	Normal morphology percentage	Normal DNA percentage
Control (peritoneal)	10.00 (6.00, 10.00)	100.00 (99.00, 100.00)	12.00 (10.00, 19.00)
Control (gavage)	10.00 (5.50, 10.00)	100.00 (99.00, 100.00)	48.00 (3.50, 50.00)
lead	50.00 (12.50, 93.50)	99.00 (98.5, 100.00)	25.00 (13.00, 45.00)
Lead + Vitamin E	30.00 (1.50, 74.50)	100.00 (99.00, 100.00)	8.00 (1.00, 19.00)
Vitamin E	1.00 (0.00, 2.50)	99.00 (100.00, 98.50)	4.00 (1.50, 33.50)
P	0.000	0.87	0.82

The findings showed a significant difference of death sperm percentage among the groups (P=0.000) and the most death sperm percentage was seen in lead group that had a significant difference with control (peritoneal), control (gavage), vitamin E groups (P=0.004, P=0.001, P=0.000). Also the findings showed a significant difference of vitamin E group with control (peritoneal), control (gavage) and lead + Vitamin E groups (P=0.000, P=0.001, P=0.024).

DISCUSSION

This study assessed the effects of vitamin E on the spermatogenesis and sperm parameters in rats. The findings showed that intraperitoneal injection of lead acetate (10 mg/kg/rat) for 30 day could increase sperm death. Similarly, Mishra et al study showed that intraperitoneal injection of lead acetate (10 mg/kg/rat) for five weeks could increase the number of abnormal sperm.[31] Moreover, the finding of Paolo Liva and Etal indicated that intraperitoneal injection of lead acetate (8 mg/kg/mouse) for 35 days could reduce the total number of sperm.[32] Dorostghol and Etal reported that adding the lead acetate 1% to rats' water for 70 days could reduce the number of total sperm.[33] Human sperm has a specific sensitivity to oxidative damage because it consists of high levels of multiple covalent non-saturated fatty acids and can produce ROS, superoxide anion, and H_2O_2. Lead can cause side effects on sperm probably by activating a pathway of producing ROS.[7] The lowest epididymitis weight was seen in the lead group and the highest in the vitamin E and (peritoneal) control groups. Reduction in epididymitis weight may be due to reduced testosterone which in turn may be due to the reduced number of sperm and epididymitis weight. Vitamin E and control (gavage) groups experienced more weight gain than other groups and a significant difference was

seen in weight gain between the control (gavage, peritoneal) and lead + vitamin E groups. Reduction in reproductive organs weight in the lead groups may be related to reduce cell germ and spermatic cells.[34] Acriding coloring showed that the samples of rats' sperm of different groups had not a significant difference in normal DNA and normal morphology. Although the heterogeneity of sample extensions prolonged fixation time in acridine coloring, and the potential error in microscopic test was a limitation of this study. This can be a simple, appropriate, and common method of evaluating chromatin in basic and clinical studies.[35,36] Lack of any difference seems to be due to the administered dose and treatment duration. The findings showed a significant difference in sperm mobility between the control (peritoneal) and lead groups. This finding is consistent with Al-Attare et al. study that showed prolonged exposure to high doses of lead could reduce sperm mobility.[37] In addition, the highest sperm death rate of the lead group was significantly higher than control, vitamin E, and lead + vitamin E groups, and the sperm death reduced noticeably in lead + vitamin E group compared to the lead group. The WHO reported several factors affecting survivability, mobility, and fertility of sperm.[36-39] In this study, an explanation may be related to sperm hypersensitivity to oxidative damage and high capability of producing ROS, which can be stimulated by environmental or physiological factors and reducing mobility and survivability of the sperm.[11] As an antioxidant and non-enzymatic agent, vitamin E can prevent peroxidation reactions in the cell wall by restricting free radicals, and therefore, protects the cell wall.[40] In this study, it was observed the positive effects of vitamin E on sperm survival in lead-exposed rats, which were caused probably by eliminating or reducing the oxidative effects of lead.

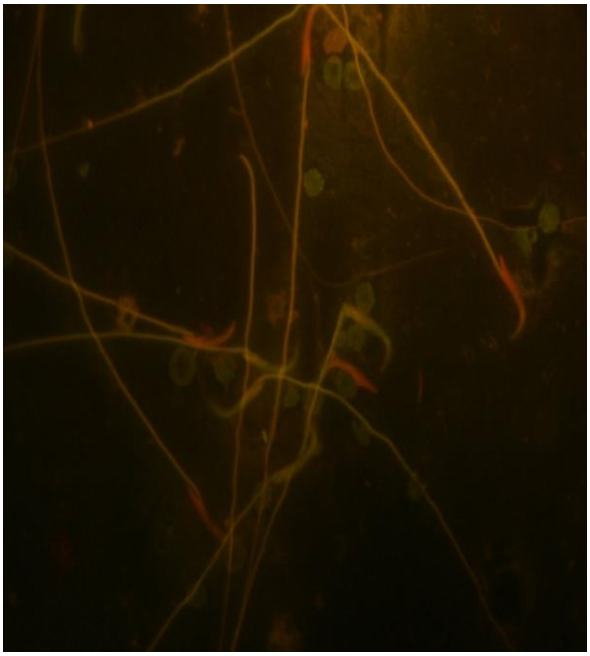

Figure 1: Evaluation of the whole DNA in sperm

Green head sperms are normal and red head sperms are abnormal in vitamin E group (100 mg/kg/rat). Orange acridine coloring (magnification x 1000).

CONCLUSION

According to the findings of this study, lead poisoning can increase the sperm death. Considering the lead side effects on several organs of the body, the researchers suggest various strategies to reduce and neutralize these side effects, including use of antioxidants in the diet that can prevent lead poisoning and even reduce the side effects of other heavy metals. According to the findings of this study, vitamin E, as an antioxidant, can cause weight gain and increases in epididymitis weight and survive sperm. It can noticeably reduce the toxic effects of lead on sperm. However, it was not observed any positive effect on other fertility criteria.

Further studies should be conducted to investigate the effects of different doses of vitamin E in the long time to find preventive approaches to lead poisoning.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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