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Research Article

Protective Effect of Nano-Vitamin C on Infertility due to Oxidative Stress Induced by Lead and Arsenic in Male Rats

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Occupational and environmental exposure to heavy metals such as arsenic (As) and lead (Pb) by inducing oxidative damage may impair male fertility. However, there is a new view that shows that the nano form of vitamins such as vitamin C, which have antioxidant activity, can be effective in improving this disorder. Therefore, this study aimed to evaluate the effect of NVC (NVC) on reproductive toxicity caused by the combination of Pb and As on testicular histology, sperm morphology, oxidative stress parameters, and hormonal changes in male rats. In this experimental study, forty-two male Wistar rats were randomly divided into six groups: control, NVC (200 mg/kg), As (50 ppm sodium arsenate), Pb (500 ppm Pb acetate), As + NVC, and Pb + NVC. FSH, LH, and testosterone levels were measured in serum. The activity of glutathione peroxidase (GPx), superoxide dismutase (SOD), catalase (CAT), carbonyl protein, malondialdehyde (MDA), and total antioxidant capacity (TAC) was measured in testis. Histological examination and sperm parameters were also evaluated. FSH, LH, and testosterone levels and sperm parameters significantly decreased, and levels of protein carbonyl, MDA, and DNA fragmentation increased in the As and Pb groups, while treatment with NVC could improve them. Histological evaluation and sperm parameters in As and Pb groups showed damage in the process of spermatogenesis and sperm parameters. The treatment with NVC could significantly improve these parameters. The activity of GPx, SOD, and CAT in testis decreased in As and Pb groups, while treatment with NVC could enhance them. It can be concluded that NVC by inhibiting oxidative damage and improving serum level of testosterone, LH, and FSH could overcome As- and Pb-induced reproductive dysfunction.

1. Introduction

Occupational and environmental exposure to heavy metals such as As and Pb has increased in recent decades due to the development of industry and can adversely affect many functional systems of the body, including the endocrine system and reproduction [1, 2]. Exposure to pesticides, working in industry, and contaminated food sources can be a reason for contamination with these metals [3]. Infertility is a medical and psychiatric disorder in which couples are unable to conceive generally during a year of unprotected sex. Infertility has been a concern of the World Health Organization (WHO) in recent years [4].

Various mechanisms, including cell dysfunction, impairment of signaling pathways, oxidative stress, apoptosis, inflammation, and changes in endocrine function, are involved in harmful reproductive effects caused by heavy metals [5]. As and Pb bind to the thiol groups of protein and disrupt their function. In mitochondria, it depletes energy [6]. It can also cause the secretion of plasma glucocorticoids and suppress the secretion of gonadotropins [7, 8]. Oxidative stress can induce the expression of Bax and Bcl-2 genes. Oxidative stress, apoptosis, and cellular toxicity induced by As and Pb can cause male infertility. Therefore, antioxidant compounds can reduce the toxicity of spermatogenesis by decreasing the expression of apoptosis-inducing genes [7, 9].

As and Pb are nonessential metals that cause biochemical, physiological, and behavioral impairment in cells and organs. Studies have also shown that As and Pb poisoning is mediated by dysfunction of the pituitary-hypothalamic axis and the function of interstitial and Sertoli cells [10, 11]. They showed that Pb and As poisoning disrupts the reproductive system by altering the serum content of LH, FSH, and testosterone. Inflammation and oxidative damage are believed to play an important role in reproductive disorders caused by As and Pb [7, 11]. In addition, other studies have identified oxidative damage and inflammation as one of the basic mechanisms of infertility in men [12–15]. Therefore, it is assumed that inhibiting oxidative damage and inflammation by antioxidant and anti-inflammatory agents such as micronutrients and vitamins is a valuable and uncomplicated treatment strategy [16].

Vitamins are essential nutrients that can be easily obtained from the diet. Studies have shown that deficiency of vitamins C, B1, and B6 plays a role in the complications of cadmium and Pb poisoning, while supplementation of these vitamins has a significant protective role against the damage caused by heavy metal poisoning [17]. Vitamins C and E are natural nonenzymatic antioxidants that can scavenge free radicals and effectively inhibit lipid peroxidation. In addition, the protective effects of vitamins C on the liver, kidneys, brain, and testes against oxidative damage caused by exposure to Pb have been well demonstrated [18–20]. Vitamin C exerted nonenzymatic antioxidant function by destroying free radicals and trapping them in the aqueous phase to protect the biological membrane [21, 22]. However, there is a report that high doses of vitamin C have been suggested to cause oxidative stress and cell death [23]. Despite its widespread use as a potent antioxidant in the field of medicine, its use is limited. The biggest challenge in using vitamin C is maintaining its stability in the body and getting it to the active site [24]. At present, oxygen pressure control during formulation and storage and maintenance of proper temperature and pH are used for this purpose [18, 25]. In addition, the use of vitamin C derivatives and the use of new drug delivery systems such as microencapsulation and nanotechnology are new solutions that have attracted special attention in the last decade [25]. Today, nanotechnology is advancing rapidly in the world. Special attention has been paid to its applications in industry, catalysts, sensors, cancer therapies, and free radical scavengers [23]. Therefore, the administration of proper doses and particle size of vitamin C can possibly exert appropriate biological effects. Due to the above explanations and the lack of a similar study, the aim of this study was to investigate the effects of nanoparticles of vitamin C (NVC) on reproductive damage caused by exposure to Pb and As.

2. Materials and Methods

2.1. Animals. The present study was an experimental-interventional study that was performed on forty-two adult male Wistar rats (weight range: 200–230 g and age range: 7–8 weeks) purchased from Pasteur Institute of Iran. All stages of this study were performed based on the guide for the care and use of laboratory animals approved by Kurdistan University of Medical Sciences with the reference no. IR.MUK.-REC.1399.6014. Animals were kept at $20 \pm 2^{\circ}C$ and 12 hours light: 12 hours dark-light cycle and had free access to chow and water. After one week of familiarization, the animals were randomly divided into controlled and treated groups.

2.2. Chemicals. Sodium arsenate, lead acetate, and vitamin C nanoparticles were purchased from Sigma-Aldrich (Germany). Sodium arsenate (dose) and lead acetate (dose) were added into drinking water; vitamin C nanoparticles were also prepared in a 10% oral solution at a dose of 200 mg/kg.

2.3. Acute Toxicity Study. An acute toxicity study was performed to determine the lethal dose (LD50) of vitamin C nanoparticles (50–1000 mg/kg). Twenty-five adult male rats (200–230 g) were randomly divided into five groups. The groups received (P.O.) 50, 100, 200, 400, and 1000 mg/kg body weight of vitamin C nanoparticles, respectively. They were monitored for abnormal symptoms and mortality for the first 24 hours after injection. After this period, this process lasted up to 14 days for chronic complications. In the study, acute oral poisoning with different doses ranging from 50 to 1000 mg/kg was investigated. No mortality was observed at the maximum dose (1000 mg/kg). Belodarased on this and earlier studies that have used an oral dose of 200 mg/kg of vitamin C (Jelodar et al., 2013, [24]), we chose a similar dose, 200 mg/kg, to continue the study [26].

2.4. Experimental Design. Animals were randomly divided into six groups of seven to evaluate the protective effects of vitamin C nanoparticles [27].

Control group (C): animals received only water and chow.

As group (As): animals received 50 ppm sodium arsenite in drinking water for 30 days.

Pb group (Pb): animals received 500 ppm lead acetate in drinking water for 30 days.

NVC (NVC): animals received nano-vitamin C (200 mg/kg/day) which was given by gavage for 30 days.

As + NVC group: animals received 50 ppm sodium arsenate via drinking water and 200 mg/kg nano-vitamin C (200 mg/kg/day) daily by gavage.

Pb + NVC group: animals received 500 ppm lead acetate via drinking water and 200 mg/kg nano-vitamin C (200 mg/kg/day) daily by gavage.

The duration of the study was 30 days to evaluate the effectiveness of nanoparticles of vitamin C in subacute toxicity dose in exposure to Pb and As on the reproductive system [28–31].

2.5. Sampling and Measurement of Hormonal Variables. The weight of the animals was measured before and at the end of the study period. After the last day of treatment,

animals were anesthetized by intraperitoneal injection of ketamine (100 mg/kg) and xylazine (10 mg/kg). Blood samples were taken from the heart, and serum was separated by centrifugation (3000 rpm, 10 min) and used to measure hormonal parameters. Serum concentrations of LH, FSH, and testosterone were measured using the radioimmunoassay (RIA) method. The hormonal diagnostic kits (Catalog# KA2240) used in this study were purchased from Pars Azmoun Co., Tehran, Iran. The hormonal kits included standard solutions of radioactive iodine, antibodies, and wash buffer. The basis of this method was the competition between the antigens in the assay sample for binding to the antibody, which used radioactive antibodies and a buffer with less than 10% free sodium [32, 33].

2.6. Preparation of Tissue Samples and Measurement of Oxidative Damage Parameters. The testes were immediately removed after blood sampling. The weight of the right and left testes was measured. After washing the testis with cold saline and placing it inside the microtube, the testicular samples were frozen in liquid nitrogen, and they were then stored at −80°C. At the time of measurement, testicular tissue was manually homogenized in phosphate buffer (0.1 M, pH: 7.4), and debris was removed by centrifugation at 3000g for 10 min. After centrifugation (2750 rpm, 10 min), the resulting supernatant was used for biochemical testing [34]. Total antioxidant capacity (TAC) was measured by the ferric-reducing antioxidant power (FRAP) method. In this method, the ability of plasma to reduce ferric ions in the presence of free radicals was measured. At acidic pH, when the FeIII-TPTZ complex was reduced to FeII, it produced a blue dye that had the highest light absorption at 593 nm. TAC values were measured using a standard solution of $FeSO_4$ that has been used for calibration of the analysis. The outcomes are expressed in μmol/L $FeSO_4$ [35]. The enzyme glutathione peroxidase (GSH-Px) catalyzes the oxidation of glutathione by the cumene hydroperoxide. The oxidized glutathione is then converted back to regenerative glutathione in the presence of the enzyme's glutathione reductase and NADPH. In this experiment, the $NADP^+$ obtained at 340 nm was measured. The basis of superoxide dismutase (SOD) activity was to inhibit the conversion of superoxide to H_2O_2 and O_2, which was measured at a wavelength of 505 nm as described by a previous study [36]. Catalase activity was measured by the Aebi method based on H_2O_2 decomposition at 240 nm [36, 37]. Malondialdehyde (MDA) measurement was determined based on the reaction with thiobarbituric acid (TBA) using spectrophotometry at 532 nm [38]. Testicular protein carbonyl content was measured by the Levine method. In this method, reagents 2,4-dinitrophenylhydrazine re-shift with the carbonyl groups present in the proteins to form a yellow complex whose color intensity was spectrophotometric at 380 nm [37]. Total protein was measured by the Bradford method [39].

2.7. Measurement of Sperm Parameters. According to the criteria of the World Health Organization (WHO), the sperm analysis was performed as follows. A hemocytometer slide was used to assess sperm count. Only sperm cells with head, middle, and tail were counted using a light microscope with a magnification of x40. Counting was done twice for each sample, and its mean was recorded. Results were expressed as the number of sperm per ml of semen [40]. To evaluate the motility of sperm, 10 μl of each sample was placed in the center of the Makler counting chamber, and it was observed and counted by a light microscope with a magnification of ×40. In this study, 200 sperm cells were counted, and the percentage of sperm motility was determined according to the guidelines of WHO. The evaluation of sperm viability was performed using eosin-nigrosin staining. First, eosin solution (1%, Merck) was added to the sperm solution (2 : 1 ratio), and after 30 s, the nigrosin solution (10%, Merck) was added to it. 10 μl of the new solution was smeared on a slide and then air-dried. Finally, sperm viability was assayed under a light microscope (100× magnification) [41].

2.8. DNA Fragmentation Assessment. Sperm DNA fragmentation was assessed by a sperm DNA fragmentation kit (Avicenna, Iran) using the sperm chromatin dispersion (SCD) method according to the manufacturer's instruction. In brief, the sperm cells were washed twice with the PBS buffer, and then a suspension containing 15–20 million sperm solutions was prepared. After that, spermatozoa were immersed in agarose microgel and smeared on the slide. In addition, denaturation by acid and lysis solution, dehydration, and staining with Diff-Quick were done. Sperm cells with large halos (that were similar or larger than the diameter of sperm's head) and sperms with medium-sized halos (halo greater than 1 : 3 of the smallest diameter of the sperm's head and less than the smallest diameter) were defined as spermatozoa having no fragmentation [42, 43].

2.9. Histological Evaluation and Study of Spermatogenesis. The right testis was wholly removed and fixed in a 10% formalin solution. It was then embedded in paraffin after a week, and sections with a thickness of 5 microns were prepared and then stained with hematoxylin and eosin. Histological features were examined with a light microscope (BX-51, Olympus Corporation, Tokyo, Japan) with the same magnification (40x). Moreover, spermatogonia, spermatocytes, spermatids, Leydig cells, and Sertoli cells were counted in 5 fields for each section under a light microscope [44]. Seminiferous tubule diameter, lumen diameter, and epithelial thickness were measured in different groups using a counting probe and Motic Images software [44, 45]. The percentage of tubule vacuolization, tubule with desquamated cells, and pyknotic cells was also measured using ZILOS-tk, version 5.9. The spermatogenesis and sperm maturation process was analyzed by histological examination of seminiferous tubules. Fifty seminiferous tubules were cross-sectionally evaluated and scored on a scale of one to ten based on defined criteria. Scoring included the following: 1—there are no germ cells and Sertoli cells; 2—there are no germ cells; 3—there are only spermatogonia; 4—there are only a few spermatocytes; 5—there are many spermatocytes but no spermatozoa or spermatids; 6—there are only a few

spermatids; 7—there are many spermatids but no spermatozoa; 8—there are only a few spermatozoa; 9—there are many spermatozoa but disorganized spermatogenesis; and 10—spermatogenesis and tubules are observed completely and perfectly [46].

2.10. Statistical Analysis. All data are presented in mean ± SEM. The normality of the data was evaluated by the Kolmogorov-Smirnov test. One-way ANOVA followed by Tukey's post hoc test were performed to analyze the parametric data. All graphs and statistical analyses were performed by GraphPad Prism version 7 software (GraphPad Software, San Diego, California, USA).

3. Results

3.1. Body Weight and Testis Index. The As and Pb groups had lower testis weight as compared to the other groups. Moreover, the testis index in As and Pb groups significantly decreased as compared to other groups (Table 1) ($p = 0.002$).

3.2. Serum Levels of Reproductive Hormones. The highest level of FSH was seen in the NVC group, and the lowest level was seen in the Pb and As groups (Table 2). Nonetheless, in the As and Pb groups which received NVC, the level of FSH, LH, and testosterone increased compared with the As and Pb groups. In some cases, these differences were not significant ($p = 0.035$). Serum testosterone concentration in the NVC and control groups was the highest level in comparison to the other groups ($p = 0.007$).

3.3. Sperm Parameters. Parameters related to the morphology of epididymal sperm cells in the different groups are presented in Table 3. Sperm concentration in control and NVC groups was significantly higher than that of the Pb and As groups ($p = 0.035$). The percentage of survival of sperm in control and NVC groups was significantly higher than that in the Pb and As groups ($p < 0.05$). The Pb and As groups had the highest percentage of dead sperm compared to other groups. However, these parameters improved in the As and Pb groups which received the NVC as compared to the As and Pb groups, and this difference was not substantial. The highest amount of motility percentage was observed in the NVC group, and the lowest level was seen in the Pb group. The highest percentage of DNA fragmentation was observed in the As group, which had a significant difference from the NVC group. DNA fragmentation level significantly improved in the As + NVC and Pb + NVC groups as compared with that in the As and Pb groups ($p < 0.05$).

3.4. Study of Spermatogenesis. The number of spermatogonia significantly decreased in the As and Pb groups compared to the control group ($p = 0.046$). The mean number of spermatocytes significantly decreased in the As treatment compared to the control and NVC groups ($p < 0.05$). These parameters significantly improved in the As and Pb groups receiving NVC compared to the As and Pb groups. The maximum number of spermatid cells was seen in the NVC group, which was significantly different from the As and Pb groups ($p < 0.001$). The decrease in the number of Leydig cells and Sertoli cells in the As and Pb groups was significantly different from those in control and NVC groups ($p < 0.01$) (Table 4).

3.5. Histological and Testicular Structure. The diameter of the seminiferous tubules in the As and Pb groups showed a significant difference compared to the control group. There is no significant difference between the control group and the NVC group. The highest epithelial thickness of seminiferous tubules was observed in the NVC group, and the lowest was in the As group. There was a significant difference between them ($p < 0.01$). The percentage of vacuolation tubes significantly increased in the As and Pb groups in comparison with the control group ($p < 0.001$), while administration of NVC in As + NVC and Pb + NVC groups could significantly improve this effect. The lowest level of tubule with desquamated cells was observed in the NVC group, and the highest level was observed in the As group. This revealed a significant difference between these groups ($p < 0.01$). The minimum level of pyknotic cells was observed in the control group, and the maximum level was in the As group describing a significant difference between these groups ($p < 0.01$). The highest Johnsen score was observed in the group which received NVC, and the lowest was in the As and Pb groups. There was a significant difference between the control group and the As and Pb groups according to this parameter. On the other hand, administration of NVC in the As + NVC and Pb + NVC groups could significantly improve this effect (Table 5 and Figure 1).

3.6. Oxidative Stress Parameters. The highest level of GPx was observed in the control and NVC groups, and the lowest was in the Pb group. In the As and Pb groups treated with NVC, the level of this enzyme had significant with the control group ($p = 0.038$). The highest activity of SOD was in the NVC group, and the lowest was in the As group. The activity of this enzyme in the As and Pb groups treated with NVC significantly improved as compared with that in the As and Pb groups ($p = 0.032$). The highest CAT activity was in the NVC group, and the lowest level was in the Pb group. There was a significant difference among the NVC group with other groups except for the control group ($p < 0.05$). The level of MDA considerably increased in As and Pb groups compared to the control and NVC groups, while the level of this parameter significantly decreased in the As and Pb groups treated with nano-vitamin C in comparison with the As and Pb groups ($p < 0.05$). Testicular carbonyl protein concentration in As and Pb groups significantly increased as compared to the control group. There was a significant difference among the carbonyl protein concentration of the control group with other groups except for NVC. The amount of carbonyl protein in the As and Pb groups treated with NVC showed a major decrease in comparison with the As and Pb groups ($p < 0.05$). The level of TAC revealed a

Table 1: Effect of NVC on body weight and testicular absolute and relative weights of As- and Pb-exposed rats.

Groups	Initial body weight (g)	Final body weight (g)	Body weight change	Right testis weight (g)	Left testis weight (g)	Testis index (%)
C	214.0 ± 3.60	239.8 ± 6.02	25.8 ± 2.42	1.08 ± 0.063	1.11 ± 0.079	0.45 ± 0.011
As	211.3 ± 3.20	215.4 ± 7.60 a*	4.1 ± 4.40 a*	0.878 ± 0.02	0.84 ± 0.031 a*, d*	0.39 ± 0.335 a*
Pb	218.8 ± 2.00	221.5 ± 4.40 a*	2.7 ± 2.41 a*	0.928 ± 0.023	0.91 ± 0.021 a*	0.41 ± 0.026 a*
NVC	213.4 ± 1.91	236.11 ± 3.40 b*	22.71 ± 1.52 b**, c**	1.10 ± 0.049 b*	1.08 ± 0.045 b*	0.46 ± 0.0.013 b*, c*
As + NVC	217.4 ± 4.5	231.6 ± 2.11 b*	14.23 ± 1.98b*, c*	0.94 ± 0.059	1.03 ± 0.046	0.42 ± 0.015a*, d*
Pb + NVC	213.4 ± 2.50	229.11 ± 6.21	15.71 ± 3.71b*, c*	1.021 ± 0.63	1.04 ± 0.048	0.44 ± 0.0.054 b*

All values are presented as mean ± SEM. * p value <0.05; ** p value <0.01. a, b, c, and d, respectively, are compared with C, As, Pb, and NVC groups. Final body weight and body weight changes significantly increased in the As + NVC and Pb + NVC groups compared to Pb and As groups ($p = 0.015$).

Table 2: Comparison of changes in the serum concentration of hormones in the studied animals.

Groups	FSH (ng/mL)	LH (ng/mL)	Testosterone (ng/mL)
C	2.11 ± 0.079	6.14 ± 0.268	5.55 ± 0.099
As	1.39 ± 0.136 a*	4.95 ± 0.254 a**	2.42 ± 0.064 a**
Pb	1.3 ± 0.071 a*	5.14 ± 0.232	2.20 ± 0.130 a***
NVC	2.42 ± 0.141 b**, c*	6.41 ± 0.223 b*	5.50 ± 0.219 b**, c**
As + NVC	1.4 ± 0.752 *a, d**	5.27 ± 0.120 b*, d*	2.80 ± 0.332 a*, b*, d
Pb + NVC	1.41 ± 0.730 a*, b*, d**	5.31 ± 0.201 b*, d*	2.21 ± 0.075 a***, d**, e*

All values are presented as mean ± SEM. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. a, b, c, and d are compared with C, As, Pb, and NVC groups, respectively.

Table 3: Effect of NVC on sperm parameters of As- and Pb-exposed rats.

Groups	Sperm concentration ($\times 10^6$/ml)	% vitality	% dead sperm	% motility	% DNA fragmentation
C	345.47 ± 6.217	85.00 ± 0.925	15.00 ± 0.925	63.14 ± 1.223	55.14 ± 0.508
As	234.42 ± 22.229 a*	44.57 ± 0.611 a**	55.42 ± 0.611 a***	30.71 ± 0.420 a*	75.13 ± 0.594 a**
Pb	214.57 ± 2.827 a**	44.28 ± 0.993 a**	55.43 ± 1.020 a***	30.00 ± 0.654 a*	69.00 ± 2.370 a**, b*
NVC	337.57 ± 20.496 b*, c*	81.42 ± 6.105 b****, c**	18.57 ± 6.105 c b**	69.28 ± 6.398 b**, c*	44.42 ± 0.895 a*, b**, c*
As + NVC	287.85 ± 15.615 c*	54.14 ± 0.857 a*, d*	45.85 ± 0.857 a*, d*	43.14 ± 0.769 a*, b*, c*, d*	55.28 ± 0.565 b*, c*, d***
Pb + NVC	278.42 ± 3.198 a*, c*	54.87 ± 0.828 a*, d*	44.28 ± 0.778 a*, b*, c*, d*	44.85 ± 0.508 a*, b*, c*, d*	56.00 ± 0.975 b*, c*, d***

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. a, b, c, and d are compared with C, As, Pb, and NVC groups, respectively.

Table 4: Effect of NVC on spermatogenic cell count in the testis of As- and Pb-exposed rats.

Groups	Spermatogonium	Spermatocyte	Spermatid	Leydig cell	Sertoli cell
C	48.00 ± 0.816	56.42 ± 0.649	59.00 ± 2.115	8.28 ± 0.420	6.13 ± 0.404
As	32.14 ± 0.508 a*	35.57 ± 1.556 a**	45.43 ± 1.231 a***	3.57 ± 0.368 a***	3.00 ± 0.308 a***
Pb	32.42 ± 0.649 a*	40.42 ± 0.996 a**, b	48.28 ± 2.043 a**	3.85 ± 0.404 a***	3.28 ± 0.285 a***
NVC	45.42 ± 1.986 b*, c*	56.85 ± 0.857 b*, c*	62.57 ± 1.324 b***, c**	7.71 ± 0.865 b**, c**	5.85 ± 0.341 b**, c**
As + NVC	40.25 ± 1.426 a*, b**, c**	46.57 ± 1.172 a*, b**, c*	52.42 ± 0.719 a*, b**, d**	5.57 ± 0.368 a**	3.14 ± 0.340 a***, d**
Pb + NVC	37.71 ± 1.209 a**, b*, c*, e*	43.00 ± 0.816 a**, b*	52.57 ± 0.649 a*, b**, d*	5.85 ± 0.508 a**, b*	3.00 ± 0.308 a***, d**

* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. a, b, c, d, and e are compared with C, As, Pb, NVC, and As + NVC groups, respectively.

decrease in As and Pb groups as compared to the control group ($p < 0.01$). Moreover, the amount of TAC in the As and Pb groups treated with nano-vitamin C significantly increased as compared to those in the As and Pb groups ($p = 0.043$) (Figure 2).

4. Discussion

4.1. Body and Testicular Weight Changes. Biological damage caused by environmental pollutants such as heavy metals such as Pb and As is a major concern of human societies that has been addressed in recent decades. Among these damages, reproductive activity defects are more important because of their relationship with the survival of the human race, and efforts to prevent and control them have always been a priority. Antioxidant defense mechanisms that are disrupted by these metals after oxidative damage are one of the main causes of reproductive failure due to exposure to these metals [47]. Our results showed that exposure to Pb and As, in addition to reproductive disorders, reduced body weight and testicular index, which was consistent with the results of other researchers. However, these results are

Table 5: Histological results of testicular tissue and spermatic tubule in different groups.

Groups	Seminiferous tubule diameter (μM)	Tubule vacuolization (%)	Seminiferous epithelium thickness (μM)	Lumen diameter (μM)	Pyknotic cell (%)	Tubule with desquamated cells (%)	Johnsen score
C	473.28 ± 1.82 2	4.71 ± 0.420	111.14 ± 1.682	230.57 ± 1.586	1.71 ± 0.285	4.71 ± 0.565	10.03 ± 0.047
As	406.71 ± 413 a**	31.37 ± 2.20 a***	97.85 ± 0.857	217.57 ± 1.937 a*	11.57 ± 1.50 a***	31.57 ± 2.67 a***	6.77 ± 0.48a***
Pb	397.14 ± 6.745 a***	30.71 ± 2.337 a***	98.83 ± 1.10	218.58 ± 2.102 a*	12.28 ± 1.53 a***	30.51 ± 1.643 a***	7.35 ± 0.034a***
NVC	439.57 ± 2.213 b*, c**	5.57 ± 0.649 b***, c***	126.42 ± 14.272 b**, c**	231.42 ± 1.445 b*, c*	3.85 ± 0.853 b*, c**	4.60 ± 0.565 b***, c**	9.85 ± 0.024b*
As + NVC	409.28 ± 5.139 a**, d*	19.57 ± 1.269 a**, b*, c**, d**	104.85 ± 1.335	221.28 ± 1.106 a*, d*	8.42 ± 1.19 a**	27.85 ± 1.994 a**, **c, d**	8.69 ± 0.028a*, c*
Pb + NVC	402.00 ± 4.654 a**, d*	17.42 ± 1.296 a**, b, c**, d**	105.57 ± 0.812	221.71 ± 2.542 a*, d*	7.78 ± 1.01 a**, d*	26.00 ± 1.327 a**, c**, d**	8.12 ± 0.094a*, c*

All values are presented as mean ± SEM. * p value <0.05; ** pp value <0.01; *** p value <0.001. a, b, c, and d, respectively, are compared with C, As, Pb, and NVC groups.

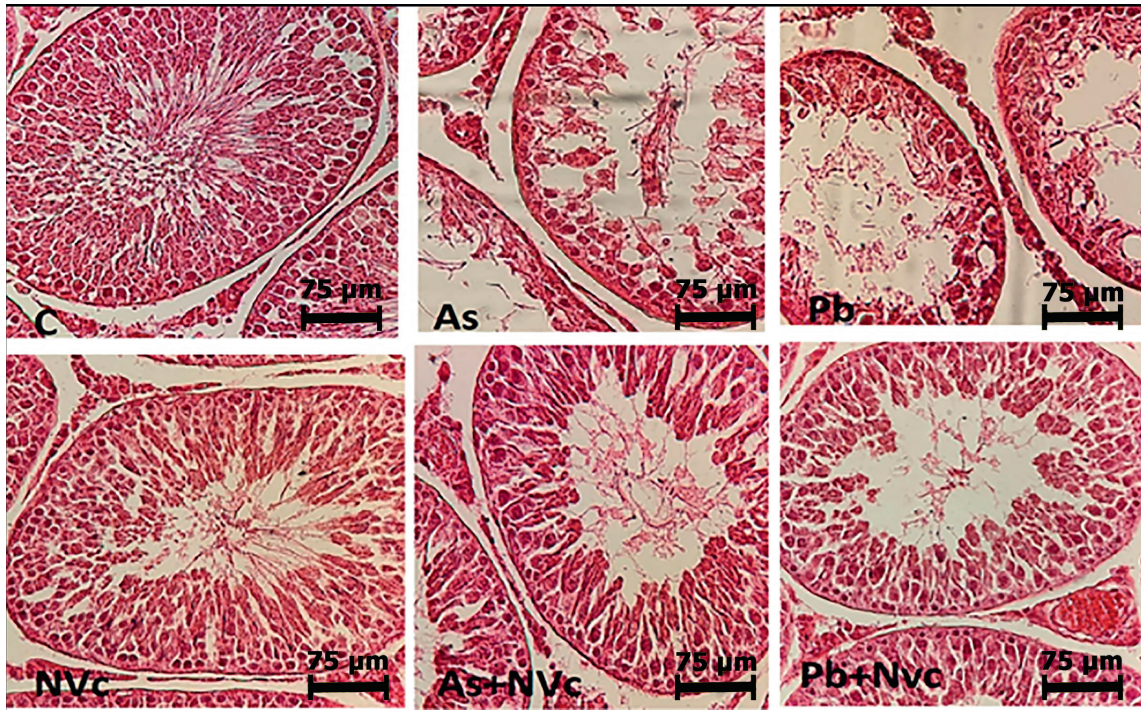

Figure 1: Histological sections of seminiferous tubules prepared from different groups. C: control group; different levels of spermatogenesis cells regularly and without changes in necrosis; normal thickness of seminiferous tubules. As: severe decrease in the mean of different cells in the process of spermatogenesis in seminiferous tubules, and decrease in the diameter of seminiferous tubules. Pb: reducing the number of the different cells in the process of spermatogenesis and reducing the diameter of the lumen and seminiferous tubules. NVC: maximum presence of the different spermatogenesis cells and no sign of cell necrosis. As + NVC and Pb + NVC: increasing the mean of the different cells of spermatogenesis process and increasing the diameter of seminiferous tubules and necrotic cells. H&E (×400). Scale bar: 75 μm.

significantly improved by the consumption of vitamin C nanoparticles. Gaskill et al. [48], Joseph et al. [49], and Bhatia et al. [50] also claimed that weight loss due to exposure to heavy metals could be due to the body's imbalance in food intake and absorption, the latter of which could be due to inhibition of enzymes involved in digestion and absorption of nutrients. Moreover, in Ezedom and Asagba et al. it was reported that prolonged exposure to heavy metals such as As could reduce the absorption of nutrients. Researchers have shown that cadmium and As can accumulate in various body tissues, including the liver, brain, kidneys, and testes, leading to the inhibition of oxidative enzymes, thus affecting several normal metabolic processes [37].

4.2. Hormonal Changes. Gonadotropins, testosterone, and their interaction with regulatory centers in the central nervous system play a very important role in controlling the function of the reproductive system. LH is a prerequisite for gonadal function, and FSH is responsible for spermatogenesis and normal testis activity [51]. Several possible mechanisms have been proposed for heavy metals and their damaging effects on the reproductive system, as well as changes in gonadotropin levels [52]. The results of this study indicate the disruptive role of As and Pb on the nerve centers that regulate the synthesis and release of FSH and LH. Pb as an endocrine disrupter can reduce the release of FSH and LH. Therefore, a decrease in LH concentration reduces the number and function of Leydig cells, secreting testosterone in groups treated with heavy metals. Moreover, oxidative damage and the decrease in FSH and LH concentration, in addition to cytological changes in testicular tissue, reduce cell lines from spermatogonium to spermatid and ultimately reduce sperm concentration [53], the results of which were well demonstrated in our study. These results showed that the concentrations of FSH, LH, and testosterone, which were reduced with As and Pb, could be increased by treatment with NVC. Karanth et al. showed that vitamin C leads to the release of LH and FSH from the pituitary gland; it increases the level of intracellular calcium ions and activates the release of these hormones [54].

4.3. Sperm Morphology. As a result of As intake, concentration, vitality, and motility in the sperm decreased significantly. ROS production as well as As binding to thiol groups in protein and chromatin of spermatozoa can cause significant changes in sperm dysfunction [55].

Pb reduces fertility by impairing the release of FSH, LH, and testosterone and leads to a decrease in sperm production and abnormal morphology. Vitamin C also increased sperm concentration, motility, and viability in a dose-dependent manner. In line with the results of this study, Okon and Uduak showed that vitamin C at a dose of 400 mg/kg could have a similar effect; they concluded that vitamin C has a strong effect on male reproductive function. Thus, the use of NVC at 200 mg/kg increases the levels of FSH, LH, and

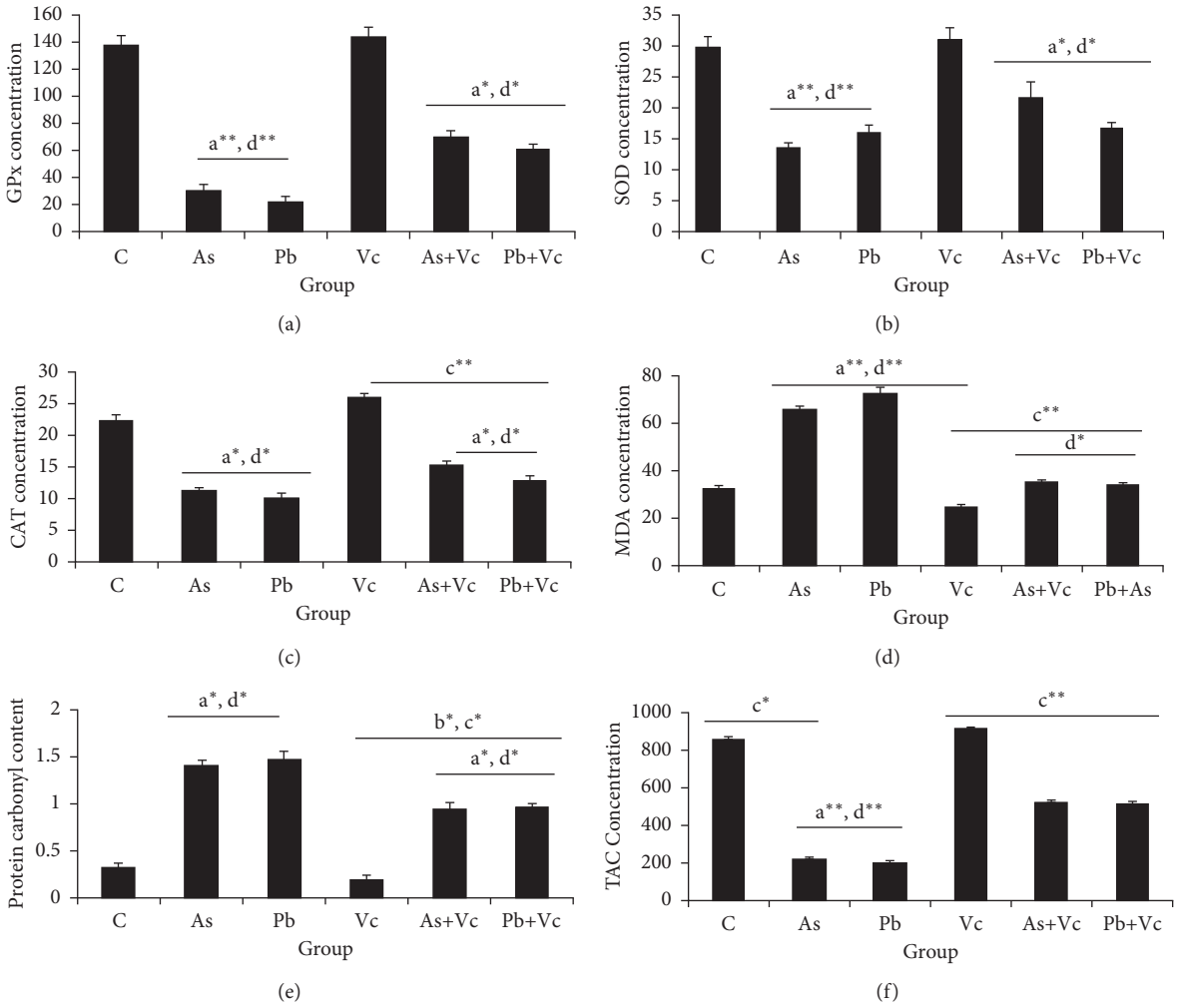

Figure 2: Mean ± SEM of testicular oxidative markers in different groups. $^{*}p < 0.05$; $^{**}p < 0.01$; $^{***}p < 0.001$. a, b, c, and d are compared with C, As, Pb, and NVC groups, respectively. (a) Glutathione peroxidase (GPx). (b) Superoxide dismutase (SOD). (c) Catalase (CAT). (d) Malondialdehyde (MDA). (e) Protein carbonyl content. (f) Total capacity antioxidant (TAC).

testosterone directly, which can subsequently control oxidative stress [56].

The effect of vitamin C on testosterone levels and sperm parameters in gentamicin intoxication was investigated by Rahayu et al. [57]. Their results, in agreement with our results, showed that NVC increased testosterone level and improved sperm parameters [57]. NVC could improve reproductive function by increasing the number of spermatozoa cells, sperm concentration, motility, and viability and neutralizing oxidative damage caused by As and Pb.

4.4. Spermatogenesis Changes. Pb also, directly and indirectly, affects interstitial and Sertoli cells in a reproductive function [28]. The results of the present study show the effects of cytotoxicity of As and Pb on sperm parameters. Testicular weight loss can be due to a decrease in the diameter of the seminiferous tubules, a decrease in the thickness of the germ membrane in the seminal vesicles, and an increase in the percentage of pyknotic cells that indicate apoptosis caused by exposure to Pb and As [33]. One of the main reasons for testicular weight loss and disruption of spermatogenesis is the formation of free radicals and reduced antioxidant capacity of testicular tissue. However, these changes were ameliorated by the antioxidant effects of NVC.

Damage that resulted from exposure to As and other heavy metals causes oxidative stress, inflammation, and apoptosis, which can be inhibited by antioxidant and anti-inflammatory compounds [33]. On the other hand, the possible accumulation and complications of As and Pb in testicular tissue induce free radicals and apoptosis in testes. Therefore, the spermatogenesis and testicular weight decreased. NVC improved body weight, gonadal hormones, and other changes in testicular tissue of As and Pb groups. It could be due to inhibiting oxidative stress, inflammation, and apoptosis [24, 58–60]. Our results, in line with previous reports, showed that administration of NVC might have improved testicular function in the As and Pb groups through its antioxidant effects [60]. El-Sayed et al. showed that vitamins C and E could improve the toxic effect of Pb on spermatogenic cell

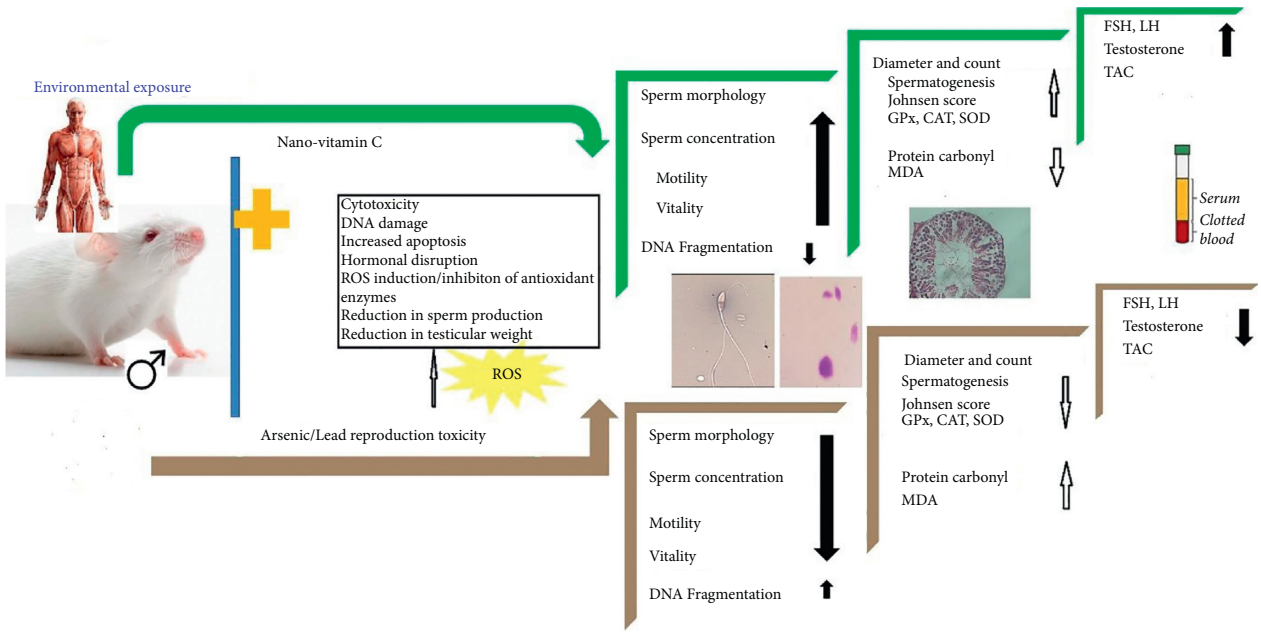

Figure 3: Diagram of the summary of the study results.

necrosis, spermatogenesis cells of tubules, edema, and interstitial hemorrhage [61].

In another study, the effect of vitamins C and E and nanoselenium on the weight and biochemical and serological parameters of ammonium-induced oxidative stress was investigated [62]. The results showed that vitamins C and E and nanoselenium increase weight, control biochemical changes, and strengthen the immune system [63]. In addition, vitamin C has been shown to reduce the accumulation of Pb in red blood cells, liver, and brain by increasing catalase levels [64]. NVC and vitamin E could also reduce inflammation and pain after surgery [65]. In another work, the antioxidant and anti-apoptotic effects of vitamin C in doxorubicin treatment showed that treatment with them reduced sperm DNA fragmentation and had protective effects on the reproductive system [66]. However, heavy metal damage may lead to oxidative stress, inflammation, and apoptosis, which induce the inhibition of antioxidant and anti-inflammatory compounds [67, 68].

On the other hand, oxidative stress increases the expression of Box and Bcl-2 and can cause apoptosis in Leydig and Sertoli cells. This condition damages their receptors (LHCGR and FSHR), leading to the formation of a negative feedback loop, which inactivates the hypothalamus to secrete GnRH. Therefore, oxidative stress can reduce the concentration of LH and FSH in Pb and As poisoning. Under these conditions, the use of antioxidants such as NVC improved reproductive performance. This is mainly achieved by reducing cellular oxidative damage and regulating this signaling pathway [53, 60].

5. Conclusions

Most male infertility can be attributed to oxidative damage due to various environmental and occupational exposures to environmental pollutants, waves, and heavy metals such as As and Pb. It can be concluded that, in addition to oxidative damage, a decrease in FSH and LH concentrations, changes in cytological testicular tissue, a decrease in cell lines from spermatogonium to spermatid, and finally a decrease in sperm parameters after exposure to As and Pb were observed in this study. Moreover, NVC improved the reproductive damage caused by exposure to Pb and As by increasing the concentration of antioxidant enzymes and reducing the level of oxidative damage parameters. In addition, NVC can increase the concentration of LH, FSH, and testosterone and improve sperm parameters and the process of spermatogenesis. The results of this study suggested that the use of antioxidant compounds containing vitamin C in the form of nanoparticles (possible to increase stability) can improve oxidative stress conditions in infertility, which can be caused by environmental pollutants in the ecosystem (Figure 3).

5.1. Study Limitations and Suggestions. According to the use of antioxidants such as vitamin C and E to treat infertility, the synthesis of NVC in the pharmaceutical market after comparative clinical studies with vitamin C is proposed to extend the results of the present study. Finding the exact toxic dose of NVC in vitro was one of the research limitations of this study, which is recommended to be considered in future studies. Moreover, investigation of the molecular path involved in oxidative damage and apoptosis is one of the limitations of the study which is recommended to complete the results.

Abbreviations

NVC: Nano-vitamin C
RIA: Radioimmunoassay
As: As group

FRAP: Ferric-reducing antioxidant power
Pb: Pb group
TBA: Thiobarbituric acid
TAC: Total antioxidant capacity
SOD: Superoxide dismutase
CAT: Catalase
GPx: Glutathione peroxidase
MDA: Malondialdehyde
ROS: Reactive oxygen species
WHO: World Health Organization
ALAD: δ-Aminolevulinic acid dehydratase.

Data Availability

The data used to support the findings of this study are available from the corresponding author upon request.

Additional Points

Highlights. (i) Nonessential heavy metals such as As and Pb are toxic to the reproductive systems. (ii) Oxidative stress is one of the major causes of infertility. (iii) Like the first report, NVC increased male reproductive function against Pb and As toxicity.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

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References

[1] S. Kumar, "Occupational and environmental exposure to lead and reproductive health impairment: an overview," *Indian Journal of Occupational and Environmental Medicine*, vol. 22, no. 3, p. 128, 2018.

[2] S. V. S. Rana, "Perspectives in endocrine toxicity of heavy metals-A review," *Biological Trace Element Research*, vol. 160, no. 1, pp. 1–14, 2014.

[3] A. Mantovani and F. Maranghi, "Risk assessment of chemicals potentially affecting male fertility," *Contraception*, vol. 72, no. 4, pp. 308–313, 2005.

[4] N. Kumar and A. Singh, "Trends of male factor infertility, an important cause of infertility: a review of literature," *Journal of Human Reproductive Sciences*, vol. 8, no. 4, p. 191, 2015.

[5] N. Lukáč, "Relationship between trace element concentrations and spermatozoa quality in rabbit semen," *Slovak Journal of Animal Science*, vol. 42, pp. 46–50, 2009.

[6] B. Sharma, S. Singh, and N. J. Siddiqi, "Biomedical implications of heavy metals induced imbalances in redox systems," *BioMed Research International*, vol. 2014, Article ID 640754, 2014.

[7] M. Zubair, M. Ahmad, and Z. I. Qureshi, "Review on arsenic-induced toxicity in male reproductive system and its amelioration," *Andrologia*, vol. 49, no. 9, Article ID e12791, 2017.

[8] M. Raeeszadeh, P. Mortazavi, and R. Atashin-Sadafi, "The antioxidant, anti-inflammatory, pathological, and behavioural effects of medicago sativa L. (Alfalfa) extract on brain injury caused by nicotine in male rats," *Evidence-Based Complementary and Alternative Medicine*, vol. 2021, Article ID 6694629, 9 pages, 2021.

[9] P. Duan, C. Hu, H. J. Butler et al., "4-Nonylphenol induces disruption of spermatogenesis associated with oxidative stress-related apoptosis by targeting p53-Bcl-2/Bax-Fas/FasL signaling," *Environmental Toxicology*, vol. 32, no. 3, pp. 739–753, 2017.

[10] J. Ramos-Treviño, S. Bassol-Mayagoitia, J. A. Hernández-Ibarra, P. Ruiz-Flores, and M. P. Nava-Hernández, "Toxic effect of cadmium, lead, and arsenic on the Sertoli cell: mechanisms of damage involved," *DNA and Cell Biology*, vol. 37, no. 7, pp. 600–608, 2018.

[11] J. Gandhi, R. J. Hernandez, A. Chen et al., "Impaired hypothalamic-pituitary-testicular axis activity, spermatogenesis, and sperm function promote infertility in males with lead poisoning," *Zygote*, vol. 25, no. 2, pp. 103–110, 2017.

[12] A. Alahmar, "Role of oxidative stress in male infertility: an updated review," *Journal of Human Reproductive Sciences*, vol. 12, no. 1, p. 4, 2019.

[13] A. Agarwal, M. Rana, E. Qiu, H. AlBunni, A. D. Bui, and R. Henkel, "Role of oxidative stress, infection and inflammation in male infertility," *Andrologia*, vol. 50, no. 11, Article ID e13126, 2018.

[14] A. Akbari and G.-A. Jelodar, "The effect of oxidative stress and antioxidants on men fertility," *Zahedan Journal of Research in Medical Sciences*, vol. 15, no. 7, pp. 1–7, 2013.

[15] S. Satarug, G. C. Gobe, D. A. Vesey, and K. R. Phelps, "Cadmium and lead exposure, nephrotoxicity, and mortality," *Toxics*, vol. 8, no. 4, p. 86, 2020.

[16] G. Ghanwat, A. J Patil, J Patil, M Kshirsagar, A Sontakke, and R. K Ayachit, "Effect of vitamin C supplementation on blood Lead level, oxidative stress and antioxidant status of battery manufacturing Workers of Western Maharashtra, India," *Journal of Clinical and Diagnostic Research: Journal of Clinical and Diagnostic Research*, vol. 10, no. 4, pp. BC08–11, 2016.

[17] S. Nayyar and R. Jindal, "Essentiality of antioxidant vitamins for ruminants in relation to stress and reproduction," *Iranian Journal of Veterinary Research*, vol. 11, no. 1, pp. 1–9, 2010.

[18] A. C. Caritá, B. Fonseca-Santos, J. D. Shultz, B. Michniak-Kohn, M. Chorilli, and G. R. Leonardi, "Vitamin C: one compound, several uses. Advances for delivery, efficiency and stability," *Nanomedicine: Nanotechnology, Biology and Medicine*, vol. 24, Article ID 102117, 2020.

[19] K. Nourian, H. Baghshani, H. Baghshani, and D. Shahsavani, "The effect of vitamin C on lead-induced plasma BiochemicalAlterations in fish, *Cyprinus carpio*," *Iranian Journal of Toxicology*, vol. 13, no. 2, pp. 25–29, 2019.

[20] S. Berenjian and M. Raeeszadeh, "Prescription of antibiotics before and after surgery at the surgical wards of isfahan amiralmomenin hospital compared with the standard guidelines," *Health Research Journal*, vol. 1, no. 3, pp. 133–140, 2016.

[21] A. Bendich, L. J. Machlin, O. Scandurra, G. W. Burton, and D. D. M. Wayner, "The antioxidant role of vitamin C," *Advances in Free Radical Biology & Medicine*, vol. 2, no. 2, pp. 419–444, 1986.

[22] S. Mumtaz, S. Ali, R. Khan et al., "Therapeutic role of garlic and vitamins C and E against toxicity induced by lead on various organs," *Environmental Science and Pollution Research*, vol. 27, no. 9, pp. 8953–8964, 2020.

[23] A. Chakraborty and N. R. Jana, "Vitamin C-conjugated nanoparticle protects cells from oxidative stress at low doses

but induces oxidative stress and cell death at high doses," *ACS Applied Materials & Interfaces*, vol. 9, no. 48, pp. 41807–41817, 2017.

[24] A. Akbari, G. Jelodar, and S. Nazifi, "An overview of the characteristics and function of vitamin C in various tissues: relying on its antioxidant function," *Zahedan Journal of Research in Medical Sciences*, vol. 18, no. 11, p. e4037, 2016.

[25] Z. Lan and W.-X. Yang, "Nanoparticles and spermatogenesis: how do nanoparticles affect spermatogenesis and penetrate the blood-testis barrier," *Nanomedicine*, vol. 7, no. 4, pp. 579–596, 2012.

[26] M. Kemp and R. Productions, *Organisation for Economic Co-Operation and Development*, OECD, Paris, France, 2009.

[27] S. Saritha, C. B. Davuljigari, K. P. Kumar, and G. R. Reddy, "Effects of combined arsenic and lead exposure on the brain monoaminergic system and behavioral functions in rats: reversal effect of MiADMSA," *Toxicology and Industrial Health*, vol. 35, no. 2, pp. 89–108, 2019.

[28] Y.-J. Kim and J.-M. Kim, "Arsenic toxicity in male reproduction and development," *Development & Reproduction*, vol. 19, no. 4, pp. 167–180, 2015.

[29] H. Abadin, A. Ashizawa, Y.-W. Stevens et al., *Toxicological Profile for Lead*, Agency for Toxic Substances and Disease Registry, Atlanta, GA, USA, 2007.

[30] D. A. Cory-Slechta, "Prolonged lead exposure and fixed ratio performance," *Neurobehavioral Toxicology & Teratology*, 1986.

[31] A. R. Chowdhury, A. Dewan, and D. Gandhi, "Key-word system of indexing," *Biochimica et Biophysica Acta (BBA) - General Subjects*, vol. 799, no. 1, p. 95, 1984.

[32] M. Raeeszadeh, A. Nadia khademi, and A. Akbari, "The effects of broccoli and caraway extracts on serum oxidative markers, testicular structure and function, and sperm quality before and after sperm cryopreservation," *Cryobiology*, vol. 99, pp. 11–19, 2021.

[33] Y. S. El-Sayed and M. S. El-Neweshy, "Impact of lead toxicity on male rat reproduction at "hormonal and histopathological levels"," *Toxicological and Environmental Chemistry*, vol. 92, no. 4, pp. 765–774, 2010.

[34] E. C. García-Díaz, L. E. Gómez-Quiroz, E. Arenas-Ríos, A. Aragón-Martínez, J. A. Ibarra-Arias, and M. d. S. I. Retana-Márquez, "Oxidative status in testis and epididymal sperm parameters after acute and chronic stress by cold-water immersion in the adult rat," *Systems Biology in Reproductive Medicine*, vol. 61, no. 3, pp. 150–160, 2015.

[35] T. Ruskovska, E. H. Jansen, and R. Antarorov, "Evaluation of assays for measurement of serum (anti)oxidants in hemodialysis patients," *BioMed Research International*, vol. 2014, p. 843157, 2014.

[36] M. Jeszka-Skowron, A. Zgoła-Grześkowiak, T. Grześkowiak, and A. Ramakrishna, *Analytical Methods in the Determination of Bioactive Compounds and Elements in Food*, Springer, Berlin, Germany, 2021.

[37] T. Ezedom and S. O. Asagba, "Effect of a controlled food-chain mediated exposure to cadmium and arsenic on oxidative enzymes in the tissues of rats," *Toxicology reports*, vol. 3, pp. 708–715, 2016.

[38] J. Limón-Pacheco and M. E. Gonsebatt, "The role of antioxidants and antioxidant-related enzymes in protective responses to environmentally induced oxidative stress," *Mutation Research: Genetic Toxicology and Environmental Mutagenesis*, vol. 674, no. 1-2, pp. 137–147, 2009.

[39] M. M. Bradford, "A rapid and sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding," *Analytical Biochemistry*, vol. 72, no. 1-2, pp. 248–254, 1976.

[40] X.-W. Cao et al., "A review of WHO laboratory manual for the examination and processing of human semen," *Zhonghua nan ke xue= National journal of andrology*, vol. 17, no. 12, pp. 1059–1063, 2011.

[41] I. Ortiz, M. Urbano, J. Dorado et al., "Comparison of DNA fragmentation of frozen-thawed epididymal sperm of dogs using sperm chromatin structure analysis and sperm chromatin dispersion test," *Animal Reproduction Science*, vol. 187, pp. 74–78, 2017.

[42] F. Khadivi, S. Razavi, and F. Hashemi, "Protective effects of zinc on rat sperm chromatin integrity involvement: DNA methylation, DNA fragmentation, ubiquitination and protamination after bleomycin etoposide and cis-platin treatment," *Theriogenology*, vol. 142, pp. 177–183, 2020.

[43] M. Seif, T. Abd El-Aziz, M. Sayed, and Z. Wang, "Zingiber officinale ethanolic extract attenuates oxidative stress, steroidogenic gene expression alterations, and testicular histopathology induced by sodium arsenite in male rats," *Environmental Science and Pollution Research*, vol. 28, no. 16, pp. 19783–19798, 2021.

[44] S. Kazemi, F Feizi, F Aghapour, G. A Joorsaraee, and A. A Moghadamnia, "Histopathology and histomorphometric investigation of bisphenol A and nonylphenol on the male rat reproductive system," *North American Journal of Medical Sciences*, vol. 8, no. 5, pp. 215–21, 2016.

[45] M. Qaderi Forough, M. Raeeszadeh, and A. Amiri, "Dose-response changes of *Brassica oleracea* var. italica hydroalcholic extract in the control of oxidative stress by induction of diazinon on the cells of testicular tissue in male adult rat," *Journal of Rafsanjan University of Medical Sciences*, vol. 16, no. 7, pp. 593–604, 2017.

[46] S. G. Johnsen, "Testicular biopsy score count—a method for registration of spermatogenesis in human testes: normal values and results in 335 hypogonadal males," *Hormone Research in Paediatrics*, vol. 1, no. 1, pp. 2–25, 1970.

[47] M. I. Yousef, F. M. El-Demerdash, and F. M. E. Radwan, "Sodium arsenite induced biochemical perturbations in rats: ameliorating effect of curcumin," *Food and Chemical Toxicology*, vol. 46, no. 11, pp. 3506–3511, 2008.

[48] C. L. Gaskill, L. M. Miller, J. S. Mattoon et al., "Liver histopathology and liver and serum alanine aminotransferase and alkaline phosphatase activities in epileptic dogs receiving phenobarbital," *Veterinary Pathology*, vol. 42, no. 2, pp. 147–160, 2005.

[49] F. M. El-Demerdash, M. I. Yousef, and F. M. E. Radwan, "Ameliorating effect of curcumin on sodium arsenite-induced oxidative damage and lipid peroxidation in different rat organs," *Food and Chemical Toxicology*, vol. 47, no. 1, pp. 249–254, 2009.

[50] A. Bhatia et al., "Dose dependent study on the effectiveness of β-carotene on the survivability of mice against lethal gamma irradiation," *Radiation Protection and Environment*, vol. 24, no. 1-2, pp. 96–101, 2001.

[51] H. Shoorei, A. Khaki, M. Shokoohi et al., "Evaluation of carvacrol on pituitary and sexual hormones and their receptors in the testicle of male diabetic rats," *Human & Experimental Toxicology*, vol. 39, no. 8, pp. 1019–1030, 2020.

[52] A. Fallah, A. Mohammad-Hasani, and A. H. Colagar, "Zinc is an essential element for male fertility: a review of Zn roles in men's health, germination, sperm quality, and fertilization," *Journal of Reproduction & Infertility*, vol. 19, no. 2, pp. 69–81, 2018.

[53] S. Ramaswamy and G. F. Weinbauer, "Endocrine control of spermatogenesis: role of FSH and LH/testosterone," *Spermatogenesis*, vol. 4, no. 2, Article ID e996025, 2014.

[54] S. Karanth, W. H. Yu, A. Walczewska, C. Mastronardi, and S. M. McCann, "Ascorbic acid acts as an inhibitory transmitter in the hypothalamus to inhibit stimulated luteinizing hormone-releasing hormone release by scavenging nitric oxide," *Proceedings of the National Academy of Sciences*, vol. 97, no. 4, pp. 1891–1896, 2000.

[55] K. Renu, H. Madhyastha, R. Madhyastha, M. Maruyama, S. Vinayagam, and A. Valsala Gopalakrishnan, "Review on molecular and biochemical insights of arsenic-mediated male reproductive toxicity," *Life Sciences*, vol. 212, pp. 37–58, 2018.

[56] U. Okon and I. Utuk, "Ascorbic acid treatment elevates follicle stimulating hormone and testosterone plasma levels and enhances sperm quality in albino Wistar rats," *Nigerian Medical Journal*, vol. 57, no. 1, p. 31, 2016.

[57] I. Rahayu, E. Usman, and M. Reza, "Effect of vitamin C on testosterone level, sperm count and sperm morphology in gentamicin-induced wistar rats," *International Journal of Research in Medical Sciences*, vol. 7, no. 2, 2019.

[58] L. Rössig, J. Hoffmann, B. Hugel et al., "Vitamin C inhibits endothelial cell apoptosis in congestive heart failure," *Circulation*, vol. 104, no. 18, pp. 2182–2187, 2001.

[59] S. I. Chang, B. Jin, P. Youn, C. Park, J.-D. Park, and D.-Y. Ryu, "Arsenic-induced toxicity and the protective role of ascorbic acid in mouse testis," *Toxicology and Applied Pharmacology*, vol. 218, no. 2, pp. 196–203, 2007.

[60] R. Z. Hamza and A. E.-A. A. Diab, "Testicular protective and antioxidant effects of selenium nanoparticles on Monosodium glutamate-induced testicular structure alterations in male mice," *Toxicology Reports*, vol. 7, pp. 254–260, 2020.

[61] M. F. El-Sayed, S. K. Abdel-Ghafar, M. A. Adly, A. A. Salim, and W. M. Abdel-Samei, "The ameliorative effects of DMSA and some vitamins against toxicity induced by lead in the testes of albino rats. II," *The Journal of Basic & Applied Zoology*, vol. 71, pp. 60–65, 2015.

[62] M. Shaban El-Neweshy and Y. Said El-Sayed, "Influence of vitamin C supplementation on lead-induced histopathological alterations in male rats," *Experimental & Toxicologic Pathology*, vol. 63, no. 3, pp. 221–227, 2011.

[63] M. Harsij, H. Gholipour Kanani, and H. Adineh, "Effects of antioxidant supplementation (nano-selenium, vitamin C and E) on growth performance, blood biochemistry, immune status and body composition of rainbow trout (*Oncorhynchus mykiss*) under sub-lethal ammonia exposure," *Aquaculture*, vol. 521, Article ID 734942, 2020.

[64] M. Raeeszadeh, M. Rezaee, A. Akbari, and N. Khademi, "The comparison of the effect of Origanum vulgare L. extract and vitamin C on the gentamycin-induced nephrotoxicity in rats," *Drug and Chemical Toxicology*, pp. 1–8, 2021.

[65] R. Patra and D. Swarup, "Effect of antioxidant ascorbic acid, l-methionine or α tocopherol alone or along with chelator on cardiac tissue of lead-treated rats," *Veterinarski Arhiv*, vol. 74, no. 3, pp. 235–244, 2004.

[66] J. González-Serrano, R. López-Pintor, R. Cecilia-Murga, J. Torres, G. Hernández, and J. López-Quiles, "Application of propolis extract, nanovitamin C and nanovitamin E to prevent alveolar osteitis after impacted lower third molar surgery. A randomized, double-blind, split-mouth, pilot study," *Medicina Oral, Patología Oral y Cirugía Bucal*, vol. 26, no. 2, pp. e118–e125, 2021.

[67] A. A. Fouad, W. H Albuali, A. S Al-Mulhim, and I Jresat, "Protective effect of telmisartan treatment against arsenic-induced testicular toxicity in rats," *Zeitschrift fur Naturforschung. C, Journal of biosciences*, vol. 70, no. 7-8, pp. 175–181, 2015.

[68] B. T. Lovaković, "Cadmium, arsenic, and lead: elements affecting male reproductive health," *Current Opinion in Toxicology*, vol. 19, pp. 7–14, 2020.