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EFFECT OF VITAMIN C ON SEMEN QUALITY OF RABBITS INTOXICATED WITH LEAD

Kabir A. Raheem[*1], Daniel C. Ifenkwe[2], Yunus A. Wahab[3] and Peter I. Rekwot[4]

[1]Department of Theriogenology and [2]Department of Physiology and Pharmacology, College of Veterinary Medicine, Michael Okpara University of Agriculture, Umudike, Abia State, Nigeria; [3]Department of Veterinary Medicine, University of Ibadan, Ibadan, Oyo State, Nigeria and [4]National Animal Production Research Institute, Shika, Zaria, Kaduna State, Nigeria.

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ABSTRACT

The use of Vitamins C in the management of reproductive dysfunction induced by lead poisoning in rabbits has not been completely elucidated. In this study, 9 adult Chinchilla rabbit-bucks were randomly assigned into three groups (groups A – C) consisting of three animals per group. The first group (group A) is the controlreceived only drinking waterthroughout the experimental period. Group B received lead (10 mg/kg) in drinking water daily for a period of 42 days while the second group (group B) received both lead (10 mg/kg) and Vitamin C (500 mg/kg) in drinking water for the same period. Semen samples were collected with the aid of an artificial vagina and evaluated for volume, colour, consistency and pH before and at the end of the treatment period for all the groups. Testes were also harvested for histopathological examination. Data generated from the study were subjected to statistical analysis using paired students' t test and ANOVA. Lead treatment caused significant ($p<0.05$) decreases in semen characteristics such as spermatozoa motility and concentration, liveability and percentage spermatozoa abnormalities compared to the control and their pre-treatment values. Testicular histopathologic lesions in the lead treated group included vacuolation, degeneration of seminiferous tubules and the absence of spermatozoa in the lumen. On the other hand, there were significant improvements on these parameters in the group that received lead and vitamin C. The results suggest that vitamin C at a dosage of 500 mg/kg is capable of suppressing and protecting against the adverse effects of lead poisoning on semen quality and testicular histopathology in rabbits.

Keywords: Lead poisoning, Vitamin C, Rabbit, Effect, Semen Quality

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INTRODUCTION

An important aspect of male infertility is of unknown aetiology which may be attributed to various environmental and occupational exposures to toxic substances. Such toxic substances include pesticides,

***Correspondence: E-mail: ka.raheem@mouau.edu.ng; Tel: +2349031310693**
ISSN: 2315 – 6856; e-ISSN: 2636 – 5553; *Website: www.jvasonline.com*

asbestos, lead, formaldehyde and cadmium with lead occupying the first position. The wider use of lead-related substances and its subsequent presence and spread in almost every part of our environment partly gives credence to this line of thought. Lead and lead compounds are used in paint, ceramics, pipes, plumbing materials, solders, gasoline, ammunition and cosmetics such as lipstic and vanish with largest use in the acid storage of motor batteries [2,3,4].

Lead can also be emitted into the environment from industrial sources and contaminated sites, such as former lead smelters. Natural levels of lead in soil may range between 50 and 400 parts per million.Economic activities such as mining, smelting and petroleum refining cause substantial increase in lead levels in the environment, especially near mining and smelting sites, thereby constituting occupational hazards to nearby residents and workers in those industries [5,6]. An incidence of acute lead poisoning that was linked to artisanal gold mining occurred in Zamfara State, North-western Nigeria in 2010 and resulted in the death and morbidity of over 400 and 1,000 children respectively [7].

Disorders associated with lead poisoning have been reported in some body systemsof human and animals. The cardiovascular, renal, nervous and reproductive systemsare mostly affected. The adversities of lead are more felt in infants and children with significant impact on brain development and associated reduced intelligence quotient [8]. Lead poison is acquired mainly through the oral route. Gastrointestinal absorption of lead was estimated to be about 40% in children, 10% in ruminants and 3% in non-ruminants [9].

Previous experimental studies have demonstrated alleviation of the adversities of lead poisoning by the administration of Vitamin C or other anti-oxidants [10,11]. Most of these studies have focussed on ratsand mice whereas the rabbit has been acknowledged to be a good model for male reproductive toxicology studies in humans [12]. Besides, the rabbit is also prone to lead poisoning from paints used to prevent rusting of the wire gauze cage. The aim of the study was to evaluate the effect of vitamin C on semen quality and testicular histopathology of rabbits experimentally intoxicated with lead.

MATERIALS AND METHODS

Experimental Animals and their management

A total of 9 maturemaleChinchilla and one female New Zealand White rabbits were used in this study. The rabbits were sourced from a commercial rabbit farm in Ikot Ekpene, Akwa Ibom State, Nigeria. They were housed individually in an iron cage of about I.5 m^2 per rabbit. The rabbits were fed with pelletized feed (Vital feed®, Nigeria) and had access to water *ad libitum.* They were acclimatization for 3 weeks before the commencement of the study. During this period, the rabbits were treated for both internal and external parasites with Ivermectin injection, (Promectin®) administered at the dose of 0.04 mg/kg through the subcutaneous route

Experimental Design

The nine male rabbits, weighing between 1.5 Kg and 2.1 kg, were randomly assigned into three groups (A – C) of three rabbits each. The first group (A) served as the control and received only drinking water throughout the experimental period. The second group (B) was treated with lead acetate (Sigma, Aldrich, UK) at 10 mg/kgin drinking water while rabbits in the third group (C) received a combination of lead acetate (10 mg/kg) and Vitamin C (500 mg/kg) in drinking water. The choice of lead dosages of 10 mg/Kg body weight and 500 mg/Kg Vitamin C were based on results of a previous study with lead acetate and vitamin C in rats [13]. Rabbits in each of the three groups received their treatments *per os* for a period of 42 days.

Semen Collection

The rabbit-bucks were trained to ejaculate into artificial vagina (AV) during the acclimatization period of three weeks. The AV was used to collect semen from all the animals for baseline data just before the

commencement of treatment and repeated at the end of the treatment period. The AV was specifically constructed using a small plastic pipe and condom as inner lining. Briefly, after the rabbit-buck had mounted the rabbit-doe, the AV was gently slipped in front of vagina and the buck penis was inserted into the AV for ejaculation as was described in previous study l14l. It was necessary to warm the instrument to 38ºC in order to perfectly mimic the body temperature of the vagina just prior to each usage while the glycerol (placed inside the condom) caused expansion of the condom and subsequent thinning of the AV lumen to exert a pressure on the buck penis when it was inserted.

Semen evaluation

Semen macroscopic parameters that included semen volume, colour, consistency and pH were evaluated according to standard technique [15]. The semen colour was graded as 3.0, 2.0 and 1.0 for creamy, milky and watery colourations respectively. The consistency scale ranged from 1 to 4 where 1 stood for watery, 2 for slightly thick, 3 for thick and 4 for very thick.Microscopic evaluation of spermatozoa motility, semen concentration, liveability and spermatozoa morphological abnormalities were also determined as was described in previous study [16].

Histology of the Testis

At the end of the treatment, the testes were harvestedand cut into strips of 1 cm long, and processed into a paraffin-embedded tissue section according to standard technique with little modification [17]. Briefly, testicular tissue sections were placed in a Bouin's solution for 24 hours before subsequent dehydration in ascending grades of ethanol. Thereafter, the samples were cleared overnight in chloroform, infiltrated and embedded in molten paraffin wax. The blocks were later trimmed and paraffin-embedded samples were sectioned at 5-6 microns on to a super frost slide..

The protocol for processing the tissue section on the slide for Haematoxylin and Eosin staining (H & E) had been described in earlier study [18].Briefly, the sections were de-paraffinised in tissue clearing reagent, Histoclear (Fisher Scientific, Loughborough, UK) for 30 minutes and rehydrated in a graded series of ethanol of 100%, 95%, 70% and 50% for 5 min each. The slides were then incubated in 10% (v/v) Gill's Haematoxylin (Park Scientific Ltd., Northampton, UK) in distilled water (dH_2O) for 4 minutes. They were then dipped in 1% (v/v) acetic acid in ethanol for 30 sec before staining in 1% (w/v) Eosin (Sigma) in dH_2O for 1 min. The slides were washed in slow running water for 5 min between each step. Finally, sections were dehydrated in graded alcohols (100%, 90% 70%, 50% and 50% for 5 minutes each) and mounted with Vecta Mount permanent mounting medium (Vector Laboratories, Peterborough, UK) before applying coverslips.

Statistical analysis of data

The data obtained from the study were presented as mean ± standard error of mean (SEM). For each group, pre-treatment data were firstly compared with post treatment data using paired student's t test while the data of all the three groups at the end of treatment were compared using analysis of variance (ANOVA) with the aid of SPSS 20 (Chicago, IL, USA). Values of P less than 0.05 are considered significant. When P is less than 0.05, Bonferroni was chosen for post hoc analysis of individual comparison of treatment groups.

RESULTS

Semen macroscopic parameters

Semen macroscopic parameters evaluated are presented in Table 1. Semen volume ranged between 0.50 ml and 1.25 ml before the treatment in all the three groups. Mean semen volume in group A before and after the treatment were 0.67 ± 0.17 ml and 0.70 ± 0.15 ml respectively, while that of group B was significantly ($p< 0.05$) reduced from 0.73 ± 0.03 ml before treatment to 0.46 ± 0.11 ml after the treatment. In group C, reduction of semen volume from 1.03 ± 0.22 ml before treatment to 0.64 ± 0.28 ml

after treatment was not significant ($p > 0.05$) and the latter was also similar to the post treatment values of control group.

Semen colour of control group was creamy before and after the treatment with average grade score of 3.00 ± 0.00. Group B had creamy coloured semen (score 2.63 ± 0.33) prior to treatment which turned milky (score 2.00 ± 0.00) at the end of the experiment, whereas the creamy colour of group C (2.66 ± 0.33) was unchanged at the end of treatment (3.00 ± 0.00 respectively) and hence, was not significantly ($p > 0.05$) different compared to the control.

Group A had semen consistency grade score of 3.00 ± 0.00 (thick) before and after the treatment. Group B had an average score of 2.83 ± 0.66 prior to treatment which was reduced to 1.00 ± 0.00 (slightly thick) at the end of the experiment. On the other hand, the semen consistency grade scores for group C before and after treatment were 2.53 ± 0.66 and 3.00 ± 0.00 (thick) respectively with no significantly difference between the two values and compared to the post treatment value of group A.

The pH of the semen before treatment in all the three groups tended towards neutrality (7.0). Group A had pH of 7.50 ± 0.00 and 7.54 ± 0.57 before and after the treatment respectively. pH of Group B significantly reduced from 7.53 ± 0.01 before the treatment to 5.04 ± 0.09 (acidic) at the end of the treatment. The pH of group C did not significantly changed before (7.54 ± 0.04) and after (7.53 ± 0.04) treatment.

Table I. Semen macroscopic parameters in rabbits intoxicated with lead and treated with vitamin C and their controls.

		Control (Group A)	Lead(Group B)	Lead + Vit C (Group C)
Semen Volume (ml)	Before treatment	0.67 ± 0.17	0.73 ± 0.03	1.03 ± 0.22
	After Treatment	0.70 ± 0.15^{a}	$0.46 \pm 0.11^{b*}$	0.64 ± 0.28^{a}
Semen colour	Before treatment	3.00 ± 0.00	2.63 ± 0.33	2.66 ± 0.33
	After Treatment	3.00 ± 0.00^{a}	$2.00 \pm 0.00^{b*}$	3.00 ± 0.00^{a}
Semen consistency	Before treatment	3.00 ± 0.00	2.83 ± 0.66	2.53 ± 0.66
	After Treatment	3.00 ± 0.00^{x}	$1.01^{y*} \pm 0.00$	$3.00^{x} \pm 0.00$
Semen pH	Before treatment	7.50 ± 0.00	7.53 ± 0.01	7.54 ± 0.04
	After Treatment	7.54 ± 0.57^{a}	$5.04 \pm 0.09^{b*}$	7.53 ± 0.04^{a}

Different superscript on the same row denotes significant difference at $p > 0.05$compared to the control while presence of '' along the column indicates significance difference at $p > 0.05$ between pre-treatment and post-treatment values of the same group.

Semen microscopic parameters

The percentage sperm motility in the control group A before (69.5 ± 7.3%) and after (69.0 ± 6.6%) the treatment did not significantly ($p > 0.05$) differ from each other. On the contrary, there was a significant ($p < 0.05$) reduction in group B from 74.0 ± 2.1% before treatment to 32.3 ± 8.3% after treatment. At the same time, no significant ($p > 0.05$) change was observed with group C before (85.5 ± 3.7%) and after (84.7 ± 8.4%) the treatment and when compared with the control. Percentage spermatozoa motility of

Group B was also significantly lower than those control and group C at the end of treatment.

The percentage viable spermatozoa of the control (A), group B and C were 79.4± 3.2%, 84.1 ± 1.05% and 91.1 ± 2.26 % before the treatment. After treatment, there was a significantly ($p < 0.05$) reduction in group B (56.3 ± 3.8) compared to the control (83.0 ± 0.6 %) whereas a significant ($p > 0.5$) increase was observed with group C at the end of the treatment (95.3 ± 7.20%) compared to the control.

Prior to the treatment, the spermatozoa concentration of group B (106.26 ± 3.03 × 10^6/ml) and group C (104.30 ± 1.03 × 10^6/ml) treatment groups were not statistically different ($p > 0.5$) from that of the control (113.10 ± 3.40 × 10^6/ml). After the treatment, there was no significant change in the sperm concentration of the control (119.00 ± 2.57 × 10^6/ml), while significant ($p < 0.05$) reduction and increment were respectively observed with group B (30.00 ± 1.32 × 10^6/ml) and group C (150.00 ± 13.00 × 10^6/ml) and these differed significantly ($p < 0.05$) from their pre-treatment values. In addition, the spermatozoa concentration of group B was significantly ($p > 0.05$) lower than that of control and group C.

Various abnormalities were observed in the spermatozoa of the experimental rabbits and these were mostly head and tail defects with a few cases involving the mid-piece. The percentage of abnormal spermatozoa was similar before and after treatment in the control (group A). In group B, there was a significant ($p < 0.05$) increase in percentage of abnormal spermatozoa from 1.6 ± 0.0.8 % before the treatment to 31.1 ± 8.2 % after the treatment, whereas, the percentage of abnormal spermatozoa of group C was not significantly ($p > 0.05$) different before (1.8 ± 0.6%) and after the treatment (3.2 ± 0.9%). Similarly, the percentage spermatozoa abnormalities in group B was significantly ($p > 0.05$) higher than those of group A (control) and group C.

Table 2. Semen microscopic characteristics in Rabbit intoxicated with lead and treated with vitamin C and their controls.

		Control (Group A)	Lead (Group B)	Lead + Vit C (Group C)
Motility Spermatozoa (Score: 1-100%)	Before treatment	69.5 ± 7.3	74.0 ± 2.1	85.5 ± 3.7
	After Treatment	69.0^{a} ± 6.6	32.3^{b*} ± 8.3	84.7^{a} ± 8.4
Percentage live spermatozoa (Score: 1-100%)	Before treatment	79.4 ± 3.2	84.1 ± 1.05	91.1 ± 2.26
	After Treatment	83.0^{a} ± 0.6	56.3^{b} ± 3.8	95.3^{a} ± 7.20
Spermatozoa Concentration (× 10^6/ ml)	Before treatment	113.10 ± 3.40	106.26 ± 3.03	104.30 ± 7.03
	After Treatment	119.00^{a} ± 2.57	30.00^{b} ± 1.32	150.00^{c} ± 13.00
Spermatozoa abnormalities (%)	Before treatment	1.9 ± 0.1	1.6 ± 0.8	1.8 ± 0.6
	After Treatment	2.7 ± 0.4^{a}	31.1 ± 8.2^{b}	3.2 ± 0.9^{a}

Different superscript on the same row denotes significant difference at $p < 0.05$ compared to the controlwhile presence of '' along the column indicates significance difference between pre-treatment and post-treatment valuesat $p < 0.05$.

Testicular histopathology

Evaluation of the testicular histology of the control group at a lower magnification showed a uniform morphology of the seminiferous tubules andSertoli cells in close association with the germ cellline in the control group (Figure IA).On the other hand, group B showed a conspicuous distorted testicular morphology with a lot of spaces within the seminiferous tubular (ST)lumen indicative of absence of spermatozoa (arrow in Figure 1B). Group C showed a better testicular histo-morphology almost nearest to that observed in the control (Figure 1C).

At higher magnification, the control animal had a normal testicular morphology comprising of ST which are active containing the germ cell lines at various stages of development from spermatogonia at the base

of the tubule which undergo division and differentiation as they progress to the luminal compartment. Spermatozoa were also observed at the central lumen of the ST as a brand of wave-like structure (Figure2A). In group B, the pathologies were very obvious and included desquamated seminiferous tubular epithelium, presence of cellular debris in the centre of tubules (arrow in Figure2B) instead of spermatozoa and disappearance of Leydig cells in the interstitium (Figure 2B). Group C had some spermatozoa in the ST and moderate degeneration of the seminiferous epithelium compared to the group B (Figure 2C).

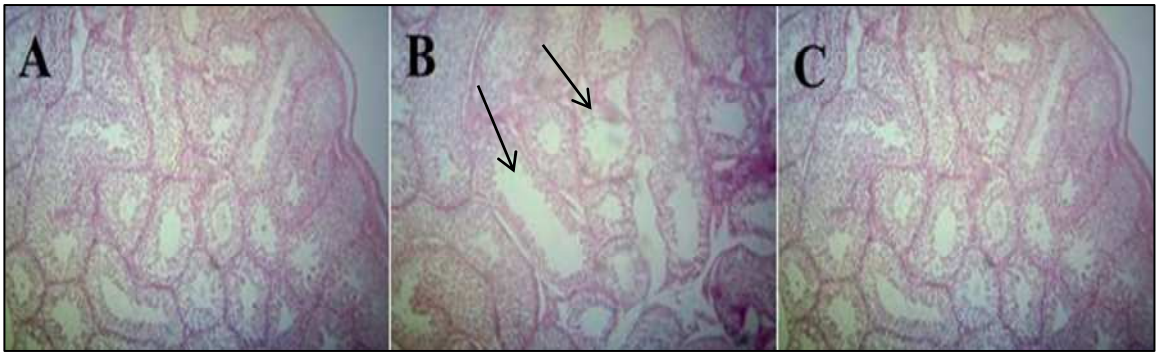

Figure 1.Histological sections of testes of rabbits treated with water (A), lead acetate (10 mg/kg) (B) and lead acetate (10 mg/kg) and vitamin C (500 mg/kg) (C) for 42 days. (× 4) Haematoxylin Eosin stains.

DISCUSSION

Lead poisoning has been recognized as a major public health risk in both developed and developing countries although emphasis has always been on developing countries where appropriate measures are not in place to contain human exposure to the metal [19,20]. Oral administration was used in this study since the oral route via drinking water has been reported as the amajor route of exposure of humans and animals to lead poisoning [21,22]. According to the National Research Council [9], gastrointestinal absorption of lead was estimated to be about 40% in children, 10% in ruminants and 3% in non-ruminants. Following absorption, almost 99% of the lead binds to erythrocytes while the remaining diffuse into soft tissues and bones, where they equilibrate with blood lead. Thereafter, the accumulation of lead in erythrocytes, soft tissues and rapidly growing bones are mostly responsible for its toxic effects [19].

The semen volumes obtained in the control, lead (prior to treatment) and lead + Vit C are at par with those of previous reports [23,24]. At the same time,reduced semen volume with the lead group in this study is consistent with observation of previous studies [25,26]. Semen volume has a direct association with total spermatozoa concentration while extremely low volume is observable with frequent ejaculate collection within a short time [23] and is usually associated with values below the minimum concentration and failure of spermatozoa to be transported into the uterus and oviduct, sequel to which is fertilization failure. pH is a measure of acidity and alkalinity with normal semen tending towards neutrality while acidity is more detrimental to semen than alkalinity [27]. The low pH of the lead group (in the range of 5.0) is detrimental to spermatozoa motility necessary to propel it to the oviduct and may also interfere with its functional capacity since too low intracellular pH does not allow for sperm capacitation [28].

The results of spermatozoa motility, viability, concentration and percentage abnormalities obtained in this study prior to the commencement of the treatment agree with previous reports in rabbits [29]. The significant reduction in spermatozoa motility observed with the lead treatment compared with the control also agrees with previous observations in the rat [30], mice [31], bull [32], deer [33] and human [25]. Alexaki *et al.* [32] demonstrated the adverse effects of lead on bull semen *in vitro*. In humans, these poor semen characteristics are similar to findings in both low and high exposure to environmental lead [34] especially when the lead plasma concentration is over 10 µg/L [35]. On the contrary, Willems *et al.* [36] failed to observe any significant deleterious effect in sperm characteristics of rabbits after lead exposure. The difference may be associated with different route of administration (subcutaneous versus oral) and the much lower dosages of 0.25 mg/kg and 0.5 mg lead acetate/kg body weight used in their study compared to 10 mg/kg body weight used in this study.

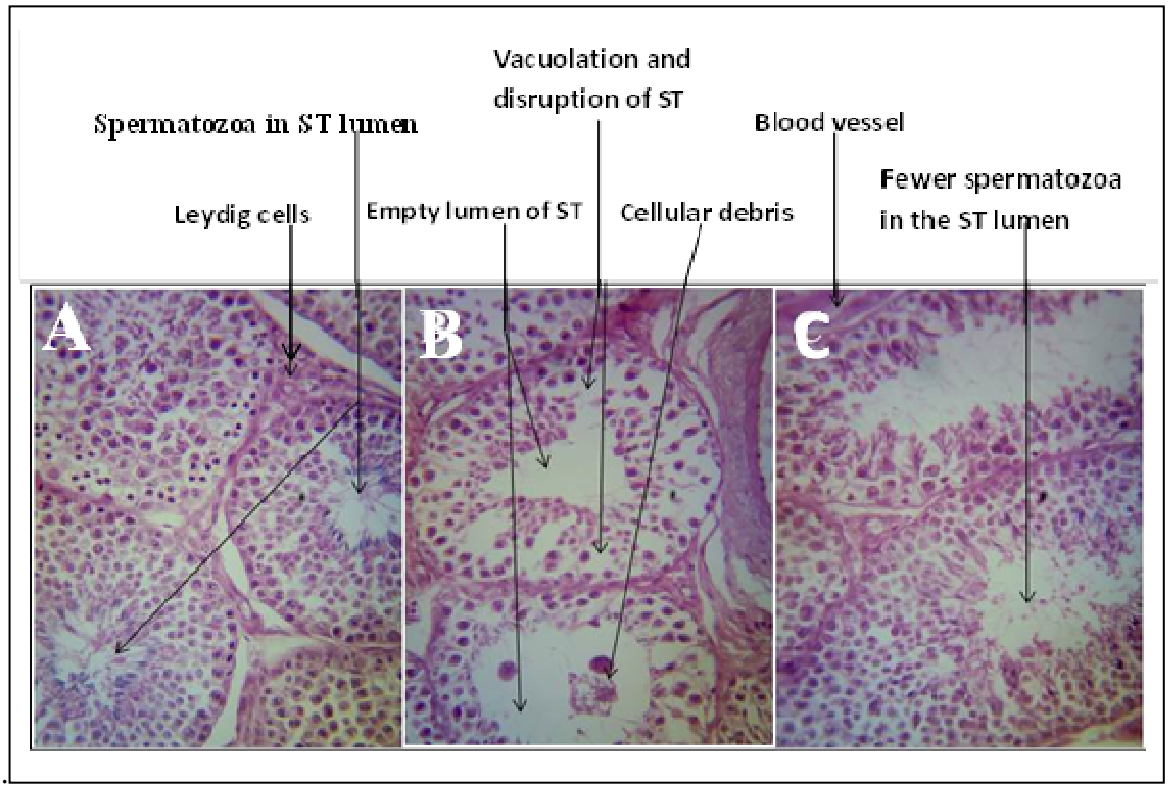

Figure 2. Testicular histopathology of rabbit testis in control (A), lead (B) and lead+ VitC (C) groups (× 200) Haematoxylin Eosin stains. Compared to the control with intact testicularhisto-morphology, vacuolation, cellular debris and absence of spermatozoa were seen in the lead group while the lead + Vit C group had few spermatozoa and moderate degeneration of the seminiferous tubular parenchyma.

Motility is an important semen characteristic that determines the potential of ejaculated spermatozoa getting to the isthmic-ampullary junction of the oviduct where fertilization usually occurs[37]. According to Zamjanis [35], there are four major categories of motility exhibited by sperm cells, namely: progressive, rectilinear motility in which the sperm cells move rapidly in straight forward direction; circling motility that involves movement in circle due to defective mid-piece or tail; reverse circling movement that denotes movement in backward direction and finally pendulation movement in which spermatozoa exhibit jerky, serpentine motility without showing marked progress. Semen is usually deposited at the vagina or the uterine cervix depending on the species. Therefore, there is need for the

spermatozoa to move through the 'hurdle' of cervix and uterus as well as uterine horn before getting to the point of fertilization in the oviduct. The clinical implication of the circular motility exhibited in the lead treated group agrees with previous observations of lead treatment in rats [39] with failure of the spermatozoa to reach the oviduct.
Sperm concentration in rabbit-buck varies slightly depending on the body weight and breed [40,41]. The value prior to treatment in the treatment groups and that of the control rabbit-bucks were within earlier reported ranges [29], while reduction in spermatozoa concentration of the lead group after treatment agrees with Naha *et al.* [25]. Reduction in spermatozoa concentration is usually associated with loss of fertilizing potential of the spermatozoa.

Sperm cells are produced within the seminiferous tubule of the testes. The presence of necrosis and other histopathological lesions within the seminiferous tubule of the lead group reported in the present study agree with previous studies [42,43,44]. Similar disruption of histo-architexture of the seminiferous tubule and the epididymis was reported in experimental lead poisoning in the rat [42]. The degeneration of the testicular cells found in this study is similar to the observation of Saxena *et al.* [43] in growing rat with consequent implication of affecting steroidogenesis vis-à-vis spermatogenesis. The absence of spermatozoa in the seminiferous tubule lumen of the lead treated group is a pointer to arrest of spermatogenesis in the present study. On the contrary, there was restoration of poor semen characteristics in the lead + Vit C group with improved spermatozoa quality and testicular histopathology compared with the lead group. These results are consistent with reports of earlier workers [45,46,47]. The restoration of steroidogenic enzymes (such as 17β-hdroxysteroid dehydrogenase) activities by vitamin C has been observed in a previous study [48].

Reproductive effects of lead are complex and involve multiple pathways. Many hypotheses have been suggested to explain the mechanisms through which lead adversely affects reproduction. One of such mechanisms is the reduction in semen parameters such as spermatozoa motility, liveability, concentration and associated increased percentage spermatozoa abnormalities as observed in this and other studies. The possible mechanism by which lead causes adverse effect on spermatogenesis may be due to anaemia which is a consistent observation in lead poisoning [49]. Lead in the blood has a direct effect on the oxygen-carrying capacity of the blood by causing reduced hemoglobin concentration [50]. Reduced supply of oxygen to the testicular cells impairs testicular cell survival and their functional capacity.
The pathological testicular lesion observed in the lead group may not be due to direct localisation of lead within the testicular tissue since previous reports suggest that blood-testis barrier is able to protect testicular cells from direct exposure to high levels of blood lead [51]. On the contrary, a study in Bangalore, India involving occupation lead exposure detected lead in the semen of lead workers which is suggestive of a compromise in the integrity of blood-testis barrier especially at higher lead doses [26].
Another mechanism by which lead may induce poor semen characteristics and causing testicular histopathology is the oxidative stress associated with generation of reactive oxygen species (ROS) and the loss of sperm defence capacity to cope with such stress [52]. Sperm cells, like other cells, contain a large number of antioxidants to prevent or repair the damage caused by ROS, as well as regulate redox-sensitive signaling pathways [53]. Reactive oxygen species inhibit the production of sulfhydryl antioxidants, inhibit enzyme reactions, damages nucleic acids and inhibit DNA repair, as well as initiating lipid peroxidation in cellular membranes. Lead intoxication induces oxidative stress and promotes the generation of hydrogen peroxide [54].

Reactive oxygen species produce adverse effects on sperm viability, motility, DNA fragmentation, membrane lipid peroxidation, capacitation, hyperactivation and acrosome reaction [33]. Lead and cadmium were reported to cause an increase in ROS by elevating testicular malondialdehydes (MDA) and decrease in activities of the testicular antioxidant enzymes, superoxide dismutase (SOD), catalase, glucose 6 phosphate dehydrogenase (G6PDH) and glutathione-S-transferase (GST) in mitochondrial and/or post-mitochondrial fraction [48].

Vitamin C is an anti-oxidant and this is a possible mechanism for restoration of all semen characteristics and testicular histopathology induced by lead poisoning in the lead + Vit C group. The administration of Vit C reportedly reduced the generation of excessive reactive oxygen species due to lead-associated oxidative stress in rat spermatozoa [52,55]. Furthermore, the administration of Vit C in drinking water significantly reduced anaemia caused by lead intoxication in experimental Sprague Dawley rats [49] possibly by elevating the haemoglobin concentration and erythrocyte antioxidant enzymes such as superoxide dismutase [45]. In addition, Lihm *et al.* [56] reported the use of high doses of vitamin C to accelerate the excretion of lead in body of rats experimentally exposed to lead poisoning. Such elimination of lead from the body system is essential to elongate the time it takes for the lead to get to the threshold of causing deleterious effects.

In conclusion, therefore, the results of this study suggest that exposure of rabbit to 10 mg/kg of lead acetate caused reduction in semen characteristics that included reduced motility, concentration and viability of spermatozoa and increased number of spermatozoa abnormalities with degeneration of the seminiferous tubule and adverse effect on spermatogenesis. The addition of Vit C at 500 mg/kg was able to reverse the poor semen characteristics with minimal pathology on the testes. This suggests that Vit C at the dosage used in this study could effectively ameliorate the adverse effects of lead poisoning on semen characteristics and testicular histology in rabbit.

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