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The Effect of Zinc and Coenzyme Q10 Supplementation in Combination with Vitamin C on Lead Acetate-Induced Testicular Injury and Oligospermia in Rats

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ABSTRACT

Background: Male infertility means that a man cannot start a pregnancy with the female partner. In this relation, the fatal impact of environment factors such as heavy metals was reported a worldwide concern. Recent studies revealed that high concentrations of lead could cause a decrease in sperm motility, volume, count, concentration and activity. Zinc, coenzyme Q10 and vitamin C as effective antioxidants were discussed in this relation. So this study evaluated the protective effect of zinc, coenzyme Q10 in combination with vitamin C against lead acetate induced testicular toxicity and oligospermia in rats.

Methods: Thirty adult male rats were allocated to five equal groups including control, induced lead (LA), Zinc, coenzyme Q10 and their combination with vitamin C as treatment groups. They were administered orally by gavage two hours before lead acetate induction. Semen profile and gonadal histopathology were assessed at the end 21 days.

Results: LA exposure could lead to oligospermia and a decrease in sperm motility, viability and abnormality in morphology. Zinc, coenzyme Q10 and their combination with vitamin C resulted in a significant higher number of normal sperms up. Histopathological findings showed protective effects of zinc, coenzyme Q10 and their combination and establishment of a normal histoarchitecture in testis with normal sperms.

Conclusion: Supplementation with zinc, coenzyme Q10 and their combination with vitamin C could overcome the lead induced testicular injuries and oligospermia and result in an increase in sperms quality of rats.

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Introduction

Infertility as a disease of the reproductive system crashes the capacity a person to be capable to have children and can affect a male, a female or both. Man infertility usually has a tough problem with his gonads and cannot start a pregnancy with his female partner (1). Potential links between exposure to environmental toxicants such as lead and oligospermia that lead to male fertility were demonstrated (2). Exposure to environmental pollutants was illustrated to affect sperm manufacturing (3). Furthermore, some chemicals can cause direct damaging effects on accessory male gonads and the testis such as 1,2-Dibromo-3-Chloropropane (DBCP) effects on germ cells (4). It is estimated that infertility disorder enrolls 8-12% of couples globally, while the male factor was shown to be the first reason to contribute approximately 50% of married men (5).

However, lead (Pb^{2+}) as a toxic element to testicles can lead to infertility (6). In this relation, the role of dietary antioxidants to maintain the testis from damage was described before (7). The fatal impact of heavy metals on fertility is still a worldwide concern. Lead acetate as a heavy metal can be naturally found in the environment (8). Lead exposure by human can impact on other body systems too such as cardiovascular system, renal system, respiratory system, nervous system and gastrointestinal system (9-13). Lead acetate (LA) as an industrial environmental pollutant can endanger many body organs such as bone, lung, liver, kidney, immune system and the gonads (14). Mechanism of testicular injury triggered by LA is an oxidative stress process which can be developed by the inequity between the capacity of the antioxidants and the free radicals in the testis (15, 16). A recent study revealed that testicular toxicity induced by LA can involve free radicals such as hydroxyl ion (OH^-), superoxide ion (O^{-2}), and nitric oxide (NO) (17). It was shown that toxicity with high concentrations of LA can decrease sperm motility, volume, count, concentration, and activity (18, 19).

Coenzyme Q10 is the main element in the chain of electron transportation in eukaryotic cell mitochondria and is important for energy production (7). Q10 supplementation was mentioned to have a beneficial role in prevention and treatment of many diseases such as cardiovascular and cancer diseases (20). Furthermore, it was documented that Q10 has anti-inflammatory and antioxidant properties and is able to avoid lipid peroxidation (21). Coenzyme Q10 found endogenously in the seminal fluid is very important, as its deficiency can cause a reduction in sperm parameters; so it is used to treat infertility in men and to improve the density, morphology, motility and count of the sperms (22). Vitamin C is a common and available antioxidant that is important in treatment of oxidative stress process and in immunomodulation and can enhance the absorption of iron (23). Zinc is an essential trace element and its deficiency can impair numerous functions in human body as it is an essential ion in reactions of a great number of proteins and enzymes. Zinc is an important ion in the proteins called (zinc finger protein) and also can increase the transcription and replication of DNA; while in contrast lead can induce DNA damage (24). A recent study revealed that zinc has a protective role against the lead-induced testicular damage through the maintenance of the physiology and the anatomy of testis and can improve spermatogenesis by down regulating Xanthine Oxidase (XO)/uric acid (UA)/caspase 3-mediated apoptosis and up-regulating the steroidogenic enzymes (25). This study was designed to evaluate the protective effect of zinc and Q10 supplementation in combination with vitamin C against lead acetate induced testicular injury and oligospermia in rats.

Materials and Methods

Lead acetate was purchased from Sigma-Aldrich and Q10 coenzyme, zinc and vitamin C were obtained from Now Company. Adult male Wistar rats (n=30) weighing approximately (200-250 g) were obtained from college of veterinary medicine\ university of Basrah, Basrah, Iraq; while they were acclimated for a period of one week before starting experiments. They were caged in a plastic cage in an air-conditioned room with standard bed, food pellets and water *ad libitum*. All animal experiments were carried out in accordance with international guidelines; while they were approved by the National Institute of Health (86/609/EEC) as guidelines for working with laboratory animals. Animal care was undertaken according to the local ethical committee at the College of Pharmacy, University of Basrah, Basrah, Iraq (Approval number of EC71).

The 30 male rats were allocated into 5 equal groups as (i) control group that received only tap water; (ii) induced LA group that received 50 mg/kg of LA daily via a gavage for 3 weeks (26); (iii) zinc group that received 20 mg/kg of zinc in combination with 100 mg/kg of vitamin C (27); (iv) Q10 group that received 100 mg/kg of Q10 in combination with 100 mg/kg of vitamin C (28); (v) combined group that received zinc, Q10 and vitamin C (100 mg/kg). A total of 50 mg/kg of LA two hours before the antioxidant feeding was administered daily by an oral tube for 21 days. The weight was determined at the starting point of the experiments every week until they were sacrificed. Water and food intake was measured daily for 21 days and the general vital signs of rats were recorded.

On day 21, all rats were sacrificed after anesthesia by diethyl ether inhalation and then their testes and epididymis were excised. Sperm evaluation such as sperm count, motility, viability was assessed from cauda epididymis. Rat testes were fixed in 10% formalin, sectioned, and embedded in paraffin and then were sectioned to 4-5 µm thickness and stained by hematoxylin and eosin stain (H&E) under a microscope. Statistical analysis was carried out using Graph Pad Prism software (version 7.0, Inc., San Diego, CA). Descriptive data were presented statistically as mean SEM for wholly estimated parameters. One-way analysis of variance (ANOVA) and Tuckey's multiple comparison tests were utilized for comparison between groups. A p value less than 0.05 was considered to be significant.

Results

Rats exposed to LA displayed a lower sperm quality than other groups based on lack of sperm count, motility and viability. Furthermore, numerous abnormal sperm morphologies such as decapitated, double-tailed, double-headed, microcephalic and macrocephalic sperms were noticed in LA group. Sperm malformation rate in LA group was higher than the control group too ($p<0.001$) (Figure 1). Sperm count in rats treated with zinc in combination with vitamin C before LA administration was significantly higher than other groups. Sperm motility in zinc+vitamin C, Q10+vitamin C and zinc+Q10+vitamin C groups was significantly higher than LA group; but still lower than the control group ($p<0.001$). Regarding sperm morphology, LA group showed lowered number of normal sperms than the control group; however, zinc+vitamin C, Q10+vitamin C and zinc+Q10+vitamin C treatment groups displayed a significantly higher number of normal sperms than LA group. The results revealed significant higher percentage of dead sperms in LA and zinc+vitamin C groups when compared with the control group; however, zinc+vitamin C group showed less percentage of dead sperms than LA group. Treatment with Q10+vitamin C revealed no dead sperms as illustrated in Figure 1.

Control group displayed normal histoarchitecture of the testis that was full of sperms (Figure 2A). Testis in LA group illustrated marked Leydig cell damage and vacuolation, in addition to thickness and depletion of spermatogonia, spermatocytes and spermatids in seminiferous tubules (Figure 2B). Testis of the zinc+vitamin C group demonstrated improvement in seminiferous tubules with sperms found in the lumen (Figure 2C). Testis of the Q10+vitamin C group showed seminiferous tubules restoration with some vacuolation (Figure 2D). Treatment with zinc+Q10+vitamin C revealed normal histoarchitecture of the testis that was full of sperms (Figure 2E).

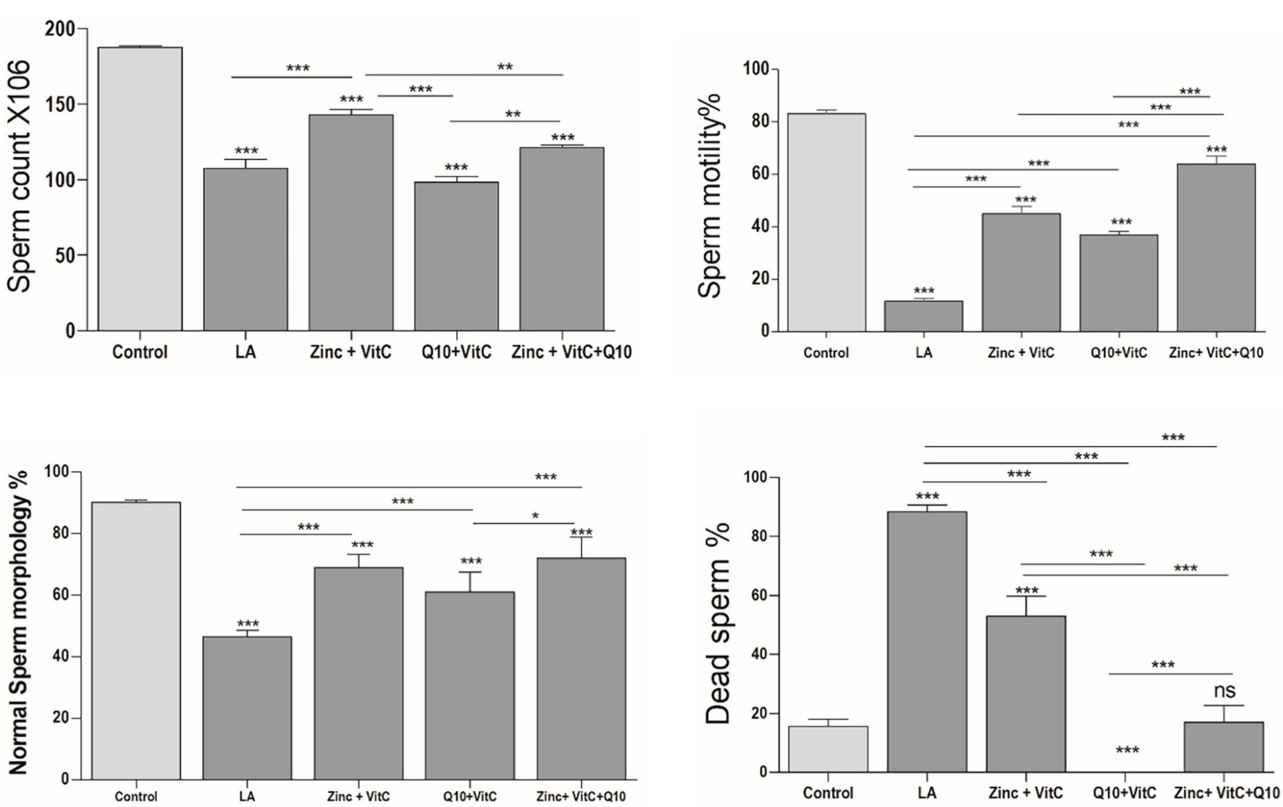

Figure 1: Effects of zinc, Q10 or both in combination with vitamin C supplementation on sperm quality, two hours before lead acetate administration (sperm count, motility, morphology and % of dead sperms). Sperms were obtained from the cauda epididymis of rats treated with lead acetate with or without above supplementations for 21 days. Data were expressed as mean±SEM (n=6) based on one-way ANOVA, *$p<0.05$, **$p<0.01$, ***$p<0.001$ when compared with the control group. The symbol * represents significant difference of $p<0.05$ between groups. The symbol ** represents a highly significant difference $p<0.01$.

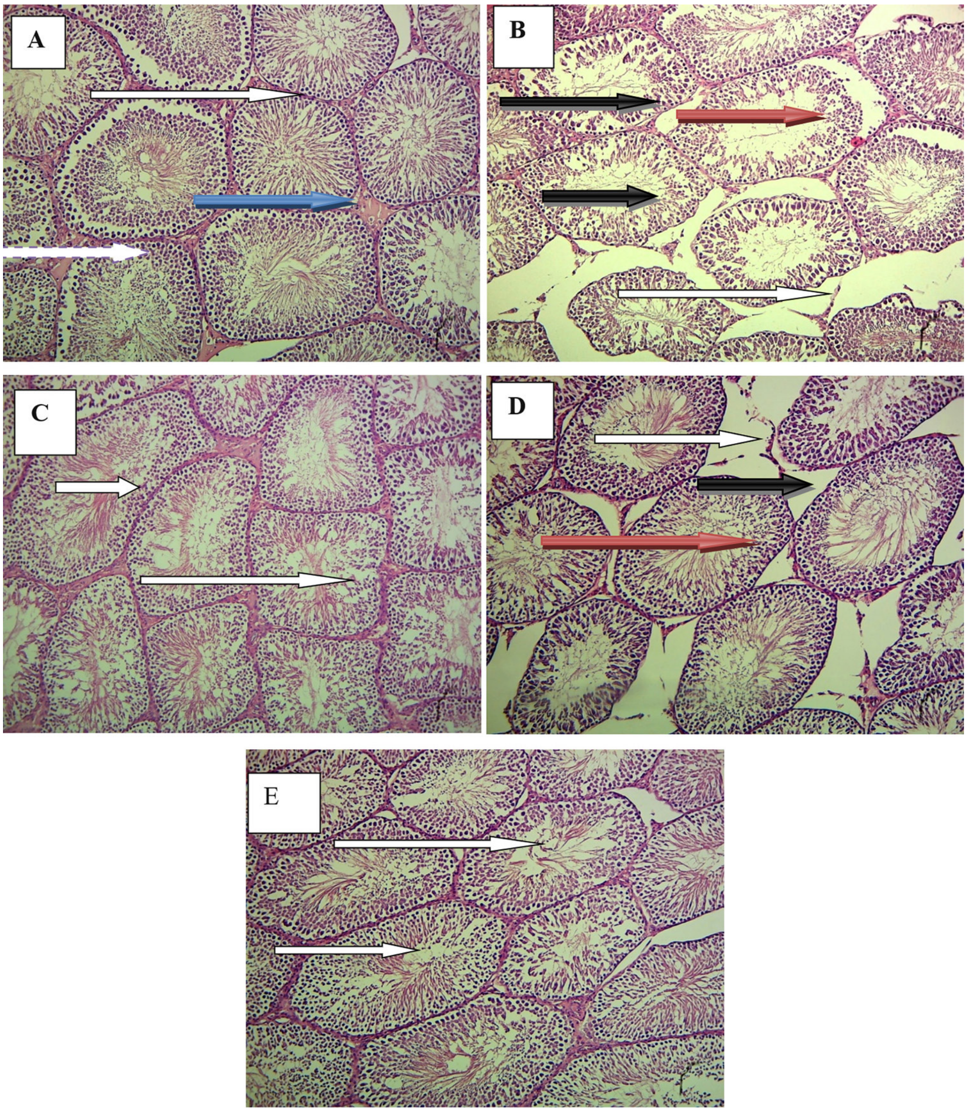

Figure 2: Light micrographic section of rat testis (stained by H& E: X200) after treatment with zinc, Q10 or both in combination with vitamin C supplementation, two hours before lead acetate (LA) administration. (A) Control group appeared normally in seminiferous tubules with full of spermatozoa that had normal architecture in germinal cell proportion and Leydig cells. (B) Lead acetate group showing severe germ cell loss and vacuolization, sloughing of germ cells into the tubular lumen, impaired spermatogenesis and edema. (C) zinc+vitamin C group demonstrated improvement in seminiferous tubules with appearance of sperms in the lumen. (D) Q10+vitamin C group showed seminiferous tubules restoration with some vacuolation and edema. (E) zinc+Q10+vitamin C group revealed normal histoarchitecture of the testis and full of sperms. (H&E, 200X magnification).

Discussion

Exposure to heavy metals among the other toxicants in the environment can result in testicular damage and sex hormone turbulences that can have a negative influence on male fertility (2, 30). Exposure to lead has extensive adverse impacts on humans and animals. In our study, long term contact to LA could reduce sperm quality and disturb testicular tissue construction and spermatogenesis, and finally reduce sperm count, motility and viability (3, 17). Furthermore, numerous abnormal sperms morphology such as decapitated, double-tailed, double-headed, microcephalic and macrocephalous sperm were reported in LA group and sperm malformation rate in LA group was higher than the control group. These effects may be related to oxidative damage by reactive oxygen species and our findings are consistent with the results of other studies (17, 23, 25, 30).

Food supplementation with zinc and vitamin C before LA administration showed a significant increase in sperm count and motility when compared to LA alone treatment group; but still it was lower than the control group (31). These results are in cope with a previous study revealing that zinc decreased testicular oxidative stress and inflammation induced by lead through downregulation of XO/UA signaling and increased the level of γ-glutamyl-cysteinylglycine (GSH); therefore, stopping the inflammation that was dependent on nuclear factor kappa-light-chain-enhancer of activated B cells (NF-κB) (32).

Also, food supplementation with Q10+vitamin C before LA exposure exhibited a significant increase in sperm motility when compared to LA alone treatment group; but it was still lower than the control group. Coenzyme Q10 that plays a main role in electrons transporting chain (33, 34) can have a protective role in testes after magnetic field exposure (35). Another study found its protective effect on the testes after ischemia/reperfusion injuries (36). The protection effect on the testes after sodium arsenite toxicity in addition to coenzyme Q10 could increase the expression and the level of antioxidant enzymes and glutathione; moreover, coenzyme Q10 could decrease oxidative stress in testes by suppressing lipid peroxidation and increasing antioxidant enzyme activity (7, 37).

Regarding sperm morphology, all treatment groups illustrated lowered number of normal sperms than the control group; however, zinc+vitamin C, Q10+vitamin C and zinc+Q10+vitamin C treatment groups displayed a significant higher number of normal sperms than LA group. These results revealed a significant higher percentage of dead sperms in LA and zinc+vitamin C groups in comparison to the control group; however, zinc+vitamin C showed less percentage of dead sperms than LA group (38). Furthermore, treatment with Q10+vitamin C revealed no dead sperms and these findings were in consistent with recent studies reporting that 180 male patients who received an antioxidant mixture containing 40 mg coenzyme Q10 daily for three months could increase the sperm density and morphology; but not sperm motility (39, 40).

Conclusion

Food supplementation with zinc, Q10 or both in combination with vitamin C was demonstrated to have protective effects against lead acetate induced testicular toxicity. Furthermore, these supplementations can increase sperm quality. Further studies on humans are needed to confirm these results.

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Authors' Contribution

All this work was done with my personal effort.

Conflict of Interest

None declared.

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