Research Journal of Medical Sciences 6 (2): 66-70, 2012
ISSN: 1815-9346
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Selenium and Zinc Attenuate Lead-Induced Reproductive Toxicity in Male Sprague-Dawley Rats

[1]B.A. Falana and [2]I.P. Oyeyipo
[1]Department of Anatomy, [2]Department of Physiology,
College of Health Sciences, Osun State University, Osogbo, Osun State, Nigeria

Abstract: Lead is a major environmental metal and a known reproductive toxicant. It causes reproductive toxicity via suppression of spermatogenesis and androgenesis in males. This study investigated the effect of lead exposure and metals supplementation on male reproductive activities in male albino rat. Forty male rats were divided into four groups and treated orally for 30 days with lead, selenium and zinc. Group A which served as the control received distilled water, group B received 2.25 mg kg^{-1} body weight of lead acetate only C received 2 mg kg^{-1} body weight of sodium selenium and 500 mg kg^{-1} BW/day zinc carbonate only group D received 2.25 mg kg^{-1} body weight lead acetate with 2 mg kg^{-1} body weight of sodium selenium and 500 mg kg^{-1} BW/day zinc carbonate. Sperm count, motility, viability, volume and morphology were evaluated while serum Luteinizing (LH) and testosterone levels were assayed. Lead treatment decreased sperm functions: count, viability, volume, motility, normal morphology and serum LH and testosterone. Co-administration of selenium and zinc with lead was found to attenuate the decrease in sperm functions and enhance serum reproductive hormones level. Selenium and zinc treatment only increased sperm count, motility, viability, volume, morphology and hormonal level. This study showed that lead apart from being a hormonal disrupter adversely affect sperm cells which contributed to the reproductive damage in the male rats. The protective effects of selenium and zinc on reproductive toxicity as evidenced by the clear restoration of sperm functions and testicular steroidogenesis indices could be attributed to its antioxidants and androgenic properties.

Key words: Metals, toxicity, testosterone, luteinizing hormone, sperm, antioxidant

INTRODUCTION

Lead is an abundant heavy toxic metal which is known to induce a broad range of physiological and behavioral dysfunction in humans (Gurer *et al*., 1999). Lead poisoning still remain an important health problem associated with several clinical symptom with limited molecular mechanism undying the toxicity. Recent studies suggests that oxidative stress is a potential contributor to lead's toxicity and that lead directly and/or indirectly change the prooxidant and antioxidant balance in biological tissue (Quinian *et al*., 1988; Lima *et al*., 1991; Samsheshekariaiah *et al*., 1992) and all toxic metals have in common the ability to cause oxidative damage. Toxic metals increase production of free radicals and decrease availability of antioxidant reserves to respond to the resultant damage (Ercal *et al*., 2001).

Selenium is an important element responsible for male fertility and testosterone biosynthesis (Hansen and Deguchi, 1996; Dimitrov *et al*., 2007) and its also a component of deiiodonidase enzyme which transform thyroxine into 3, 5, 3 triiodothyronine (Athur *et al*., 1997) and plays a vital role in oxidative stress control as a component of glutathione peroxidase (El-Sisy *et al*., 2008). Selenium deficiency has also been associated with nutritional muscular dystrophy (Abou-Zeina, 1996). Selenium is present in both prokaryotic and eukaryotic cells. It has been linked to regulatory function in cell growth, cell survival, cytotoxicity and transformation (Borek *et al*., 1986). Selenium deficiency have also been shown to give rise to testicular structural and functional disturbances (Shalini and Bansal, 2008) and oxidative stress in the organ due to diminished antioxidant property of the elememt as a co-factor of glutathione peroxidase (Shen *et al*., 1999).

Zinc is a trace element essential for living organism. More than 300 enzymes require zinc for their activity. Zinc plays an important role in DNA replication, transcription and protein synthesis influencing cell division and differentiation (Anderson and Desnick, 1979). Studies have shown that zinc can prevent cell damage through the activation of antioxidant defense system (Angel *et al*.,

Corresponding Author: I.P. Oyeyipo, Department of Physiology, College of Health Sciences, Osun State University, P.M.B. 4494 Osogbo, Nigeria

1986; Arroyo *et al*., 1987) and it is an essential component of the oxidant defense system (Powell, 2000; Ozturk *et al*., 2003). Zinc is a critical element in male reproductive system for proper hormonal metabolism, sperm formation and motility. Its deficiency has also been associated with impotence and reduced sexual performance (Modupe *et al*., 2009). However, studies have shown that lead toxicity has been associated with relative Zinc (Zn) deficiency (Martin *et al*., 1994; Agarwal and Prabakaran, 2005). Thus, keeping all these in view, the present study was designed to investigate the combined effect of selenium and zinc supplementation on lead-induced reproductive toxicity in Sprague-Dawley rats.

MATERIALS AND METHODS

The experiment was conducted in the Animal House of Osun State University, Osogbo, Osun State, Nigeria in 2011. Experiments were performed on forty Sprague-Dawley rats (180-200 g) obtained from the animal house College of Health Sciences, Osun State University Osogbo, Nigeria. The animals were housed in wire meshed cages and maintained in a well ventilated room at 25±2°C on a 12 h light and dark cycle with free access to rat chow (Bendel Feeds and Floor Mills Ltd., Benin City, Nigeria) and drinking water. The experiments were conducted in conformity with NIH guideline for the care and use of laboratory animals, the animals were allowed to acclimatize for a period of 2 weeks before the commencement of the experiments. The animals were randomly divided into four groups (A-D, n = 10) and were treated for 30 days. Group A received distilled water (vehicle), group B received 2.25 mg kg^{-1} body weight of lead acetate only C received 2 mg kg^{-1} body weight of sodium selenium and 500 mg kg^{-1} BW/day zinc carbonate only, group D received 2.25 mg kg^{-1} body weight lead acetate with 2 mg kg^{-1} body weight of sodium selenium and 500 mg kg^{-1} BW/day zinc carbonate.

Blood sample collection: Blood samples (2-3 mL) was collected via ocular sinus into a sterilized sample bottle and was allowed to clot at room temperature, the clot was retracted and sample centrifuged at 2647×g for 15 min and serum separated. The serum samples were stored frozen at -20°C.

Sperm collection and analysis: Each testis was removed along with its epididymis. The epididymis was carefully separated from the testis and the cauda severed from its remaining part. The cauda was quickly transferred to a pre-warmed slide (27°C) and lacerated with razor. Sperm characteristics analysis was done then carried out. Progressive motility was tested immediately. The semen was squeezed onto the microscope slide and two drops of warm 2.9% sodium citrate was added (Raji *et al*., 2006). This was then covered with a cover slip, examined and scored under the microscope using the x40 objective of the microscope. A viability study (percentage of live spermatozoa) was done using eosin-nigrosin stain. Semen was squeezed onto a microscope slide and two drops of the stain was added (Raji *et al*., 2006). The motile (live) sperm cells were stained. The stained and unstained sperm cells were counted using x40 objectives of the microscope and an averge for each were taken from which percentage viability was calculated. Sperm morphology was done by staining the sperm smears on microscope slides with two drops of Walls and Ewas stain and dried (Raji *et al*., 2006). The slides were examined under microscope using x100 objective under oil immersion. The abnormal sperm cells were counted and the percentage calculated. The epididymis was immersed in 5 mL normal saline in a measuring cylinder and the volume of fluid displaced was taken as the volume of epididymis. Sperm count was done under a microscope with the aid of the improved Neubauer haemocytometer. Count was done in five large throma square and adjustment was made for volume of normal saline added.

Testosterone assay: An Enzyme-based Immunoassay (EIA) system was used to measure testosterone level in serum samples collected. The EIA kit was obtained from immunometrics (London, UK) and contained a testosterone EIA enzyme label, testosterone EIA substrate reagent and EIA quality control sample. A quality control was carried out at the beginning and at the end of the assay to ascertain the acceptability with respect to bias and within batch variation. The EIA kit used had a sensitivity of approximately 0.3 nmol M^{-1} (0.1 g mL^{-1}) of testosterone. The intra and inter assay variations were 10.02 and 10.12%, respectively.

Luteinizing hormone assay: Plasma concentration of Luteinizing Hormone (LH) was determined by EIA tests. The assay kits for LH were supplied by Diagnostic Automation Inc., (Calabases, CA, USA) and MP Biomedicals (Orangeburg, NY, USA), respectively. The EIA is based on the principle of solid phase Enzyme-Linked Immunosorbent Assay (ELISA). The assay test for LH utilizes a mouse monoclonal anti-α-LH antibody for solid phase (micro titre wells) immobilization and a mouse monoclonal anti-β-LH antibody in the antibody-enzyme (horseradish peroxidase) conjugate solution.

Statistical analysis: Data were expressed as mean±SEM, Statistical significance between various groups was determined using impaired t-test and ANOVA. The p-values of <0.05 were accepted as significant. Analysis of data was done using the SPSS software for windows (SPSS Inc, Chicago IL, USA).

RESULTS AND DISCUSSION

Sperm parameter: The sperm count, motility and viability in the caudal epididymis were significantly reduced ($p<0.01$) after treatment with lead when compared with the control (Table 1). However, it was observed that there was a significant increases ($p<0.05$) in these sperm parameters in selenium and zinc only treated group. Administration of lead, zinc and selenium attenuated the decrease in sperm count, motility, viability and volume when compared with the lead treated group while a decrease in sperm abnormal morphology was observed in the animals treated with selenium and zinc only.

There was also a significant increase ($p<0.05$) in sperm abnormal morphology in lead treated rats. Nonetheless, co-administration of lead with zinc and selenium significantly reduced ($p<0.05$) the percentage abnormal morphology in comparison with lead treated rat though the number did not reach the control value as shown in Table 2.

Table 1: Effect of lead, zinc and selenium on sperm analysis in experimental animals

Groups	Motility (%)	Live/dead ratio (%)	Volume (mL)	Count (10^6 mL^{-1})
Control	91.60±4.00[a]	90.80±4.35[a]	4.95±0.02[a]	103.60±6.80[a]
Lead treated	46.00±5.12[b]	53.50±3.42[b]	4.66±0.04[b]	51.80±7.20[b]
Selenium+ Zinc treated	97.20±4.45[c]	96.20±4.15[c]	5.18±0.05[c]	130.40±4.88[c]
Lead+Zinc+ Selenium treated	87.60±4.48[a]	92.44±3.74[a]	4. 84±0.04[a]	110.80±7.12[a]

Table 2: Effect of lead, zinc and selenium on morphological characteristics of sperm in experimental animals

Sperm parameters	Control	Lead treated	Selenium+ Zinc treated	Lead+Selenium +Lead treated
Tailless head	6.00	5.00	3.00	3.00
Headless sperm	5.00	7.00	5.00	4.00
Rudimentary tail	8.00	14.00	6.00	6.00
Curved tail	15.00	65.00	8.00	24.00
Curved midpiece	4.00	6.00	4.00	6.00
Bend midpiece	5.00	8.00	2.00	6.00
Bent-Tail	9.00	33.00	3.00	12.00
Swapped-Tail	2.00	7.00	2.00	1.00
Total number of abnormal sperm	50.00	142.00	34.00	62.00
Total number of normal sperm	407.00	306.00	425.00	400.00
Abnormal cells (%)	10.94[a]	31.70[b]	7.41[c]	13.42[a]

Values are expressed as means±SEM of 8 rats per group. Means in columns not sharing common superscript letters are significantly different; $p<0.05$

Serum testosterone and luteinizing hormone: Mean serum level of testosterone and luteinizing hormone were significantly ($p<0.05$) decrease in lead treated rats compared with the control as shown in Fig. 1 and 2. The co-administration of lead with selenium and zinc increased the serum levels of testosterone and luteinizing hormone above values of lead treated group but values were still less than control value. Selenium and zinc treated group had a significant increase ($p<0.05$) in the mean serum level of testosterone and luteinizing hormone when values were compared with the other groups as shown in Fig. 1 and 2

The results of the present study suggested that lead has a deleterious effect on male reproductive function in rat that could be ameliorated by selenium and zinc supplementation while selenium and zinc supplementation possesses pro-fertility properties in male rats.

To the best of the knowledge this is the first study that evaluates the combined protective effects of zinc and selenium supplementation against male reproductive damage induced by lead in experimental animals.

Reproductive toxicity in this study has been shown as evidences by a clear attenuation of lead-induced sperm function, damage and testicular steroidogenesis. The decrease in the sperm count, motility, viability, normality and volume caused by lead observed in their study is in

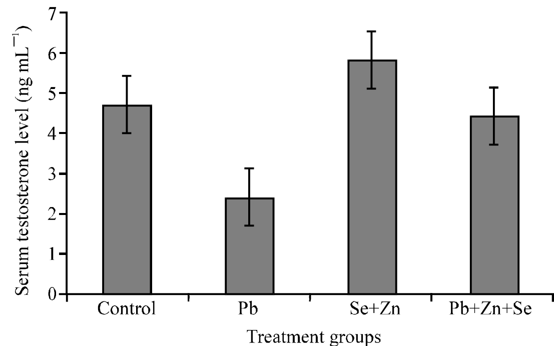

Fig. 1: Effect of lead, zinc and selenium on serum testosterone level in experimental animals

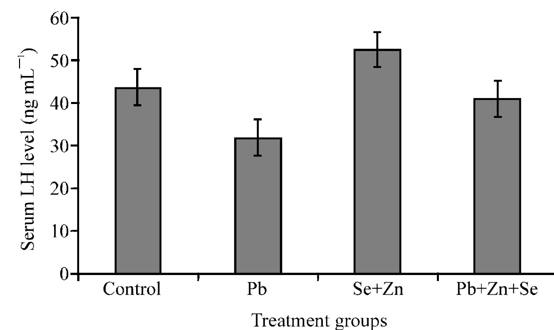

Fig. 2: Effect of lead, zinc and selenium on serum luteinizing level in experimental animals

agreement with other studies (Benoff *et al*., 2003). Sperm parameters such as count, motility and morphology are key indices of male fertility as these are the pure markers in testicular spermatogenesis and epididymal maturation (Morakinyo *et al*., 2010). The reduced sperm characteristics caused by lead administration may be due to the low testosterone concentration observed in this study as high level of testosterone is critically required for normal spermatogenesis, development and maintenance of sperm morphology and normal morphology and physiology of seminiferous tubules (Sharpe *et al*., 1992). The decrease in sperm count denotes that lead could adversely affect cell division since, testosterone is essential for completion of meiotic division during spermatogenesis.

The reduced sperm viability in rat agrees with the reduction in the progressive sperm motility because immobile sperms are considered dead. This is confirmed by the fact that the dead sperm took up the Eosin/Nigrosin stain when smeared; a histological evidence that they are dead. This result may also be due to the effect of lead on the epididymal site by acting as a spermatoxic agent on maturing or matured spermatozoa (Chinoy *et al*., 1985). The most prominent morphological abnormality observed in the lead treated rats was in the form of bent tail and curve tail. These secondary abnormalities usually occur during epididymal transport, maturation and storage of sperm. It is during this period that the spermatozoa develop motility (Tulsiani *et al*., 1998). These could also give explanation to the reduced sperm motility because the sperm tail is responsible for motility.

Low serum testosterone level observed in lead treated group in the study may occur due to the reduced level of LH (Shaw *et al*., 1979; Kerr and Sharpe, 2006) as circulating LH is responsible for maintaining normal plasma testosterone concentration. Co-treatment of lead with selenium and zinc attenuated spermatogenesis damage induced by lead treatment as shown by the return of the sperm count, motility and normal morphology toward normal control values. Zinc has been reported to boost testosterone level (Saxena *et al*., 1989) while its deficiency has been associated with lead treatment which causes reduction in testosterone secretion and impairs the responsiveness of leydig cells to gonadotropin (Martin *et al*., 1994) which could be due to a malfunction of the receptor mechanism controlling storage and release of testosterone (Kellokumpu and Rajaniemi, 1981).

The restoration of testosterone and luteinizing hormone levels towards control value when zinc and selenium was administered with lead might have stimulated the production of both quantitatively and structurally normal sperm since spermatogenesis requires LH for initiation and maintenance in male rats. LH stimulates Leydig cell to secrete testosterone which is absolutely require for normal spermatogenesis.

In addition, the results of this study suggest that combined supplementation of selenium and zinc has beneficial effect on male reproductive function in rat. These data are confirmed by the observation on increase sperm counts, motility, testosterone, luteinizing hormone and decreased percentage sperm morphological anomalies which are in consonance with earlier study (El-Sisy *et al*., 2008).

CONCLUSION

The combined protective effect of zinc and selenium against lead induced reproductive toxicity might be mediated through its androgenic activities. This study showed that lead apart from being a hormonal disrupter adversely affects sperm cells which contribute to reproductive toxicity in male Sprague-Dawley rats while the protective effect of selenium and zinc was clearly indicated in the results of the study in reversing the effect of lead poisoning.

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