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Effects of zinc on male sex hormones and semen quality in rats

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Summary: This study assessed the effects of zinc on male sex hormones and semen quality in male albino wistar rats. Forty rats weighing between 150- 210g, grouped into 5 of 8 rats each, were used for the research that lasted for six weeks. Group I, the control group, received normal rat chow and water *ad libitum*. The four test groups II-V, received 20g, 40g, 60g and 80g of zinc sulphate mixed with their rat chow respectively in addition to water for six weeks. Blood samples were collected and assayed for Luteinizing hormone (LH), follicle stimulating hormone (FSH), Prolactin (PL), testosterone (T), progesterone and oestradiol. Semen was also analysed for sperm motility, sperm count and morphology. Results showed statistically significant decrease in serum levels of follicle stimulating hormone (FSH) ($p< 0.05$) in groups II and IV with mean values of 0.10±0.00 and 1.20±0.00 respectively when compared with the control (1.10±0.10). The results also revealed statistically significant increase in the serum levels of testosterone in groups II, III and IV with mean values of 3.60±1.40, 4.5±0.30 and 0.80±0.70 respectively when compared with the control with a value of 0.35±0.15. The increase in testosterone levels were dose dependent as there were consistent increment in groups II and III after which the levels decreased with increasing zinc concentrations. There was statistically significant dose dependent decrease in sperm motility and morphology in the test groups compared with the control ($p<0.05$). In conclusion, zinc sulphate has some significant positive effects on male sex hormones and sperm quality at doses within physiological levels but harmful at higher doses.

Keywords*:* Zinc sulphate, Male sex hormones, Sperm quality

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INTRODUCTION

Zinc is classified as a group 11B post-transition metal. In biological systems, it exists as Zn^{2+} and is present in all tissues and fluids in the body (European Commission,2003). It is the second most abundant transition metal after iron and it is the only metal which appears in all enzyme classes (Broadley et al,2007).It has structural, regulatory or catalytic roles in many enzymes (Vallee and Galdes,1984; Hambridge et al,1986;Cousins et al,2006). Zinc is an essential mineral of exceptional biologic and public health importance (Harmbidge and Krebs, 2007). Zinc as an essential trace element is necessary for plants (Wiaux et al, 2007); animals (Prasad, 2008) and microorganisms (Sugarman, 1983).). It plays a role in immune function (Prasad, 1998; Solomon,1998); protein and DNA synthesis and cell division (Prasad, 1995); wound healing (Heyneman,1996); normal growth and development during pregnancy, childhood and adolescence (Maret et al, 2006), is required for normal sense of taste and smell (Prasad et al, 1997) and as antioxidant (Milbury and Richer, 2008). Zinc is required for normal growth and development, testicular maturation, neurological function, wound healing and immunocompetence (European Commission,2003). It exists under physiological conditions in the divalent. The structure and function of cell membranes are also affected by zinc hence loss of zinc from biological membranes can increase their susceptibility to oxidative damage and impair their function (O'Dell, 2000). A daily intake of zinc is essential to maintain a steady state of health since the body has no specialized zinc storage system (Rink and Gabriel,2000).The recommended Dietary Allowances/Intake of zinc is sex and age dependent, with a recommended daily intakes of 11mg/day and 8 mg/day for adult men and women respectively. However, 40 milligrams has been set as the tolerable

upper limit (UI) for daily intake of zinc. This limit applies to all individuals age 19 and over (Institute of Medicine, 2001).

Red meats, especially beef, lamb and liver have some of the highest concentrations of zinc in food (Berdanier et al, 2007). Milk, fruit and vegetables are low in zinc (Sandstead and Smith Jr,1996). Zinc is included in most single tablet over-the-counter daily vitamin and mineral supplements (DiSilvestro,2004) and in men the prostate gland stores high amount of zinc (Chesters 1978).

Infertility is a major clinical problem, affecting people medically and psychologically (Raghuveer et al, 2010). It can also affect their economy, peace and harmony. Sperm counts are falling and male fertility is in the decline (Carlson et al, 1992). In Southeastern Nigeria, a positive male factor alone was found in 133 (42.4%) couples and female factor alone in 81 (25.8%) couples of the three hundred and fourteen couples evaluated for the cause of infertility (Ikechebelu et al, 2003).

In the male reproductive system, zinc is essential for optimal performance and output. Zinc in seminal fluid helps to stabilize the cell membrane and nuclear chromatin of spermatozoa (Chvapil 1973; Kvist,1980). Its other roles in male reproduction include: may have a regulatory role in the process of capacitation and acrosome reaction (Riffo et al, 1992); can protect the testis against degenerative changes (Batra et al, 2004) and it may also be used as an index of prostatic function (Caldamone et al,1979). Men with infertility due to an abnormally low sperm count may benefit from taking supplemental zinc (Wong et al, 2002). Zinc deficiency leads to gonadal dysfunction, decreases testicular weight, and causes shrinkage of seminiferous tubules (Bedwal and Bahuguna, 1994).

Zinc (Zn) has many biologically significant interactions with hormones(Abdella et al, 2011). Bishop and co-workers(1996) observed that zinc has a role in the production, storage and secretion of individual hormones as well as in the effectiveness of receptor sites and end-organ responsiveness. Among the most notable effects of Zn deficiency on hormone production and secretion are those related to testosterone, insulin and adrenal corticosteroids, spermatogenesis and the development of the primary and secondary sex organs in the male and all phases of the reproductive process in the female can be adversely affected by Zn deficiency (Insler and Lunenfeld,1993).

Infertility is on the increase especially in the developing countries. Intake of zinc is also on the increase and many are unaware of the toxic effects at higher doses. Thus, we set out to explore the effects of zinc at varying concentrations on male sex hormones and semen quality in rats.

MATERIALS AND METHODS

Forty male albino wistar rats, weighing between 150-210g used for this study were procured from the Animal House at Enugu State University, Enugu. The rats were acclimatized for two weeks during which they were fed normal rat chow(Topfeed Ltd. Sapele, Delta State Nigeria) and water *ad libitum*. The rats were housed under normal temperature and their beddings were changed every two days. The experiment lasted for six weeks. Zinc as zinc sulfate ($ZnSO_4$)(JT Baker, Philipsburg, New Jersey) was added to the feed. The control group rats received 10kg of feed, the test group rats also received 10Kg of feed. The feed for the test group was divided into four of 2.5Kg and each was supplemented with 20g, 40g, 60g and 80g of zinc sulphate before it was palletized. The zinc concentrations were gotten based on daily dietary allowance of 11mg/day and 8mg/day for male and females respectively(IOM, 2001).
The rats were grouped into 5, with 8 rats in each group. Group 1 served as control and were fed normal rat chow and water *ad libitum*. Groups II-V were given water, normal rat chow mixed with 20g, 40g, 60g and 80g zinc sulphate respectively for six weeks.

Blood samples were collected at the end by cardiac puncture after anaesthetising with chloroform. 2ml of blood were collected from four rats in each group including the control group. The samples were carefully introduced into lithium containers free from anticoagulant and were labelled for example 1a, 1b, 1c and 1d. The blood samples were allowed to clot, retract and then centrifuged for 5minutes at a speed of 5000 revolutions per minute. The plasma was then collected, refrigerated at -20C and later assayed for Prolactin, oestradiol, progesterone, LH, FSH and testosterone using ELISA Hormone Test kits, the average was recorded.

The rats were sacrificed at the end of the six weeks by cervical dislocation and the testis and caudal epididymis were excised to obtain the caudal epididymal sperm. The samples were kept at below room temperature and allowed to liquefy and analyzed within one hour of exposure.

A 20-µl sample to assay sperm motility (% vigorously swimming at 5 and 30mm post collection) was transferred to a glass slide that was covered with a cover slip and assessed by visual analysis with a microscope. Progressivity was determined by the grading system as described by WHO (1999).

To examine for sperm count,(WHO,1999), 0.1 ml sample of semen was placed in 0.9 ml of normal saline for sperm cells to swim out in a petri-dish. It was well shaken and the sample was taken to a counting chamber (haemocytometer). After the sperm cells have settled on the grid, it was then viewed under the microscope and they were counted in five

square used for counting RBC. Sperm in five squares were multiplied by 10^6 to determine the number of sperm per ml. Eosin stain was used for sperm viability determination, while Evans and Walls stains were used for morphology determination.The morphological characteristics of the sperm cells are important for the complete assessment of the seminal fluid. The differential count of morphologically normal and abnormal cells was done.The presence of abnormal primordial and mature cells above 20% respectively was used as the criteria for measuring abnormal morphology.

Statistical analysis.

Data were analysed statistically for testosterone, LH, FSH, Oestradiol, prolactin, progesterone, sperm count, sperm motility and morphology by two-way Analysis of Variance (ANOVA) using SPSS version 17.0 with significant level fixed at $p<0.05$.

RESULTS

This study shows that after six weeks of oral zinc supplementation in albino wistar rats, statistically significant decrease in serum levels of follicle stimulating hormone (FSH) was observed($p< 0.05$) in groups III and IV with mean values of 0.10±0.00IU/L and 1.20±0.00IU/L respectively when compared with the control(1.10±0.10IU/L). The results also revealed statistically significant increase in the serum levels of testosterone in groups 1, 11 and IV with mean values of 3.60±1.40ng/ml, 4.5±0.30ng/ml and 0.80±0.70ng/ml respectively when compared with the control with mean values of 0.35±0.15ng/ml. The increase in testosterone levels were dose dependent as there were consistent increment in groups 1 and 11 after which the levels decreased with increasing zinc concentrations. No significant differences were observed in the other sex hormones studied (P>0.05) (Table 1).

Table 2 showed statistically significant dose dependent decrease in sperm motility and morphology ($P <0.05$) in the test groups when compared with the control. Increasing doses of zinc supplementation led to decrease in sperm motility and morphology. Sperm count also increased with increasing doses of zinc supplementation though not significant statistically (P>0.05).

DISCUSSION

This study was done to assess the effects of oral zinc supplementation on male sex hormones and sperm quality in male albino wistar rats over a period of six weeks.

The results showed statistically significant increase in serum levels of testosterone in the test groups II, III and V that ingested 20g, 40g and 80g of zinc respectively when compared with the control, group I. The observed increase in testosterone levels in group V was less when compared with groups II and III that were given lower doses of zinc supplementation. This result agrees with the works of Ratnasooriya et al (2004) and Abdella et al (2011). The tolerable upper limit of 40mg of daily intake of zinc has been recommended (Institute of Medicine, 2001). Zinc supplementation activates secretion and action of testosterone and can lead to increased efficiency of spermatogenic machinery and increased number of germ cells in the seminiferouse tubules (Pizent et al, 2003 and Abdella et all, 2011). Thus oral zinc supplementation within tolerable level has beneficial effects. Earlier, Prasad (1996) had noted that zinc deficiency lowers plasma testosterone levels but over supplementation has no effect on testosterone level (Koehler et al, 2009). Testosterone deprivation has negative impact on the structure of penile tissues and erectile nerves (Grahl et al, 2007).

The results also revealed statistically significant decrease in serum concentrations of Follicle stimulating hormone (FSH) in groups supplemented with 20mg, 40mg and 60mg/kg body weight of zinc ($P < 0.05$).The reduction in serum levels of FSH and

Table 1. Effect of Zn supplementation on male sex hormones after six weeks.

	I (Control)	II (20g)	III (40g)	IV (60g)	V (80g)
LH (IU/L)	0.70±0.35	0.60±0.05	0.60±0.50	0.40±0.00	0.40±0.15
FSH (IU/L)	1.10±0.10	0.75±0.65	0.10±0.00*	0.30±0.10	1.20±0.00*
PLT (IU/L)	1.20±0.70	0.90±0.00	0.75±0.05	0.70±0.00	0.65±0.05
Prog (ng/ml)	0.10±0.00	1.10±1.00	0.10±0.00	0.10±0.00	0.10±0.00
Est (pg/ml)	19.0±5.0	23.65±1.95	21.80±3.90	26.60±3.10	22.90±0.20
Test (ng/ml)	0.35±0.35*	3.60±1.40*	4.50±0.30*	2.00±0.90	0.80±0.70*

LH=Leuteinizing hormone, FSH=Follicle stimulating hormone, PLT=Prolactin, Prog=Progesterone, Est=Estradiol, Test=Testosterone. * P<0.05 level.

Table 2. Effect of Zn supplementation on sperm parameters after six weeks.

	I (Control)	II (20g)	III (40g)	IV (60g)	V (80g)
Count ($x10^6$)	23.50±0.00	15.90±0.50	17.05±1.15	19.95±0.90	24.50±0.50
Motility (%)	85.00±0.00	82.50±2.50*	67.50±2.50*	62.50±2.50*	42.50±2.50*
Morphology (%)	55.00±5.00	37.50±2.50*	30.00±0.00*	22.50±2.50*	20.00±0.00*

*P<0.05

non significant effect on Luteinizing hormone (LH) following the increased levels of testosterone may be due to the negativefeedback effect of testosterone on the hypothalamus which in turn causes decrease in the secretion of FSH and LH by the gonads of the anterior pituitary gland (Guyton and Hall, 2006). Gonadotropins production is under the feedback control of sex hormones (Ganong, 2003).

It has been observed that zinc is required for normal functioning of the hypothalamic -pituitary-gonadal-axis (Miller et al, 1960; Lei et al, 1976). Hypogonadism and lack of secondary sexual characteristics have been noted in severely undernourished young men and these abnormalities tend to respond to dietary supplementation of zinc (Prasad, 1991). Zinc deficiency leads to gonadal dysfunction, decreases testicular weight, and causes shrinkage of seminiferous tubules (Bedwal and Bahuguna,1994). Zinc is an essential element in some reproductive process (Mills, 1988; Motossian 1991).

This work also revealed that increasing doses of oral zinc supplementation led to decreased motility and increased morphology of the sperm cells ($P<0.05$). This result agrees with the works done by Danscher et al (1978); Carpino et al,(1998); and Günfer et al,(2003). Degenerative changes, including spermatic arrest, degeneration of seminiferous tubules, and fibrosis in interstitial tissues have been observed following high doses of zinc supplementation and this can significantly alter sperm motility (Turgut et al, 2003). Zinc in higher concentrations has negative effects on sperm motility (Chyb et al, 2000). These observed pathological changes due to high zinc concentration can impair spermatogenesis and /or lead to production of abnormal spermatozoa. Evidence abound that infertile males have a higher percentage of abnormal spermatozoa (Menkveld et al,1990; Liu and Baker, 1992).

Zinc can also affect sperm quality through its effect on calcium ions. Ebrahimi et al (1996) reported that zinc ion can eliminate calcium ions from the specific binding sites in sperm and Busselberg (1995) showed that calcium channels are the targets of zinc ions. Because the motility of spermatozoa can be blocked by calcium deficiency (Maisse et al, 1995), it is therefore possible that one of the effects of zinc on sperm is the displacement of calcium necessary for the activation of spermatozoa(Chyb et al, 2000). This can lead to reduced sperm motility.

In conclusion, zinc supplementation within tolerable limits can improve fertility but detrimental at higher doses. Evaluation of dietary supplements containing trace elements such as zinc should form part of the management strategies in cases of infertility.

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