INSUFFICIENT SLEEP MODEL AND MALE REPRODUCTIVE FUNCTION; THE ROLE OF CONCOMITANT ZINC SUPPLEMENTATION

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ABSTRACT

Background: Insufficient sleep (IS) in today's society is gaining recognition as a public health concern, with increasing evidences that linked it to poorer health outcomes. Coincidentally, there is remarkable decline in fertility rates, in these industrialized societies which is believed to be due to lifestyle modifications. We therefore set up IS model of sleep deprivation (SD), to study its effects on male reproductive functions and the influence of concomitant Zinc supplementation on those effects.

Methods: Twenty four (24) male Wistar rats (aged 12- 14 weeks) were randomly grouped into three: Control, IS and ISZ models. IS and ISZ models were subjected to SD for 18 hours (07:00pm – 01:00pm next day) using Modified Multiple Platform Method (MMPM). The rats in ISZ model were given Zinc sulphates (5mg/animal/day) while those in control and IS models were given distilled water (1ml/animal/day) by gavage daily for 56 days respectively. Serum corticosterone, testicular tissue Malondialdehyde (MDA) and Total Antioxidant Capacity (TAC), male reproductive hormones (FSH, LH, Testosterone and Estradiol) and Sperm counts, morphology and motility were evaluated and statistically compared.

Results: Sleep deprivation in IS model resulted in significant increase ($p<0.05$) in serum Corticosterone, testicular tissue MDA, serum FSH and significant decrease ($p<0.05$) in testicular tissue TAC, serum Testosterone, serum Estradiol, sperm count and percentage of sperm with active progressive motility compared to the control. On the other hand, concomitant Zinc supplementation (ISZ model) significantly ($p<0.05$) increases testicular tissue TAC, serum Estradiol and significantly ($p<0.05$) decreases testicular tissue MDA and serum FSH compared to the IS model.

Conclusion: The IS model of SD deteriorates male reproductive functions, while concomitant Zinc supplementation ameliorates some of these functions.

Keywords: Insufficient Sleep**;** Sleep Deprivation; Total Anti-oxidant Capacity; Corticosterone; Testosterone

INTRODUCTION

Sleep is a universal, dynamic brain process that is present in organisms ranging from invertebrates to mammals (Brown and Naidoo, 2010) associated with important restorative functions for every organ in the body (Liu *et al.,* 2017). For optimal health, the American Academy of Sleep Medicine (AASM, 2014) and the Sleep Research Society (SRS) have recommended a regular seven or more hours of night sleep for adults aged 18 to 60 years (Watson *et al.*, 2015). But Modernization and industrialization resulted in global 24/7 society characterized by increased physical activities, reduced rest and sleep (Rodrigues *et al.,* 2015). Insufficient sleep (IS) occurs when a person chronically fails to obtain the amount of sleep required to maintain normal alertness and wakefulness (AASM, 2014).

Insufficient sleep is becoming increasingly common in today's society compared to few decades ago (CDC 2014). It was estimated that over 20-30% of adults are chronically sleep deprived, hence, SD has been declared to be modern-day 'public health epidemic' (Bixler, 2009: CDC, 2014).

Coincidentally, remarkable decline in fertility rates was reported in industrialized areas (Pearce *et al.,* 1999). Infertility affects approximately 15% of all couples trying to conceive, and Male factor infertility accounts for roughly half of these cases (Sharlip *et al.,* 2002). A bidirectional relationship between SD and oxidative stress has been documented (Noguti *et al.,* 2013; Hill *et al.,* 2018). Oxidative stress is postulated as one of the major factors that negatively affects male fertility status (Sharlip *et al.,* 2002). Many studies conducted both in vitro and in vivo demonstrated the beneficial effects of antioxidants on fertility and recommend their use for the treatment of male infertility (Gambini *et al.,* 2015). It was documented that Zinc supplementation promotes spermatogenesis and sperm motility (Cheah and Yang, 2011) thus, improves fertility.

Most previous studies focussed on total sleep deprivation which is hardly obtainable in real human lifestyle (Goel *et al.,* 2013). Therefore, we set up model to simulate duration and timing of SD in insufficient sleepers, to study its effects on male reproductive function and the influence of concomitant Zinc supplementation on those effects

MATERIALS AND METHODS

Materials and Chemicals

Ten plastic cages measuring (55×35×35 cm) with installed MMPM structure and five addition plastic home cages of same sizes, ketamine hydrochloride (Ketajet ®, Sterfil Laboratories, India), Diazepam (Valium, Roche LTD, Basel, Switzerlands), Commercially available dispersible Zinc sulphate tablet 20mg (Emzor Pharmaceuticals, Nigeria). The Zinc sulphate ($ZnSO_4$) was reconstituted in deionised double distilled water to form 5mg/mL suspension.

Animals

Twenty four male Wistar rats (aged 10- 12 weeks) were obtained from the animal house of Department of Human Physiology, Bayero University Kano, where the study was carried out. The rats were housed in plastic cages, adequate ventilation and natural light/dark cycle maintained, with food and water *ad libitum* in accordance with the National and International Regulations on Use of Animals for Research and Teaching 2017. Animal Research Committee, Ahmadu Bello University Zaria, granted ethical clearance for the study (ABUCAUC/2020/65).

Animal Groupings

The twenty four male Wistar rats were randomly divided into three groups of eight animals each as follows.

Control model: distilled water (1ml/animal/day) only

IS model: 18 hours SD + distilled water (1ml/animal/day)

ISZ model: 18 hours SD + $ZnSO_4$ at dose of 5mg/animal/day (Dissanayake *et al.*, 2009)

Experimental Design

The research is a longitudinal interventional study designed to simulate IS which is among the most common cause of SD in our global 24/7 society. However, to cope with the modern-day societal demands, some degree of SD is almost inevitable, so we set up Zinc supplemented SD model (ISZ) receiving Zinc sulphate as intervention. Each rat was given either distilled water (Control and IS models) or $ZnSO_4$ (ISZ model) by gavage between 07:00 - 08:00am daily for the 56 days of the study. IS and ISZ models were subjected to 18 hours of SD (07:00pm – 01:00pm next day) all through their biological day-time extending to first half of their biological night-time. After each SD episode, the animals were returned back to their home cages (01:00pm-07:00pm) for six hours of sleep/rest window during their biological night-time.

The 12 hours of SD (07:00pm – 07:00am) during the biological day-time of the rats, and the 6 hours of SD (07:00am – 01:00pm) during the biological night-time of the rats, simulates the timing and duration of SD in IS.

Sleep deprivation induction

SD was induced using our customized modified multiple platform method (MMPM). It consist of a plastic tank (55×35×35 cm) containing 10 round platforms (made from metallic pipe with plastic cap welded to iron base) of 7cm height, 5cm diameter, and placed 7cm apart, improvised from Zager *et al.,* (2009) and Choi *et al.,*(2016) descriptions (plate 1). The tank was filled with water to about 1 cm below the platform surface. The rats can move around by leaping from one platform to another. Whenever the rat sleeps, it falls into the water as a result of muscle atonia and then wakes up. The water in the tank was changed daily throughout the period of the experiment. The Control rats were placed in similar plastic tank, but filled with saw dust instead of water, so they can sleep well on it.

Figure 1: Customized MMPM

Animals sacrifice and blood samples collection

Each rat was anaesthetized by intraperitoneal injection of cocktail of diazepam (2 mg/kg) and ketamine (20 mg/kg) (Flecknell, 1993). Blood was collected via cardiac puncture and then discharged into plain sample bottle. The collected blood sample was allowed to clot at room temperature and then centrifuged at 2000g for 15 minutes. The serum collected was stored frozen for hormonal assays.

Testicular tissue handling

Testes were removed, cleared of adhering connective tissue and weighed. The left testicle of each rat was frozen for testicular oxidative stress biomarkers assessment. The right cauda epididymis of each rat was used for seminal fluid analysis.

Seminal Fluid Analysis

The right cauda epididymis of each rat was cut into pieces in a Petri dish containing 5 ml warm saline solution and incubated for three minutes, with frequent shaking to yield semen suspension.

1. Assessment of total sperm count; About ten microliter of the semen suspension was loaded on to the Neubauer hemocytometer (Deep 1/10 mm, Labart, Germany) for semen analysis at x100 magnification. Spermatozoa were counted in five random squares in triplicate per sample according to WHO laboratory manual for the Examination and processing of human semen (WHO, 2010). Total sperm count was calculated and reported as millions of sperm cells/ml.

2. Assessment of sperm motility; A small drop of the semen suspension was smeared on a warmed glass slide and covered with a glass slip. A minimum of three different fields were examined to determine the mean percentage of sperm motility and reported as percentage of motile sperm according to WHO laboratory manual for the Examination and processing of human semen (WHO, 2010).
3. Assessment of sperm morphology; the relative proportions of abnormal sperms were analyzed according to WHO laboratory manual for the Examination and processing of human semen (WHO, 2010). The relative proportions of normal and abnormal sperm cells were expressed in terms of percentages.

Hormonal assays

Serum Testosterone, Estradiol, FSH, LH and Corticosterone were measured using individual commercial enzyme-linked immunosorbent assay (ELISA) kits (Sunlong Biotech Co.,Ltd: tel: 0086-571-56623320: China.) according to manufacturer's instructions. ELISA works on the principle that specific antibodies bind the target antigen and detect the presence and quantity of antigens binding.

Testicular Oxidative Stress Biomarkers

The left testicular tissues were individually homogenized in ice cold phosphate buffer saline (10 mM pH - 7.4) using potter-Elvenhjem tissue homogenizer. The crude tissue homogenate was centrifuged at 10,000 rpm, for 15 minutes in cold centrifuge, and the resultant clear supernatant was divided into two aliquots for MDA and TAC assays.

1. MDA Determination

MDA was determined using Colorimetric TBARS as described by Das *et al.,* (1990). The principle of this method was described as follows; 2-Thiobarbituric acid reactive substances (TBARS) are naturally present in biological specimens and include lipid hydroperoxides and aldehydes, which increase in concentration as a response to oxidative stress. TBARS assay are reported in MDA equivalents. One molecule of MDA reacts with 2 molecules of 2-thiobarbituric acid via a Knoevenagel-type condensation to yield a chromophore with absorbance maximum at 532 nm.

2. Total Antioxidant Capacity (TAC) determination

Rat total antioxidant status Elisa kit was obtained from (Sunlong Biotech Co.,Ltd: tel: 0086-571-56623320: China. SL1402Ra). This method is based on the decolourization of ABTS (2-2 azinobis (3-methybenzothiazoline-6-sulfonate)) radical cation, Antioxidants present in the sample accelerate the bleaching rate to a degree proportional to their concentrations, which can be monitored spectrophotometrically, and the bleaching rate is inversely related with the Total anti-oxidant capacity (TAC) of the sample.

Data Analysis

The collected data was analyzed using the Statistical Package for Social Sciences (SPSS for Windows, Version 23, SPSS Inc., Chicago, IL, USA). Values were recorded as mean ± standard error mean (SEM). Mean values were compared using one-way ANOVA analysis, followed by Bonferonni's post hoc test for multiple comparisons. The significance level was set as $p< 0.05$.

RESULTS

Our study simulates insufficient sleepers, which is the most common mode of SD in global 24/7 society. We compared IS model with control and ISZ models to bring out the effects of SD on male reproductive function and role of concomitant Zinc supplementation on the induced effects, respectively.

Table 1. Comparison of serum corticosterone (Cort.) and testicular tissue of oxidative stress biomarkers among the SD models

Parameters	Control	IS	ISZ	*f*	*p*
Cort. (pg/ml)	221.80±5.80	302.04±2.93[a]	275.79±3.96[b]	86.648	0.000
TAC (U/ml)	3.88±0.12	2.05±0.09 [a]	3.09±0.18[b]	52.103	0.000
MDA (µmol/l)	5.77±0.88	33.39±2.05 [a]	21.57±2.47[b]	52.103	0.000

Mean ±S.E.M, n=8, $p>0.05$, [a] = significant compared to control, [b] = significant compared to IS; IS (Insufficient Sleep), ISZ (Insufficient Sleep with Zinc) MDA (Malondialdehyde), TAC (Total Antioxidant Capacity)

Table 2: Comparison of LH, FSH, Testosterone (Testost) and Estradiol hormones concentrations of among the SD models

	Control	IS	ISZ	*f*	*p*
LH(mIU/L)	1.62±0.15	3.04±0.39[a]	1.21±0.09[b]	15.75	0.000
FSH(mIU/L)	1.31±0.03	9.28±0.72[a]	1.25±0.07[b]	123.01	0.000
Testost (ng/ml)	3.88±0.04	2.22±0.05[a]	2.54±0.05	372.30	0.000
Estradiol (pg/ml)	25.48±0.30	19.51±0.19[a]	23.64±0.96[b]	26.94	0.000

Mean ±S.E.M, n=8, $p>0.05$, [a] = significant compared to control, [b] = significant compared to IS; LH (Luteinizing Hormone), FSH (Follicle Stimulating Hormone), IS (Insufficient Sleep), ISZ (Insufficient Sleep with Zinc)

Table 3: Comparison of Sperm Count (SC), percentage Sperm with Normal Morphology (SNM%) and percentage Sperm with Active Progressive Motility (SAPM%) among the SD models

Parameters	**Control**	**IS**	**ISZ**	***f***	***p***
SC × 10^6/ml	60.50±1.52	45.63±1.80[a]	48.38±1.01	28.616	0.000
SNM%	76.13±2.92	32.63±2.21[a]	48.51±0.68[b]	104.803	0.000
SAPM%	35.00±2.49	10.13±1.68[a]	14.38±2.01	43.058	0.000

Mean ±S.E.M, n=8, $p>0.05$, a = significant compared to control, [b] = significant compared to IS; IS (Insufficient Sleep), ISZ (Insufficient Sleep with Zinc)

DISCUSSION

Sleep is very important for most of the biological processes in the body, so invariably, sleep deprivation (SD) adversely affects health (Medic *et al.,* 2017). Our study revealed the extents to which IS model of SD caused negative changes on different parameters of male reproductive functions, through induction of oxidative stress in testicular tissue and systemic stress response in the body, and the ameliorative role of concomitant Zinc supplementation on those negative changes.

SD is a known physiological stressor (Meerlo *et al.,* 2008; Wu *et al.,* 2008) that results in an increase in plasma glucocorticoids (mainly corticosterone in rodents) (Cirelli *et al.,* 2006; Machado *et al.,* 2010; Herman, 2016). In our study the IS model recorded significantly higher serum Corticosterone level compared to that of the control model. This is suggestive of induction of stress response as a possible mechanism through which SD affects male reproductive function. Our finding parallels previous studies that reported increase in Cortisol, Corticosterone or both in sleep deprived subjects (Olayaki *et al.,* 2015; Choi *et al.,* 2016; Rizk *et al.,* 2020). Contrary to our finding, some studies reported no difference in either serum Corticosterone or serum Cortisol following periods of sleep deprivation/restriction (Schmid *et al.,* 2007; Zager *et al.,* 2007; Nedeltcheva *et al.,* 2009).

A small amount of ROS is essential for sperm functions, but High level of ROS can affect sperm function through oxidation of lipids, proteins, and even DNA (Agarwal and Prabakaran, 2005; Ebisch, *et al.,* 2007). When the intricate balance between ROS and antioxidants is disrupted, oxidative stress occurred (Agarwal *et al.,* 2014). Total antioxidant capacity (TAC) measures the total antioxidant capacity of all antioxidants in a biological sample. In our study, SD resulted in significant reduction in levels of TAC in IS model compared to that in the control model. This indicates that, there is marked reduction in both enzymatic and non-enzymatic anti-oxidants activities in testicular tissue due to SD. Similarly, Rizk *et al*. (2020) reported significant reduction in TAC levels of SD groups when compared to the control group. De Oliveira *et al.* (2002) also recorded significant decrease of GSH level in testicular tissue of sleep deprived rats. Everson *et al* (2005) reported decrease in GSH activity in liver after SD. GSH plays multiple roles in cellular antioxidant defence system, hence it decrease implies decrease in TAC (Debnath and Mandal, 2000). In similar development, Pasqualotto *et al.* (2000) reported that Control subjects had seminal TAC values 1.41 fold higher than that found in infertile males.

Reimund (1994) hypothesised that free radicals, which accumulate during wakefulness are removed during sleep. The removal of excess free radicals during sleep is accomplished by decreased rate of formation of free radicals and increased efficiency of antioxidant mechanisms. Interestingly, Zinc supplementation significantly increased the level of TAC more than both IS and Control models. This finding reaffirmed that Zinc is a potent antioxidant.

Spermatozoa in contrast to other cells are particularly susceptible to lipid peroxidation due to the high percentage of polyunsaturated fatty acids (PUFA) in their membrane (Talevi *et al.,* 2013; Agarwal *et al.,* 2014). MDA is a stable end product of lipid peroxidation widely used in biomedical research as an index of lipid peroxidation (Tavilani, 2008; Collodel *et al.,* 2015). In our study, the level MDA in IS model is significantly higher than that of the control model, indicating increased lipid peroxidation in testicular tissues. Our finding is in keeping with that of Rizk *et al.* (2020) where they reported significant increase in testicular MDA level after sleep deprivation. In addition, other studies reported significant increase in MDA levels in stressed Wistar rats (Nirupama and Yajurvedi, 2013; Garcia-Diaz *et al*., 2015).

In our study, Zinc supplementation significantly reduces the MDA level, signifying its role in reducing the rate of lipid peroxidation in testicular tissues. Our finding goes well with that of Nagalakshmi *et al*., (2013). However, Singh (2008) and Juneet *et al.* (2018) reported increased oxidative stress with use of excessive zinc in diet. Although the exert mechanism of action of Zinc is not fully known, but it was postulated that Zinc influence anti-oxidants activity via its role as a cofactor and enhancer of Cu, Zn-SOD, (Davis *et al.,* 2000; Colagar *et al.,* 2009). Zinc was also believed to serves through several other mechanisms; reduction of hydroxyl radical (OH•) production, due to its ability to displace Cu and Fe from membrane binding sites (Michalska-Mosiej *et al.,* 2016) and stabilization of sperm membrane against oxidative stress challenge (Chia *et al.,* 2000; Ebisch *et al.,* 2007). It was also reported that Zinc deficiency can impair antioxidant defences and makes the spermatozoa more susceptible to OS and inflammatory reactions (Colagar *et al.,* 2009). Zinc supplementation also increased catalase activity in seminal plasma within tolerable limits, and can improve fertility (Egwurugwu *et al.*, 2013).

Spermatogenesis depends on pituitary gonadotropins, follicle stimulant hormone (FSH) and luteinizing hormone (LH) (Hess and de Franca, 2008). These hormones are secreted from gonadotrophs of the anterior pituitary gland. In the testes LH binds to receptors on Leydig cells stimulating synthesis and secretion of Testosterone hormone. FSH regulates the mitotic proliferation growth and function of Sertoli cells. Sertoli cells in turn, support many aspects of sperm cell maturation (Simoni *et al.,* 1999). Gonadotropins production is under the feedback control of sex hormones (Ganong, 2003).

In our study SD induced significant increase in serum FSH of IS model compared to Control. The increase in serum FSH may be secondary to decrease in serum testosterone level as evident in our study since, Serum LH and FSH are well known to be under the negative feedback control of serum testosterone (Ganong, 2005). Our results are in accordance with other researches that reported elevated blood levels of FSH and/or LH due to lack of steroid negative feedback from the testes, which are indicators of testicular dysfunction, sub-fertility or infertility (Babu *et al.,* 2004; Martin-du, 2009). Contrary to our finding, Breen and Karsch, (2006) reported inhibition of the hypothalamus-pituitary-gonadal (HPG) axis with decreased LH and FSH secretion in response to stress.

Our study also revealed significant reduction in serum FSH concentration with Zinc supplementation. The reduction in serum FSH is an indicator about the improvement of testicular function, as decreased levels of gonadotropins usually reflect increase in sex hormones negative feedback regulation. This is in accordance to the findings of Egwurugwu *et al*., (2013) and Mohamad and Hassan, (2014) that reported significant decrease in serum levels of FSH and LH with Zinc supplementation. This is not surprising because previous researches have demonstrated that Zn is needed for the normal functioning and regulation of the hypothalamus-pituitary gonadal axis (Fallah *et al.,* 2018), all though the exact mechanism was not yet clear. The secretions of all the sex steroid hormones are in synchrony with the circadian rhythm and sleep patterns (Empson and Purdie. 1999). Hence, SD may disrupt hormone balance and affects male reproductive functions (Akindele *et al.,* 2014; Alvarenga *et al.,* 2015). In our study, the serum testosterone of IS model was significantly lower than that of control, indicating that SD resulted in reduction in testosterone production. Our finding is in keeping with many previous SD studies in both humans and animals (Nirupama and Yajurvedi, 2013; Choi *et al.,* 2016; Victor *et al.,* 2018; Rizk *et al.,* 2020). Longstanding decrease in circulating testosterone can consequently affect fertility in apparently healthy men (Alvarenga *et al.,* 2015). The exact mechanism by which SD decrease testosterone level is not known (Chen *et al.,* 2012). Invariably, it was presumed that SD as a potent stressor, increase circulating level of glucocorticoids in the body. It was reported that Glucocorticoid induce gonadal resistance to gonadotrophins and inhibit testosterone synthesis by inducing apoptosis of Leydig cells and decreases its sensitivity to LH (Wang, 2011; Chen *et al.,* 2012) leading to decrease Testosterone production. McEwen (2007) further explained that, the decrease in testosterone level during SD is due decrease in the blood flow to the testicles (less vital organs) leading to suppression of its activity during stress in order to maximally increase the resources supplied to organs critically important for adaptation.

Never the less, Alvarenga *et al.,* (2015) revealed no significant difference in Testosterone concentration in 21 days Sleep-Restricted group compared to the control group. In our study zinc supplementation resulted in slight increase in Testosterone level, which is not statistically significant.

This is contrary to the findings Egwurugwu *et al.*, (2013) that reported dose dependant increase in testosterone level with Zinc supplementation.

Traditionally, testosterone and estrogen have been considered to be male and female sex hormones, respectively. But the demonstration of wide expression of the aromatase enzyme, Estrogens receptors throughout the male reproductive system and within human sperm underlines the role of estrogens in human male reproductive function (Rochira *et al.,* 2005; Carreau *et al.,* 2010; Carreau *et al.,* 2012). In our study, SD resulted in significant decrease in serum Estradiol in IS model compared to the control model. Our finding is in accordance with that of Andersen *et al.,* (2004) that reported significant reduction in serum Estradiol of SD group compared to the control group. Conversely, Lan *et al.,* (2018) showed significant increase in serum Estradiol of male rat subjected to traumatic stress. Testosterone is the main substrate for the synthesis of these estrogens and therefore any alteration of its concentration may alter the synthesis of estrogens. In our study, the serum Estradiol value in IS model was insignificantly lower than that of control. Never the less Zinc supplementation significantly improved the Estradiol level.

Sperm concentration, motility, and morphology are the most important predictors of an individual's potential to produce viable sperm (Kao *et al*., 2008). Male fecundity reduces with decrease in sperm concentration (Maya, 2010). Our study revealed significant reduction of SC in IS model compared to control model. This is in keeping with the findings of previous studies (Victor *et al*., 2018; Wise *et al*., 2018; Rizk *et al.*, 2020), And contrary to the findings of Alvarenga *et al.,* (2015) and Choi *et al.,* (2016). It was documented that sperm count negatively associate with level reactive oxygen species (ROS) (Pasqualotto *et al.,* 2000). High levels of ROS disrupt the inner and outer mitochondrial membranes, inducing the release of cytochrome C and activating apoptosis of sperm cells, resulting in reduced sperm count (Wang, *et al*, 2003; Agarwal *et al.,* 2014).

Sperm motility and morphology are indicators of qualitative spermatogenesis. In our study, SD significantly decreases the percentages of sperm with normal morphology and sperm with active progressive motility in IS model compared to that of the control model. Our findings are in keeping many previous studies that linked SD to impaired sperm motility, concentration, and morphology (Choi *et al.,* 2016; Victor *et al.,* 2018; Wise *et al*., 2018; Rizk *et al.,* 2020). Although the precise mechanism was not fully elucidated, but, it was postulated that oxidative stress plays an injurious role with consequent reduction in sperm quality and quantity (Sarkar *et al.,* 2011; Wagner *et al.,* 2017). As evident in our study, the IS model recorded high level of MDA and low level of TAC, which connotes high level of oxidative stress. Collodel *et al.,* (2015) reported that MDA level in seminal plasma negatively correlate with sperm motility, morphology and concentration, while TAC level, positively correlates with sperm concentration, motility, and morphology (Subramanian *et al*., 2018).

It was documented that exposure to excess glucocorticoids either endogenously or exogenously can negatively affects spermatogenesis. In this context, our study also recorded high concentration of corticosterone in IS model hence, the decrease in both quality and quantity of the sperm. Rats subjected to chronic stress stimuli showed impaired sperm production in previous studies (Priya and Reddy, 2012; Garcia-Diaz *et al* 2015). Interestingly, with zinc supplementation the percentages of sperm with normal morphology and active progressive motility were significantly improved. On the contrary, Chyb *et al*, (2000) reported that Zinc affect sperm quality through its ability to displacement of calcium necessary for the activation of spermatozoa, which can lead to reduced

motility. Zhao *et al.* (2016) reported that Zn is essential for maintaining the stability of sperm chromatin and membrane, and also for inhibiting apoptosis of sperm cells with normal morphology. Hence, Zinc supplementation appears to be worthy for management of male infertility (Sinclair, 2000). However, Egwurugwu *et al.,* (2013) reported that beyond 50mg/kg of zinc there is significant dose dependent decrease in sperm motility and normal morphology in the test groups.

The SD in IS model induced significant alterations in male reproductive hormones and sperm parameters. The induced alterations were attributed to the activation of HPA axis evidenced by increased serum corticosterone, and induction of oxidative stress evidenced by increased MDA and decreased TAC in testicular tissue. These alterations may result in male reproductive hypo-function and inferentially male infertility. However, concomitant Zinc sulphates supplementation at dose of 5mg/animal/day) significantly ameliorates most of the alterations induced by SD.

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