Research Article

Mifepristone Ameliorates Sleep Deprivation - Induced Oxidative Stress in the Testis of Rats

Ajibare A.J.[1], Ayodele O.D.[2] and Olayaki L.A.[2]

[1]*Department of Physiology, College of Medicine, Ekiti State University, P.M.B. 5355, Ado – Ekiti 36001, Nigeria*
[2]*Department of Physiology, College of Health Sciences, University of Ilorin, Ilorin, Nigeria.*

ABSTRACT

Sleep deprivation is becoming an everyday experience and has been associated with generation of chronic stress and low level of testosterone. This study aimed to evaluate the influence of mifepristone on testicular oxidative stress and serum inflammatory markers in sleep deprivation induced chronic stress in rats. Twenty five rats were divided into five groups (n=5) and designated as follows: Group 1: Control, Group 2: sleep deprived (SD), Group 3: sleep deprived and sleep recovery (SD+SR), Group 4: sleep deprived mifepristone treated (SD+MIF), Group 5: sleep deprived and recovery mifepristone treated (SD+SR+MIF). Rats were sleep deprived for five days, mifepristone 10mg/kg was given orally for mifepristone treated groups while recovery groups were allowed to recover for five days. At the end of the experiments cortisol, testosterone, interleukin 6 (IL-6) and c reactive protein (CRP) were analysed while testicular malondialdehyde (MDA), catalase (CAT), and glutathione (GSH) were also evaluated. Rats in SD group had significantly increased level of MDA and cortisol, IL-6 and CRP levels (p<0.05), while showing significantly reduced the levels of testosterone, GSH, and CAT (p<0.05). Treatment with mifepristone reversed the changes in the MDA, GSH, CAT, cortisol, testosterone levels (p<0.05), while only sleep deprived recovery (SD+SR) reversed changes in IL-6 and CRP. The present findings indicate that mifepristone possesses antioxidant properties which may ameliorate the imbalance between reactive oxygen species (ROS) and production of antioxidant enzymes in the testis. However, sleep recovery is important in reversing inflammatory changes due to sleep deprived induced chronic stress.

Keywords: *Chronic Stress; Inflammation; Mifepristone; Oxidative Stress; Sleep deprivation*

INTRODUCTION

A stressor may be a physical or psychological threat to safety, status, or wellbeing; physical or psychological demands that exceed available resources. However, although stressful events are inevitable in daily life and overcoming obstacles is inherent to success, chronic stressful events are detrimental (Hannibal and Bishop, 2014). In addition, chronic stress can be either a triggering or aggravating factor for many diseases and pathological conditions (Yaribeygi *et al*., 2017) . Previous studies in rats correlated stress-induced glucocorticoid secretion with alterations in the structure of the testis and sub-fertility (Mingoti *et al*., 2003; Swami and Jeganathan Ramanathan, 2007; Yazawa *et al*., 2000).

Sleep deprivation is known to be an indicator of stress factors in the living environment and is on the rise with the advent of technological advances and also shift work and on-call duties during night are necessities in our modern world (Hardeland, 2018). Sleep deprivation is said to be responsible for a significant production of oxidants and invariably reactive

oxygen species (Villafuerte *et al*., 2015) also, chronic stress exposure has been implicated in the induction of oxidative stress by excessive production of free radicals and reactive oxygen species (ROS), which can cause alterations in both cell membranes and constituents ending by cell mutation or damage (Juliet *et al*., 2004; Yang and Lin, 2002). For example, testicular membranes are rich in polyunsaturated fatty acids and therefore are susceptible to oxidative stress(Ghosh *et al*., 2002). Furthermore, a correlation was noted between free radical production and gonadal steroidogenesis (Manna *et al*., 2003).

Sleep deprivation is a chronic stressor and can cause prolonged activation of the hypothalamic-pituitary-adrenal axis and excessive cortisol release, which is believed to contribute to inflammation by impairing the function of glucocorticoid receptors (down-regulation, reduced expression, nuclear translocation) (Mackin and Young, 2004; Silva *et al*., 2004)]. Glucocorticoid receptor abnormalities reduce the immune system's capacity to respond to cortisol

and lower inflammation, resulting in concomitantly sustained levels of cytokine and cortisol release (Chrousos, 1995; Miller *et al*., 2002).

Mifepristone an anti-glucocorticoid has been demonstrated to have use in Alzheimer's disease (Blasey *et al*., 2009) and psychotic depression (Belanoff *et al*., 2002) it has also been used in the treatment breast cancer (Im and Appleman, 2010). Since both oxidative stress and inflammation has been linked to the high level of glucocorticoids caused by chronic stress, we hypothesize that mifepristone an anti-glucocorticoid could modulate the chronic stress induced by sleep deprivation.

MATERIALS AND METHODS

Animals: Twenty five male Wistar rats of weight 180- 200g purchased from Ekiti State University, Ado –Ekiti, Ekiti-State, Nigeria were used for the study. The rats were housed and maintained in standard conditions of light, feeding and temperature in the Animal House of College of Medicine, Ekiti State University, Ado –Ekiti, Ekiti-State, Nigeria. The study was conducted in accordance with the standards established by the Guide for the Care and Use of Laboratory Animals (Research, 1996). Rats had unrestricted access to standard rat chow and tap water. The experimental protocol lasted twenty four days. The first seven days were set aside for acclimatization to laboratory environment, the animals were randomly assigned to one of the following experimental groups (n = 5 per group) and treated accordingly. Rats in Group I (Control) received olive oil (10ml/kg, orally) daily. Rats in Group II received olive oil (10ml/kg, orally) daily; designated as Sleep Deprived group (SD). Those in Group III received olive oil (10ml/kg, orally) daily; designated as Sleep Deprived, Sleep Recovery group (SD + SR). Those in Group IV received mifepristone (10mg/kg, orally) daily; designated as Sleep Deprived with Mifepristone (SD + Mif). Group V received mifepristone (10mg/kg, orally) daily; designated as Sleep Deprived, Sleep Recovery with Mifepristone treatment (SD + SR + Mif).

Drug: Mifepristone tablets 200mg with code number HR/DRUGS/325-OSP (H), batch number: NMS-01 was purchased from Naman Pharma Drugs Princess Street Mumbai -2 India. Mifepristone was dissolved in olive oil and was given by oral gavage at 10mg/kg/d as described by (Khalil *et al*., 2018).

Sleep-deprivation model: Twenty-five rats of weight 180-200g were used in the research. The rats were acclimatized for seven days under ambient temperature of 25oC and standard photoperiod of 12hr light-dark cycle after which they were subjected to Paradoxical sleep deprivation for 20hrs (11:00am-7:00am next morning) in the for 5 days with 4hr (7:00am-11:00am) rest each day using Modified Multiple Platform (MMP) method. The MMP has been previously described (Oh *et al*., 2012). The rats were sleep deprived for 5 days. The water in the tank was changed daily throughout the SD period. The control group was allowed sleep in their cages. For sleep recovery model, after 5 days of sleep deprivation, the animals were given a sleep recovery period of 5 days.

Experimental procedure:
Rats were sacrificed in stages: Sleep deprived groups at the end of five days sleep deprived and sleep recovery groups at the end of five days sleep recovery. The rats were anaesthetized using a mixture of 25% (w/v) urethane and 1% (w/v) alpha chloralose (5ml/kg; intraperitoneally., BDH chemicals Ltd., Poole, England). The animals were humanely sacrificed and samples were collected via cardiac puncture with 5mls syringes after opening of the upper abdominal region. Blood samples were collected into plain bottles. The blood samples were centrifuged at 3000r/p for 10 minutes. The serum samples were micro-pipetted into plain bottles and were immediately stored at -4_0c.

Testosterone: Testosterone content was determined using an enzyme-linked immunosorbent assay kit (Accubind, Monobind Inc. Lake Forest, USA). This assay is based on the principle of competitive binding. Competition occurs between unlabeled antigen (present in the samples) and enzyme-labeled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. After incubation (1 h), the microplate was washed four times and substrate solution was added. Optical density was measured and testosterone concentration was estimated as described previously (Aebi, 1984).

Cortisol: After sacrifice, blood was collected by cardiac puncture and serum was obtained by centrifugation ($3000 \times g$ for 10 min). Cortisol content was determined using an enzyme-linked immunosorbent assay kit (Accubind, Monobind Inc. Lake Forest, USA). This assay is based on the principle of competitive binding. Competition occurs between unlabeled antigen (present in the samples) and enzyme-labeled antigen (conjugate) for a limited number of antibody binding sites on the microwell plate. After incubation (1 h), the microplate was washed four times and substrate solution was added. Optical density was measured at 450nm and cortisol concentration was estimated (Kinn Rød *et al*., 2017).

Serum Interleukin 6 (Il-6): This assay employs the competitive inhibition enzyme immunoassay technique. A monoclonal antibody specific to interleukin 6 (IL6) has been pre-coated onto a microplate. A competitive inhibition reaction was launched between biotin labelled IL6 and unlabelled IL6 (Standards or samples) with the pre-coated antibody specific to IL6. After incubation the unbound conjugate was washed off. Next, avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. The amount of bound HRP conjugate was reverse proportional to the concentration of IL6 in the sample. After addition of the substrate solution, the intensity of colour developed was reversed proportional to the concentration of IL6 in the sample(Yoga *et al*., 2009).

Serum C-Reactive Protein (CRP): This assay employs the competitive inhibition enzyme immunoassay technique. A monoclonal antibody specific to c reactive protein (CRP) has been pre-coated onto a microplate. A competitive inhibition reaction was launched between biotin labelled CRP and unlabelled CRP (Standards or samples) with the pre-coated

antibody specific to CRP. After incubation the unbound conjugate was washed off. Next, avidin conjugated to Horseradish Peroxidase (HRP) was added to each microplate well and incubated. The amount of bound HRP conjugate was reverse proportional to the concentration of CRP in the sample. After addition of the substrate solution, the intensity of colour developed was reversed proportional to the concentration of CRP in the sample (Dimitrov *et al*., 2014).

Homogenization of Testicular Tissue Samples: Testis tissue samples were quickly excised and thereafter, washed in cooled 0.15M NaCl and were homogenized in 2ml of ice-cold potassium phosphate buffer (0.1M, pH: 7.4) using an improvised homogenizer. Samples were centrifuged at 5000r/m for 15 minutes to obtain supernatant. The supernatant obtained was micro pipetted into 3 different plain bottles and stored at -4$_0$c prior use for the estimation of MDA, GSH, and CAT.

Testicular Malondialdehyde.: Malondialdehyde (MDA) results from degradation of polyunsaturated lipids. The production of this substance is used as a biomarker to measure the level of lipid peroxidation. MDA reacts with thiobarbituric acid (TBA) as a thiobarbituric acid reactive substances (TBARS) to form a 1: 2 MDA-TBA adduct, which is absorbed at 532 nm. Thus, the quantity of TBARS is proportionate to the amount of MDA. The concentration of TBARS was calculated using MDA standard curve and was expressed as μmol/mg of protein (Devasagayam *et al*., 2003).

Testicular Gluthatione*:* GSH was measured by the method of. Briefly, to 0.1mL of sample, 0.9mL distilled water and 1.5mL of precipitating reagent were added (3.34 g metaphosphoric acid, 0.4 g EDTA, and 60.0 g sodium chloride). Tubes were shaken and allowed to stand for 5min at room temperature (25 $\pm$ 1 $\circ$C). The mixture was centrifuged for 15min at 4000 rpmat 4$\circ$C. In 1.0mL supernatant, 4.0mL of phosphate solution (0.3M disodium hydrogen phosphate) and 0.5mL 5-50-dithiobis-(2-nitrobenzoic acid) (DTNB) (80mg in 1% sodium citrate) were added. Development of yellow color

complex was read immediately at 412 nm on a spectrophotometer. A standard curve using GSH was prepared and GSH concentration in the experimental samples was extrapolated from the standard curve. GSH concentration was calculated and expressed as mM of GSH/mg protein.

Testicular Catalase Enzyme: CAT activity was assayed by H2O2 consumption, following (Aebi, 1984) method. Briefly, using UV spectrophotometric method, which depends on monitoring the change of 240nm absorbance at high levels of hydrogen peroxide solution ($\geq 30mM$). High levels of hydrogen peroxide (H2O2) immediately lead to inhibition of the catalase enzyme by altering its active site structure, although there is variation in the extent to which this occurs. Catalase was expressed in mmol/min/ml.

Statistical Analysis
Data are expressed as means $\pm$ standard error of the mean (SEM). Statistical group analysis was performed with Graph pad (Prism 7) statistical software. Test of variance was done using ANOVA, followed by Bonferroni's multiple comparisons test. Statistically significant differences were accepted at $p < 0.05$.

RESULTS

Hormones: The result shows a significant increase (P<0.05) in the level of cortisol (Fig 1A) in SD, SD+SR and SD+MIF when compared with CTRL. There was however a significant reduction (P<0.05) in the cortisol level of SD+SR, SD+MIF and SD+SR+MIF when compared to the Group SD. Furthermore, there is a significant decrease (P<0.05) in the level of cortisol in SD+SR+MIF compared to SD, SD+SR and SD+MIF. However, there was a significant reduction (P<0.05) in the level of testosterone (Fig 1B) in SD, SD+MIF, SD+SR+MIF when compared to the CTRL. Furthermore, SD+SR, SD+MIF, SD+SR+MIF all showed significant increase in testosterone level (P<0.05) compared to SD. There is a significant decrease in testosterone level (P<0.05) in SD+MIF when compared to SD+SR.
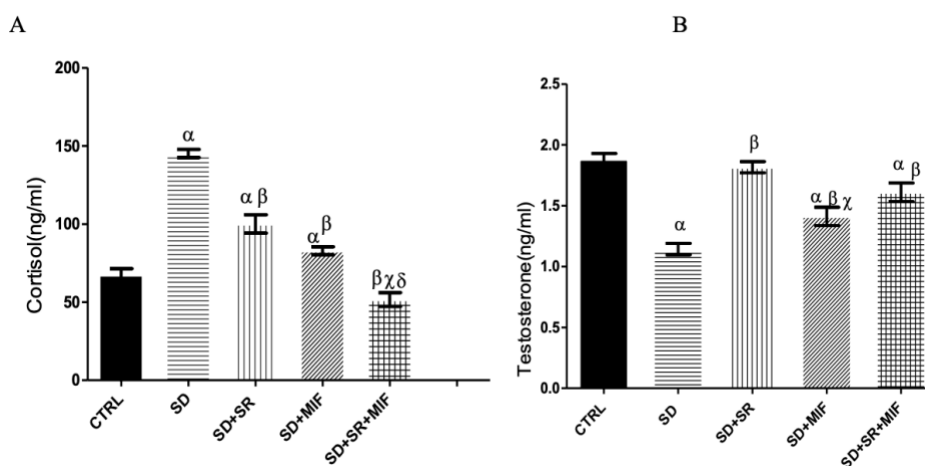

Figure.1
Effect of Mifepristone on hormonal parameters. A: Cortisol B: Testosterone. n = 5 in all groups. αp < 0.05, vs CTRL; βp < 0.05, vs SD; γp < 0.05, vs SD+SR; δp<0.05, vs SD+MIF. CTRL: control; SD: sleep deprived; SD+SR: sleep deprived and sleep recovery; SD+MIF: sleep deprived mifepristone treated; SD+SR+MIF: sleep deprived and sleep recovery mifepristone treated.

Figure 2.
Effect of Mifepristone on testicular oxidative stress parameters. A: malondialdehyde (MDA) B: glutathione (GSH) and C: catalase (CAT) n = 5 in all groups. $\alpha p < 0.05$, vs CTRL; $\beta p < 0.05$, vs SD; $\gamma p < 0.05$, vs SD+SR; $\delta p < 0.05$, vs SD+MIF. CTRL: control; SD: sleep deprived; SD+SR: sleep deprived and sleep recovery; SD+MIF: sleep deprived mifepristone treated; SD+SR+MIF: sleep deprived and sleep recovery mifepristone treated.
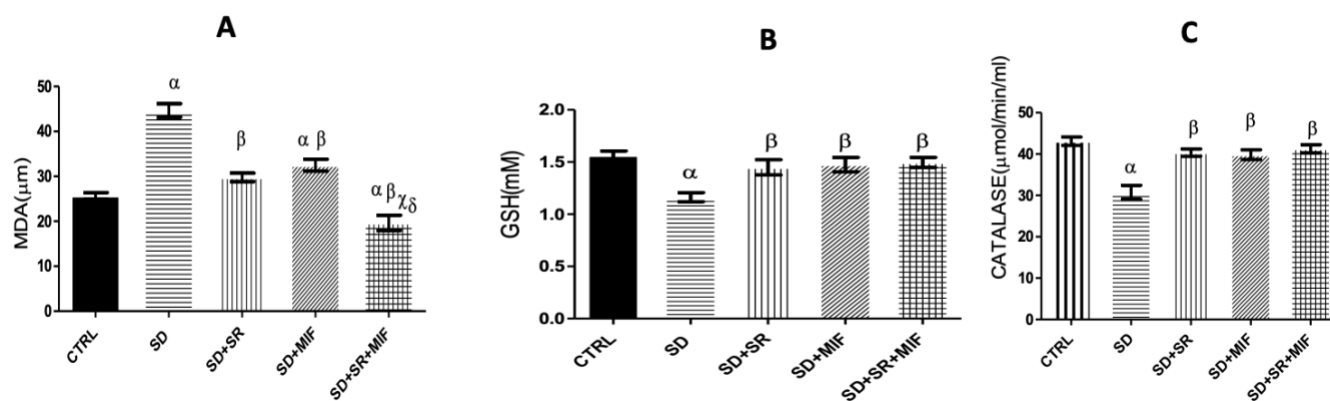

Figure 3.
Effect of Mifepristone on serum inflammatory markers. A: Interleukin-6 (IL-6) B: c reactive protein (CRP). n = 5 in all groups. $\alpha p < 0.05$, vs CTRL; $\beta p < 0.05$, vs SD; $\gamma p < 0.05$, vs SD+SR; $\delta p < 0.05$, vs SD+MIF. CTRL: control; SD: sleep deprived; SD+SR: sleep deprived and sleep recovery; SD+MIF: sleep deprived mifepristone treated; SD+SR+MIF: sleep deprived and sleep recovery mifepristone treated.
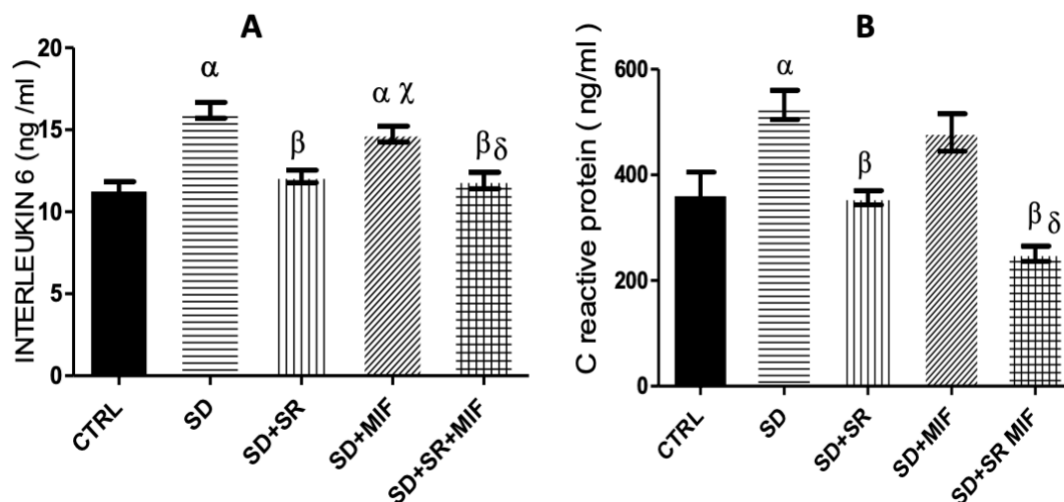
Serum Inflammatory Markers: The results of our study In Fig 2A showed a significant rise in the level of IL-6 (P<0.05) in SD compared to the CRTL. There was a significant reduction in the IL-6 level of SD+SR, SD+SR+MIF compared to SD, there was a significant increase in IL-6 level in SD+MIF compared to SD+SR, while SD+SR+MIF revealed a significant reduction of IL-6 level compared to SD+MIF. However, the results in Fig 2B showed a significant rise in the level of CRP (P<0.05) in SD compared to the CRTL. There was a significant reduction in the CRP levels of SD+SR, SD+SR+MIF compared to SD, while SD+SR+MIF revealed a significant reduction of CRP level compared to SD+MIF.

DISCUSSION

The results of the present study revealed the ability of mifepristone to ameliorate the imbalance between the reactive oxygen species and production of antioxidant enzymes in the testis of sleep deprived male wistar rats while improving their testosterone profile. In this present study, cortisol levels are significantly raised in the SD group, this alludes to the fact that sleep deprivation is a biological stressor (Olayaki *et al*., 2015). One of the proposed mechanisms for the lowering of cortisol with mifepristone (RU 486) treatment is that the drug has a direct effect at decreasing rat adrenal 3 - hydroxysteroid dehydrogenase / isomerase, 21-hydroxylase, and 11-hydroxylase production, which are all involved in glucocorticoid synthesis. Thus, by inhibiting these enzymes, a reduction in serum level of cortisol was observed (Albertson *et al*., 1994). Our findings are also consistent with other models of chronic stressed animals with glucocorticoid receptor blockade (Glavas *et al*., 2006). In the SD+SR group, the reduction in cortisol could be because during chronic stress, corticosterone levels are sustained via a positive feed-forward loop and once the stress episode is over, systemic corticosterone and ACTH may be abnormally low (Dallman *et al*., 2005).

Male rats exhibit marked hormonal alterations when subjected to paroxysmal sleep deprivation, with decreases in testosterone and estradiol concentrations, as well as increases in progesterone and glucocorticoid levels (Tufik *et al*., 2009). Furthermore, in the mechanism of stress-induced infertility, expression of glucocorticoid receptors (GRs) has been described in multiple cell types of the testis and expression is conserved across species (Zelena and Makara, 2015).

This current study revealed reduction in serum testosterone levels after SD. This agrees with the work of (Dong *et al*., 2004) (for mice), (Almeida *et al*., 2000; Manna *et al*., 2004) (for rats), and (Cote *et al*., 2013) (humans). Indeed, both endocrine and neuronal signals are important in the secretion of testosterone and they are significantly influenced by conditions such as chronic stress produced by sleep deprivation(Rajaratnam and Arendt, 2001; Román *et al*., 2006). Furthermore, (El-Sayed *et al*., 2012; Gao *et al*., 2002) reported that it is possible that the chronic stress induced lowering of testosterone could be because there may be a centrally mediated inhibition of gonadotropin releasing hormone (GnRH) by glucocorticoids or that there is a glucocorticoid tissue resistance to testosterone which also decreases the sensitivity of leydig cells to Luteinizing hormone. Other possible mechanisms could be that the decreased testosterone levels associated with SD group may be in part due to serotonin-related inhibition of testosterone production (El-Sayed *et al*., 2012; Gao *et al*., 2002). Furthermore, both serotonin and serotonin receptors have been localized in Leydig cells isolated from the testes of golden hamsters and serotonin has also been demonstrated to inhibit testosterone production (Frungieri *et al*., 2002). In addition, (Honess and Marin, 2006; McEwen, 2007) reported that the decrease in testosterone level during sleep deprivation may be due to a decrease in the blood flow to the testes during chronic stress in order to supply order vital organs.

According to (Whirledge and Cidlowski, 2010) high levels of glucocorticoids as seen in chronic stress suppresses testosterone and that the degree to which glucocorticoids inhibit Leydig cell function is determined by the amount of GR in the cell, the intracellular concentration of glucocorticoids, and the oxidative activity of 11β-hydroxysteroid dehydrogenase (11β-HSD), an enzyme that catalyzes both oxidative and reductive reactions of glucocorticoids. In addition, cumulative in vivo and in vitro evidence equally support a role of 11 beta hydroxysteroid in reversing the suppression of testosterone biosynthesis by glucocorticoids (Monder *et al*., 1994). Lowered 11β-HSD oxidative activity increases glucocorticoid-dependent inhibition of testosterone production in Leydig cells. Interestingly, (Damayanthi *et al*., 2011) demonstrated that mifepristone treatment lowered 11β-HSD oxidative activity but increased testosterone levels in rats It can therefore be said that the increase in testosterone level in SD+MIF group might be by a direct receptor mediated effect. Furthermore, (Nirupama and Yajurvedi, 2015) using restraint stress and forced swimming exercise reported that mifepristone treated stressed rats showed higher testicular 3β-HSDH activity and testosterone levels (As against significantly lower levels of both in untreated stressed rats) thereby suggesting a role of mifepristone in modulating enzymes involved in leydig cell steriodogenesis.

The results of cortisol and testosterone obtained in the SD+SR group may be due to the ability of the rats in recovery period to accommodate the HPA axis derangements that accompany sleep deprivation and allowing them to return to a normally functioning HPA axis during periods of sleep recovery (Nirupama and Yajurvedi, 2015). Similarly, it has been reported that there is a sleep dependent increase in testosterone that requires about 3 hours of slow wave sleep (El-Aziz and Mostafa, 2012; Leenaars *et al*., 2011). Of note in this study, the increase in testosterone in SD+SR group was higher than that of both SD+SR MIF and SD+MIF group. This difference though not significant might be due to the weak anti-androgenic activity demonstrated by mifepristone (Wittert, 2014). This is probably a reason why long term use of mifepristone should be discouraged.

The testis is extremely sensitive to oxidative stress because the membranes of the testis are very rich in polyunsaturated fatty acids and there is a resultant lipid peroxidation in the aftermath of oxidative stress on this organ (Kakade and Kulkarni, 2014) and this is presumed to be secondary to the elevation of glucocorticoid (Manna *et al*., 2003). Malondialdehyde (MDA) is formed as a result of lipid peroxidation and in this present study MDA was raised after sleep deprivation, antioxidants like glutathione (GSH) and catalase (CAT) were all reduced in the SD group. Accordingly,(Nirupama and Yajurvedi, 2015) noticed a reduction in the level of CAT and GSH and an increase in MDA in rats exposed to forced swimming exercise and restraint stress, and also the subsequent significant reversal in the group treated with mifepristone. Therefore it could be said that the glucocorticoid receptor blockade action of mifepristone is responsible for the reduction in MDA levels and increase in CAT and GSH seen in the SD+MIF group. Furthermore, (Dalal *et al*., 2019) reported that mifepristone decreased cholesterol efflux, reactive oxygen species production and lipid peroxidation in sperm due to its antioxidant activity during cryopreservation. They also found that ferric reducing ability of mifepristone increases in a dose-dependent manner further proving the antioxidant activity of mifepristone, in addition to its anti-glucocorticoid action. Additionally, mifepristone consists of a progesterone-like steroid ring with a non-steroid moiety named di-methylaminophenyl and the antioxidant activity of mifepristone resides in the non-steroid moiety (Kumar *et al*., 2017). In the sleep recovery group the significant reduction in MDA level and increase in level of CAT and GSH can be attributed to the reduction in high glucocorticoid level precipitated by sleep deprivation induced chronic stress and as such, sleep recovery restores or accentuates antioxidant level and leads to a decrease in free radical production. These findings are in consonant with the study of (El-Aziz and Mostafa, 2012; Olayaki *et al*., 2015).

Exposure to severe stressors such as sleep deprivation may alter the interplay between inflammation and cortisol and contribute to negative health outcomes (Wolkow *et al*., 2015). Reports from different labs have confirmed pro-inflammation induced by stress, including C-reactive protein (CRP), interleukin 6 IL-6, TNFα, IL-1β and the transcription factor

of ''nuclear factor kappa B (NF-κB)'' (Miller *et al.*, 2009). Our present study further reinforces these results because there was there was significant increase in the level of both IL-6 and CRP amongst the SD group. This maybe as a result of the fact that chronic stress may activate the sympathetic nervous system to release norepinephrine and neuropeptide Y and these two stress hormones promote the phosphorylation of mitogen-activated protein kinases (MAPKs) or high mobility group box-1 (HMGB1) release, therefore inducing systematic inflammation (IL-6, CRP). In addition, the β2-adrenergic receptor (β2-ARs) are expressed on multiple cell types involved in immune-regulation, including not only immune cells (Padro and Sanders, 2014; Theron *et al.*, 2013), but also non-immune cells with a bystander role in the immune response (e.g., glia cells, fibroblasts, endothelial cells (Johnson, 2006; Mantyh *et al.*, 1995). Chronic stress-induced epinephrine binds to β2-ARs, and then results in the activation of p38 MAPK, which in turn enhances nuclear factor kappa B (NF-κB) DNA binding and cytokines and chemokines expression (Kolmus *et al.*, 2015).

There is potential for glucocorticoid receptor antagonists to aggravate (Miech, 2005) and this is a major concern limiting the use of glucocorticoid receptor antagonists for the treatment of diabetes, depression, and other conditions. However, in this study, there was no significant change in the SD+MIF group as earlier suggested by (Li *et al.*, 2008) who examined the effect of mifepristone on mouse challenged with lipopolysaccharide this is probably because there are other mechanisms involved other than high levels of glucocorticoids.

Among the SD+SR group the significant reduction in inflammatory response we noticed could be due to the gradual stabilization of the hypothalamic – pituitary gonadal axis with subsequent gradual increase in testosterone since its production starts rising during recovery in the first three hours of slow wave sleep (Leenaars *et al.*, 2011). Interestingly, existing literature suggests that testosterone may possess anti-inflammatory effects (magnitude debatable) in-vivo based on findings that testosterone deficiency is associated with increased inflammatory cytokine levels and testosterone supplementation reduces inflammatory cytokines levels (Mohamad *et al.*, 2019) So, in this study, it is poised that testosterone has a role to play in the reduction in inflammatory response in sleep recovery.

In conclusion, Mifepristone pre-treatment ameliorates testicular oxidative stress, improves testosterone profile caused by sleep deprivation induced chronic stress but had no significant effect on inflammatory markers both in sleep deprived and sleep recovery male Wistar rats.

REFERENCES

Aebi, H., (1984):. Catalase in vitro, in: Methods in Enzymology. Elsevier, pp. 121–126.

Albertson, B.D., Hill, R.B., Sprague, K.A., Wood, K.E., Nieman, L.K., Loriaux, D.L., (1994): Effect of the antiglucocorticoid RU486 on adrenal steroidogenic enzyme activity and steroidogenesis. Eur. J. Endocrinol. 130, 195–200.

Almeida, S.A., Petenusci, S.O., Franci, J.A., Silva, A.R.E., Carvalho, T.L., (2000): Chronic immobilization-induced stress increases plasma testosterone and delays testicular maturation in pubertal rats. Andrologia 32, 7–11.

Belanoff, J.K., Jurik, J., Schatzberg, L.D., DeBattista, C., Schatzberg, A.F., (2002): Slowing the progression of cognitive decline in Alzheimer's disease using mifepristone. J. Mol. Neurosci. 19, 201–206.

Blasey, C.M., DeBattista, C., Roe, R., Block, T., Belanoff, J.K., (2009): A multisite trial of mifepristone for the treatment of psychotic depression: a site-by-treatment interaction. Contemp. Clin. Trials 30, 284–288.

Chrousos, G.P., (1995): The hypothalamic–pituitary–adrenal axis and immune-mediated inflammation. N. Engl. J. Med. 332, 1351–1363.

Cote, K.A., McCormick, C.M., Geniole, S.N., Renn, R.P., MacAulay, S.D., (2013): Sleep deprivation lowers reactive aggression and testosterone in men. Biol. Psychol. 92, 249–256.

Dalal, J., Kumar, P., Chandolia, R.K., Pawaria, S., Rajendran, R., Sheoran, S., Andonissamy, J., Kumar, D., (2019): A new role for RU486 (mifepristone): it protects sperm from premature capacitation during cryopreservation in buffalo. Sci. Rep. 9, 6712.

Dallman, M.F., Pecoraro, N.C., la Fleur, S.E., (2005): Chronic stress and comfort foods: self-medication and abdominal obesity. Brain. Behav. Immun. 19, 275–280.

Damayanthi, D., Azman, M.A.B., Aminuddin, A.H.K., Hamid, A., Nwe, K.H.H., (2011): Effects of Smilax myosotiflora on testicular 11?-hydroxysteroid dehydro-genase oxidative activity and plasma hormone levels in rats.

Devasagayam, T.P.A., Boloor, K.K., Ramasarma, T., (2003): Methods for estimating lipid peroxidation: an analysis of merits and demerits. Indian J. Biochem. Biophys. 40, 300–308.

Dimitrov, I., Kamenov, V., Angelova, P., Petrov, M., (2014): Concentration of C-reactive protein in white adipose tissue, liver and blood serum of male Wistar rats. Trakia J. Sci. 12, 29–33.

Dong, Q., Salva, A., Sottas, C.M., Niu, E., Holmes, M., Hardy, M.P., (2004): Rapid glucocorticoid mediation of suppressed testosterone biosynthesis in male mice subjected to immobilization stress. J. Androl. 25, 973–981.

El-Aziz, E.A.A., Mostafa, D.G., (2012): Impact of sleep deprivation and sleep recovery on reproductive hormones and testicular oxidative stress in adult male rats. AAMJ 10, 1.

El-Sayed, A.-F.M., Abdel-Aziz, E.-S.H., Abdel-Ghani, H.M., (2012): Effects of phytoestrogens on sex reversal of Nile tilapia (Oreochromis niloticus) larvae fed diets treated with 17α-Methyltestosterone. Aquaculture 360, 58–63.

Frungieri, M.B., Zitta, K., Pignataro, O.P., Gonzalez-Calvar, S.I., Calandra, R.S., 2002. Interactions between testicular serotoninergic, catecholaminergic, and corticotropin-releasing hormone systems modulating cAMP and testosterone production in the golden hamster. Neuroendocrinology 76, 35–46.

Gao, H.-B., Tong, M.-H., Hu, Y.-Q., Guo, Q.-S., Ge, R., Hardy, M.P., (2002): Glucocorticoid induces apoptosis in rat leydig cells. Endocrinology 143, 130–138.

Ghosh, D., Das, S., Maiti, R., Jana, D., Das, U.B., (2002): Testicular toxicity in sodium fluoride treated rats: association with oxidative stress. Reprod. Toxicol. 16, 385–390.

Glavas, M.M., Yu, W.K., Weinberg, J., (2006): Effects of mineralocorticoid and glucocorticoid receptor blockade on hypothalamic–pituitary–adrenal function in female rats prenatally exposed to ethanol. Alcohol. Clin. Exp. Res. 30, 1916–1924.

Hannibal, K.E., Bishop, M.D., (2014):Chronic stress, cortisol dysfunction, and pain: a psychoneuroendocrine rationale for stress management in pain rehabilitation. Phys. Ther. 94, 1816–1825.

Hardeland, R., (2018): Melatonin and inflammation—Story of a double-edged blade. J. Pineal Res. 65, e12525.

Honess, P.E., Marin, C.M., (2006). Behavioural and physiological aspects of stress and aggression in nonhuman primates. Neurosci. Biobehav. Rev. 30, 390–412.

Im, A., Appleman, L.J., (2010): Mifepristone: pharmacology and clinical impact in reproductive medicine, endocrinology and oncology. Expert Opin. Pharmacother. 11, 481–488.

Johnson, M., (2006): Molecular mechanisms of β2-adrenergic receptor function, response, and regulation. J. Allergy Clin. Immunol. 117, 18–24.

Juliet, P.A., Hayashi, T., Daigo, S., Matsui-Hirai, H., Miyazaki, A., Fukatsu, A., Funami, J., Iguchi, A., Ignarro, L.J., (2004): Combined effect of testosterone and apocynin on nitric oxide and superoxide production in PMA-differentiated THP-1 cells. Biochim. Biophys. Acta BBA-Mol. Cell Res. 1693, 185–191.

Kakade, A.S., Kulkarni, Y.S., (2014): Mifepristone: current knowledge and emerging prospects. J. Indian Med. Assoc. 112, 36–40.

Khalil, R.B., Smayra, V., Saliba, Y., Hajal, J., Bakhos, J.-J., Souaiby, L., Richa, S., Tamraz, J., Farès, N., (2018): Mifepristone reduces hypothalamo-pituitary-adrenal axis activation and restores weight loss in rats subjected to dietary restriction and methylphenidate administration. Neurosci. Res. 135, 46–53.

Kinn Rød, A.M., Harkestad, N., Jellestad, F.K., Murison, R., (2017): Comparison of commercial ELISA assays for quantification of corticosterone in serum. Sci. Rep. 7.

Kolmus, K., Tavernier, J., Gerlo, S., (2015): β2-Adrenergic receptors in immunity and inflammation: stressing NF-κB. Brain. Behav. Immun. 45, 297–310.

Kumar, P., Saini, M., Kumar, D., Bharadwaj, A., Yadav, P.S., (2017): Estimation of endogenous levels of osteopontin, total antioxidant capacity and malondialdehyde in seminal plasma: application for fertility assessment in buffalo (Bubalus bubalis) bulls. Reprod. Domest. Anim. 52, 221–226.

Leenaars, C.H., Dematteis, M., Joosten, R.N., Eggels, L., Sandberg, H., Schirris, M., Feenstra, M.G., Van Someren, E.J., (2011): A new automated method for rat sleep deprivation with minimal confounding effects on corticosterone and locomotor activity. J. Neurosci. Methods 196, 107–117.

Li, A., Felix, J.C., Yang, W., Jain, J.K., (2008): Effect of mifepristone on the expression of endometrial secretory leukocyte protease inhibitor in new medroxyprogesterone acetate users. Fertil. Steril. 90, 872–875.

Mackin, P., Young, A.H., (2004: Rapid cycling bipolar disorder: historical overview and focus on emerging treatments. Bipolar Disord. 6, 523–529.

Manna, I., Jana, K., Samanta, P.K., (2004): Intensive swimming exercise-induced oxidative stress and reproductive dysfunction in male Wistar rats: protective role of α-tocopherol succinate. Can. J. Appl. Physiol. 29, 172–185.

Manna, I., Jana, K., Samanta, P.K., (2003): Effect of intensive exercise-induced testicular gametogenic and steroidogenic disorders in mature male Wistar strain rats: a correlative approach to oxidative stress. Acta Physiol. Scand. 178, 33–40.

Mantyh, P.W., Rogers, S.D., Allen, C.J., Catton, M.D., Ghilardi, J.R., Levin, L.A., Maggio, J.E., Vigna, S.R., (1995): Beta 2-adrenergic receptors are expressed by glia in vivo in the normal and injured central nervous system in the rat, rabbit, and human. J. Neurosci. 15, 152–164.

McEwen, B.S., (2007): Physiology and neurobiology of stress and adaptation: central role of the brain. Physiol. Rev. 87, 873–904.

Miech, R.P., (2005):. Pathophysiology of mifepristone-induced septic shock due to Clostridium sordellii. Ann. Pharmacother. 39, 1483–1488.

Miller, A.H., Maletic, V., Raison, C.L., (2009): Inflammation and its discontents: the role of cytokines in the pathophysiology of major depression. Biol. Psychiatry 65, 732–741.

Miller, G.E., Cohen, S., Ritchey, A.K., (2002) Chronic psychological stress and the regulation of pro-inflammatory cytokines: a glucocorticoid-resistance model. Health Psychol. 21, 531.

Mingoti, G.Z., Pereira, R.N., Monteiro, C.M.R., (2003): Fertility of male adult rats submitted to forced swimming stress. Braz. J. Med. Biol. Res. 36, 677–682.

Mohamad, N.-V., Wong, S.K., Wan Hasan, W.N., Jolly, J.J., Nur-Farhana, M.F., Ima-Nirwana, S., Chin, K.-Y., (2019): The relationship between circulating testosterone and inflammatory cytokines in men. Aging Male 22, 129–140.

Monder, C., Sakai, R.R., Miroff, Y., Blanchard, D.C., Blanchard, R.J., (1994): Reciprocal changes in plasma corticosterone and testosterone in stressed male rats maintained in a visible burrow system: evidence for a mediating role of testicular 11 beta-hydroxysteroid dehydrogenase. Endocrinology 134, 1193–1198.

Nirupama, M., Yajurvedi, H., (2015): Efficacy of Ashwagandha (Withania somnifera L.) root extracts in preventing stress induced testicular damage in rat. Eur. J. Biomed. Pharm. Sci. 2, 413–424.

Oh, M.M., Kim, J.W., Jin, M.H., Kim, J.J., Moon, D.G., (2012): Influence of paradoxical sleep deprivation and sleep recovery on testosterone level in rats of different ages. Asian J. Androl. 14, 330–334.

Olayaki, L.A., Sulaiman, S.O., Anoba, N.B., (2015): Vitamin C prevents sleep deprivation-induced elevation in cortisol and lipid peroxidation in the rat plasma. Niger. J. Physiol. Sci. 30, 5–9.

Padro, C.J., Sanders, V.M., (2014): Neuroendocrine regulation of inflammation, in: Seminars in Immunology. Elsevier, pp. 357–368.

Rajaratnam, S.M., Arendt, J., (2001): Health in a 24-h society. The Lancet 358, 999–1005.

Research, I. for L.A., (1996): National Research Council: Guide for the Care and Use of Laboratory Animals. National Academy Press Washington DC.

Román, V., Hagewoud, R., Luiten, P.G., Meerlo, P., (2006): Differential effects of chronic partial sleep deprivation and stress on serotonin-1A and muscarinic acetylcholine receptor sensitivity. J. Sleep Res. 15, 386–394.

Silva, R.H., Abilio, V.C., Takatsu, A.L., Kameda, S.R., Grassl, C., Chehin, A.B., Medrano, W.A., Calzavara, M.B., Registro, S., Andersen, M.L., (2004): Role of hippocampal oxidative stress in memory deficits induced by sleep deprivation in mice. Neuropharmacology 46, 895–903.

Swami, C.G., Jeganathan Ramanathan, C., (2007): Noise exposure effect on testicular histology, morphology and on male steroidogenic hormone. Malays. J. Med. Sci. MJMS 14, 28.

Theron, A.J., Steel, H.C., Tintinger, G.R., Feldman, C., Anderson, R., (2013): Can the anti-inflammatory activities of β2-agonists be harnessed in the clinical setting? Drug Des. Devel. Ther. 7, 1387.

Tufik, S., Andersen, M.L., Bittencourt, L.R., Mello, M.T. de, (2009): Paradoxical sleep deprivation: neurochemical, hormonal and behavioral alterations. Evidence from 30 years of research. An. Acad. Bras. Ciênc. 81, 521–538.

Villafuerte, G., Miguel-Puga, A., Murillo Rodríguez, E., Machado, S., Manjarrez, E., Arias-Carrión, O., (2015): Sleep deprivation and oxidative stress in animal models: a systematic review. Oxid. Med. Cell. Longev. 2015.

Whirledge, S., Cidlowski, J.A., (2010): Glucocorticoids, stress, and fertility. Minerva Endocrinol. 35, 109.

Wittert, G., (2014): The relationship between sleep disorders and testosterone in men. Asian J. Androl. 16, 262.

Wolkow, A., Aisbett, B., Reynolds, J., Ferguson, S.A., Main, L.C., (2015): Relationships between inflammatory cytokine and cortisol responses in firefighters exposed to simulated wildfire suppression work and sleep restriction. Physiol. Rep. 3, e12604.

Yang, C.-Y., Lin, M.-T., (2002): Oxidative stress in rats with heatstroke-induced cerebral ischemia. Stroke 33, 790–794.

Yaribeygi, H., Panahi, Y., Sahraei, H., Johnston, T.P., Sahebkar, A., (2017): The impact of stress on body function: A review. EXCLI J. 16, 1057.

Yazawa, H., Sasagawa, I., Nakada, T., (2000): Apoptosis of testicular germ cells induced by exogenous glucocorticoid in rats. Hum. Reprod. 15, 1917–1920.

Yoga, R., Theis, J.C., Walton, M., Sutherland, W., (2009): Interleukin-6 as an early marker for fat embolism. J. Orthop. Surg. 4, 18.

Zelena, D., Makara, B.G., (2015): Steroids: The physiologic and pharmacologic effects of glucocorticoids. Orv. Hetil. 156, 1415–1425.