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Effects of vitamin E and melatonin on serum testosterone level in sleep deprived Wistar rats

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Abstract

Background: Sleep deprivation affects a significant proportion of the global population. It has been reported to induce oxidative stress in the testes and reduce serum testosterone levels. Exogenous anti-oxidants have been known to prevent damages and diseases associated with oxidative stress but there is dearth of knowledge on their effectiveness during sleep deprivation. *Aim:* This study was designed to investigate the effects of two anti-oxidants; melatonin and vitamin E on serum testosterone concentration in sleep deprived male Wistar rats.

Methods: Thirty (30) male Wistar rats were used for this study. Animals were divided into six (6) groups (n=5). Group 1 was the control, group 2 rats were sleep deprived, group 3 received vitamin E (200mg/kg bwt) only, group 4 rats received vitamin E and were sleep deprived, group 5 received melatonin only (10mg/kg bwt), and group 6 rats received melatonin (10mg/kg bwt) and were sleep deprived. Sleep deprivation was induced using the modified multiple platform technique. Body weights were taken on days 7, 14 and 21. Blood was collected at sacrifice and serum was obtained for analyses of testosterone, corticosterone and melatonin. Testicular malondialdehyde, superoxide dismutase and catalase levels were determined by the methods of Adam-Vizi and Seregi (1982), Misra and Fridovich (1972), and Sinha, (1972) respectively. Data obtained were analyzed using one way ANOVA and $p<0.05$ was considered significant.

Results: Serum testosterone (nmol/l) of the sleep deprived animals (0.6±0.3) reduced significantly ($p<0.05$) compared with control group (3.3±0.04), sleep deprived+vitamin E group (2.8±0.5) and sleep deprived+melatonin group (2.0±0.3). Also, melatonin+sleep deprived group had reduced testosterone compared with control. There were no significant changes in the serum corticosterone (nmol/l) and melatonin levels in all the groups compared with the sleep deprived group. However, corticosterone was increased in the sleep deprived+vitamin E group (51.6±20.5) compared with control (6.3±0.6) Sleep deprived group had increased testicular malondialdehyde (MDA) (1.6±0.1unit/mg), superoxide dismutase (SOD) (3.2±0.2unit/mg), and catalasel evels(44.3±1.1unit/mg) compared with control (0.9±0.0μ/mg). MDA, and catalase were significantly reduced in sleep deprived+vitamin E (1.1±0.2, 2.4±0.3, 39±1.0unit/mg)compared with sleep deprived while melatonin alone had increased MDA level (1.7±0.2unit/mg) compared with control. SOD in the sleep deprived+melatonin group (2.7±0.2μ/mg) as compared with control increased ($p<0.05$) while MDA and catalase levels as compared with control and sleep deprived groups showed no difference. Histological findings showed that the pathology in the testes of sleep deprived rats was ameliorated by vitamin E.

Conclusion: Vitamin E had a more potent effect than melatonin in maintaining testosterone level in sleep deprived Wistar rat.

Keywords: *Sleep deprivation, testosterone, oxidative stress, melatonin, malondealdehyde*

Résumé

Contexte: La privation de sommeil affecte une proportion importante de la population Global. Il a été rapporté pour induire un stress oxydatif dans les testicules et de réduire les niveaux du sérum testostérone. Les anti-oxydantes exogènes ont été connus pour prévenir les dégâts et les maladies associées au stress oxydatif, mais il ya une insuffisance de connaissances sur leur efficacité lors de la privation de sommeil.

Objectif: Cette étude a été conçue pour étudier les effets de deux anti-oxydantes; la mélatonine et la vitamine E sur la concentration du sérum testostérone chez les rats Wistar mâles privés de sommeil.

Méthodes: Trente (30) rats Wistar mâles ont été utilisés pour cette étude. Les animaux ont été divisés en six (6) groupes (n = 5). Groupe 1 était le contrôle, les rats du groupe 2 ont été privés de sommeil, le

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groupe 3 a reçu la vitamine E (200 mg / kg de poids corporel) seulement, groupe 4 les rats ont reçu la vitamine E et ont été privés de sommeil, groupe 5 a seulement reçu la mélatonine (10 mg / kg de poids corporel), et les rats de groupe 6 ont reçu de la mélatonine (10 mg / kg de poids corporel) et ont été privés de sommeil. La privation de sommeil a été induite en utilisant la technique à multiple plate-forme modifiée. Les poids corporels ont été prélevés aux jours 7, 14 et 21. Le sang a été recueilli au moment du sacrifice et le sérum a été obtenu pour les analyses de la testostérone, la corticostérone et la mélatonine. La malondealdehyde testiculaire, la super- oxyde dismutase et la catalase ont été déterminées par les méthodes d'Adam-Vizi et Seregi (1982), Misra et Fridovich (1972), et Sinha, (1972) respectivement. Les données obtenues ont été analysées en utilisant ANOVA à un facteur et $p < 0,05$ était considérée comme significative.

Résultats: Le sérum testostérone (nmol / L) des animaux privés de sommeil (0,6 ± 0,3) réduit de façon significative ($p < 0,05$) par rapport au groupe témoin (3,3 ± 0,04), groupe de manque de sommeil + vitamine E (2,8 ± 0,5) et groupe privé de sommeil + mélatonine (2,0 ± 0,3). En outre, le groupe, mélatonine + privé de sommeil avait réduit de testostérone par rapport au témoin. Il n'y avait pas de changements significatifs dans le sérum corticostérone (nmol / l) et les niveaux de mélatonine dans tous les groupes par rapport au groupe de manque de sommeil. Cependant, la corticostérone a été augmenté dans le groupe privé de sommeil + vitamine E (51,6 ± 20,5) par rapport au témoin (6,3 ± 0,6). Le groupe privé de sommeil avait la malondealdehyde testiculaire (MDA) (1,6 ± 0.1µ / mg), le super-oxyde dismutase (SOD) (3,2 ± 0.2µ / mg), les niveaux de catalase (44,3 ± 1.1µ / mg) augmentées par rapport au témoin (0,9 ± 0.0µ / mg). MDA, SOD et la catalase avaient significativement réduit dans le group privé de sommeil + vitamine E (1,1 ± 0,2 ; 2,4 ± 0,3 ; 39 ± 1.0µ / mg) tandis que seulement la mélatonine avait un niveau augmenté en MDA (1,7 ± 0.2µ / mg) par rapport au contrôle. SOD dans le groupe privé de sommeil + mélatonine (2,7 ± 0.2µ / mg) par rapport au contrôle a augmenté ($p < 0,05$), tandis que les niveaux de MDA et de catalase par rapport aux groupes de contrôle et privés de sommeil n'ont pas montré de différence. Les résultats histologiques ont montré que la pathologie des testicules des rats privés de sommeil a été améliorée par la vitamine E.

Conclusion: La vitamine E a eu un effet plus puissant que la mélatonine dans le maintien du niveau de testostérone dans le rat Wistar privé de sommeil.

Mots-clés: *privation de sommeil, testostérone, stress oxydatif, l mélatonine, malondealdehyde*

Introduction

The current global population works and lives under a 24/7 lifestyle that increases physical activities and imposes less sleep [1]. Sleep is a restorative process that conserves energy, detoxifies brain, and controls thermoregulation [2]. Since sleep is so essential to the human body, scientists recommend approximately eight hours of sleep a day to promote efficient performance [3].

It is estimated that about 20% of adults experience sleep deprivation globally and the last two decades have recorded significant decreases in duration of sleep [4]. This is a public health concern as sleep duration less than 7 hours/day has led to increased risks of obesity, morbidity and mortality [5]. Among the several negative effects caused by sleep deprivation on the body is the reduction in circulating testosterone in healthy men [6,7]. This is imperative since decreased testosterone level could impair gonadal and sexual functions, potentially resulting in decreased fertility and sexual inadequacy [8].

Increasing experimental evidence has indicated that sleep deprivation causes oxidative damage in some vital organs such as heart, brain, kidney etc [9-11]. One possible mechanism is that glucocorticoids, which are known to enhance ROS level [12,13] are increased during sleep deprivation [14]. Oxidative stress causes damage to almost all macromolecules of the cell, including polyunsaturated fatty acids which are major contents of testicular membranes [15]. This puts leydig cells, sertoli cells and sperm cells at high risk of being destroyed. Oxidative stress occurs as a result of imbalance between Reactive Oxygen Species (ROS) and endogenous antioxidants such as superoxide dismutase, glutathione peroxidase, glutathione reductase, vitamins (A, E, C), copper, zinc, selenium etc. found in body tissues [16].

Melatonin, an endogenous hormone secreted by the pineal gland has receptors located in the testes [17] and plays critical role in the reproductive functions of several seasonally breeding mammals [18]. It has been reported to play a role in free radical scavenging, upregulation of antioxidant and downregulation of prooxidant enzymes, improvement of mitochondrial metabolism and potentiation of the effects of other antioxidants [19] although it is also a conditional pro-oxidant [20]. There have been reports that reinforcement of the antioxidant system with exogenous melatonin during sleep deprivation protected the brain against ROS [21,22] and also generated effective resistance against oxidative testicular damage [23,24]

Aside from exogenous melatonin that has proven effective, another potent antioxidant which often times has been used by researchers to reinforce the testicular antioxidant system is vitamin E.

Vitamin E prevents lipid peroxide production, confers a major protective role against oxidative stress induced tissue damage [25] and plays a vital role in protecting Leydig cells steroidogenesis.

The above evidence has demonstrated that testicular redox status during sleep deprivation may be compromised and the effects of these exogenous antioxidant against testicular oxidative stress during sleep deprivation have not been reported.Thus, in this present study,the effects of exogenous melatonin and vitamin E on testicular redox status and testosterone concentration in sleep deprived male Wistar rats were investigated.

Materials and methods

Animals

All procedures used in this study conformed to the guideline of the care and use of animals in research and teaching [26] and were approved by the departmental Committee on Use and Care of Animals. Adult male Wistar rats weighing 150-200g were purchased from the Central Animal House, College of Medicine, University of Ibadan. The animals were allowed to acclimatize for two weeks before the commencement of the study.They were given pelletized feeds (Ladokun Feeds Limited) and water *ad libitum.*

Melatonin

Melatonin (Walgreens Chemical, USA) was administered at a dose of 10mg/kg body weight daily. Since the studied animals are known to secrete endogenous melatonin during the night, the exogenous melatonin was also administered at early night [27].

Vitamin E

Vitamin E (Sinopharm Xingsha Pharmaceuticals, China) was administered at a dose of 200mg/kg body weight daily.

Sleep deprivation chamber design and protocol

The modified multiple platform technique was used [28]. It consists of a glass chamber containing 16 multiple circular platforms of about 6.5cm in diameter with water filled up to 1cm of the upper surface. Animals were placed on top of the narrow platform where they freely jumped from one platform to another.The loss of muscle tone associated with the onset of sleep resulted in the animals falling into the water and awakening. The control chamber was designed in a similar way but had a glass barrier which was made to rest on top of the multiple circular platforms so the animals had free access to sleep. The feeders and the drinkers were attached to the grid which formed the cover of the chamber.

Experimental design

Thirty (30) male rats (150-200g) were randomly divided into six (6) groups and treated orally for 21 days as described below:

Group 1 rats received 1 ml of distilled water and served as control

Group 2 rats were sleep deprived and received 1 ml of distilled water

Group 3 rats received 200mg/kg body weight vitamin E only

Group 4 rats were sleep deprived and received 200mg/kg body weight vitamin E

Group 5 rats received 10mg/kg body weight melatonin only

Group 6 rats were sleep deprived and received 10mg/kg body weight melatonin

Both the control and experimental animals were placed in the chamber to acclimate to the chamber for about 4 hours every day (10:00– 14:00hrs) of the last 3 days of acclimatization. After the acclimation period, animals were placed in the chamber daily at 14:00hr and removed from the chamber at 10:00hr the next day. The animals were allowed to sleep in home cage for the remaining four (4) hours in the day. This particular time interval (10:00- 14:00hrs) was chosen because it is when paradoxical sleep is at its greatest incidence, thus, this created partial compensation for sleep loss [22]. Vitamin E and melatonin were administered as described earlier.

Sacrifice and specimen collection

At the end of 21 days of experiment, blood was collected from each animal through retro-orbital sinus with 70µl heparinized capillary tube [29] into plain serum bottle. Animals were thereafter sacrificed by cervical dislocation.The animals were dissected and the testes were harvested, cleared of adherent tissues. Testicular weight was immediately determined.The blood sample was centrifuged at 3000 rpm for fifteen minutes. Serum was decanted and serum hormone level was measured.

Measurement of serum hormone level

ELISA kits were used to determine serum levels of testosterone (Rapid Labs, UK), corticosterone (Cloud Clone, USA) and melatonin (Cloud Clone, USA). The procedure for assay were followed as written in the manual.

Determination of redox status

The harvested testes were homogenized in phosphate buffer (pH=7.4) and the homogenate was centrifuged at 10,000g x 15 minutes at 4°C. The supernatant was collected for biochemical assays

Assessment of malondealdehyde (MDA) reaction
Lipid peroxidation was determined by measuring the thiobarbituric acid reactive substances (TBARS) produced during lipid peroxidation. This method is based on the reaction between 2-thiobarbituric acid (TBA) and malondialdehyde, an end product of lipid peroxide during peroxidation. On heating in acidic pH, the product is a pink complex which absorbs maximally at 532nm and which is extractable into organic solvents such as butanol. The MDA level was calculated according to the method of Adam-Vizi and Seregi [30]. Lipid peroxidation in units/mg protein was computed with a molar extinction coefficient of 1.56×10^5 $M^{-1}Cm^{-1}$.

Determination of superoxide dismutase (SOD) activity
Determination of testicular superoxide dismutase (SOD) activity was done by measuring the inhibition of autooxidation of epinephrine at pH 10.2 according to the method of Misra and Fridovich [31]

Procedure
The reaction was started by adding 20µL of epinephrine (30mM) and the activity was measured at 480 nm for 4 min. An aliquot of 0.2ml of the diluted testes homogenate sample was added to 2.5ml of 0.05M carbonate buffer (pH 10.2). The reaction was started by the addition of 0.3ml of freshly prepared 0.3mM adrenaline to the mixture which was quickly mixed by inversion. The activity was measured at 480nm every 30 seconds for 150 second.The reference cuvette contained 2.5ml buffer, 0.3ml of substrate (adrenaline) and 0.2ml of water. 1 unit of SOD activity was given as the amount of SOD necessary to cause 50% inhibition of the oxidation of adrenaline and the results were expressed as U mg^{-1} protein

Determination of catalase activity
Catalase activity was determined according to the method of Sinha *et al.*, 1971[32].This method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as an unstable intermediate. The chromic acetate then produced is measured at 570nm.

Procedure
0.1 ml of 5% testicular homogenate was incubated with 0.5 ml of H_2O_2 (0.2 M) at 37°C for 90 sec in the presence of 0.01 M phosphate buffer (pH 7.4). Reaction was stopped by adding 5% dichromate solution. Further, samples were incubated at 100°C for 15 min in boiling water bath. Amount of H_2O_2 consumed was determined by recording absorbance at 570 nm.

Histological studies
The testis was fixed in 10% formalin, dehydrated stepwise in graded ethanol, cleared in xylene and then embedded in paraffin wax. A section of 5µm thickness paraffin section was taken from the mid portion of each testicular tissue and stained with Hematoxylin and Eosin stain followed by examination under a light microscope at X400 magnification

Statistical analysis
Data were expressed as mean ± SEM. Means were compared using one way Analysis of Variance (ANOVA) followed by least significant difference. $P<0.05$ was considered significant.

Results

Effects of vitamin E and melatonin on body weight and relative organ weight
Control, vitamin E and melatonin groups had increase in weight throughout the experiment while sleep deprived, sleep deprived+vitamin E and sleep deprived+melatonin gained weight during the second week but lost weight during the first and third week (Figure 1a). Sleep deprived+melatonin group significantly lost more weight than the sleep deprived+vitamin E and sleep deprived groups (Figure1a). Relative testicular weight was increased in the sleep deprived+melatonin group compared with all other experimental groups. Sleep deprived+vitamin E rats also had increased relative testicular weight compared with sleep deprived group.

Effects of vitamin E and melatonin on serum testosterone, corticosterone and melatonin concentration
Testosterone level was significantly reduced in the sleep deprived compared with all other groups.Sleep deprived+melatonin showed decreased testosterone compared with control.Concentrations of corticosterone and melatonin were comparable in the control and sleep deprived groups. However, corticosterone level was higher ($p<0.05$) in the sleep deprived+vitamin E group compared with control (Table 1).

Effects of vitamin E and melatonin on testicular redox status
Testicular malondialdehyde was increased in sleep deprived and melatonin only groups compared with

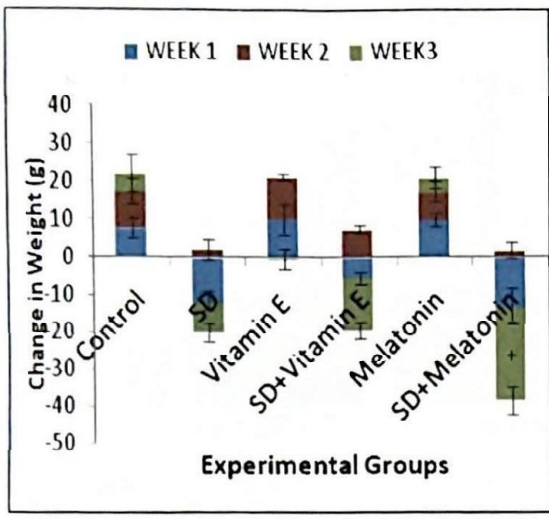

Fig. 1a. Effects of vitamin E and melatonin on body weight of sleep deprived Wistar rats Column represent mean + SEM; n=5.
*P<0.05 when compared with control,
+P<0.05 when compared with sleep deprived.
SD= Sleep deprived.

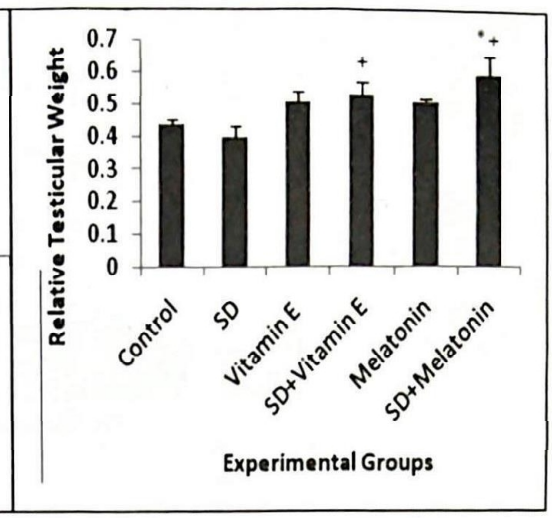

Fig. 1b. Effects of vitamin E and melatonin on relative testicular weights of sleep deprived Wistar rats. Columns represent mean±SEM; n=5
*P<0.05 when compared with control,
+P<0.05 when compared with sleep deprived.
SD = Sleep deprived.

Table 1: Effects of vitamin E and melatonin on testosterone, corticosterone and melatonin levels in sleep deprived wistar rats.

	Control	Sleep Deprived	Vitamin E	Sleep Deprived+ Vitamin E	Melatonin	Sleep Deprived+ Melatonin
Testosterone (nmol/ml)	3.32±0.4	0.64±0.3*	3.9±0.6^{+}	2.8±0.5^{+}	3.6±0.6^{+}	2.0±0.3*$^{+}$
Melatonin (nmol/l)	19.3±5.6	15.5±7.0	29±3.9	14.7±7	8.6±1.9	28.3±12.4
Corticosterone (nmol/l)	6.3±0.6	24.1±13.2	16.5±7.2	51.6±0.5*	36.6±29.2	10.3±6.6

Values expressed as mean±SEM; n=5
**P<0.05 when compared with control, +P<0.05 when compared with sleep deprived*
SD = Sleep deprived

Table2: Effects of vitamin E and melatonin on testicular redox Status in sleep deprived wistar rats

Redox Marker Groups	Malodialdehyde (unit/mg protein)	Superoxide Dismutase	Catalase (unit/mg protein)
Control	0.9 ± 0.0	1.9 ± 0.1	44.3±1.1
Sleep deprived	1.6 ± 0.1*	3.2± 0.2*	49.1 ± 1.1*
Vitamin E	1.1 ± 0.1^{+}	2.2 ± 0.2^{+}	44.1 ± 1.7^{+}
Sleep deprived + Vitamin E	1.1 ± 0.2^{+}	2.4 ± 0.3^{+}	39.4 ± 1.0 *$^{+}$
Melatonin	1.7 ± 0.2*	3.5± 0.4*	46.2±1.6
Sleep deprived + Melatonin	1.3 ± 0.1	2.7 ± 0.2*	45.5 ± 1.8

Values expressed in mean±SEM; n=5
**P<0.05 when compared with control, +P<0.05 when compared with sleep deprived*
SD = Sleep deprived

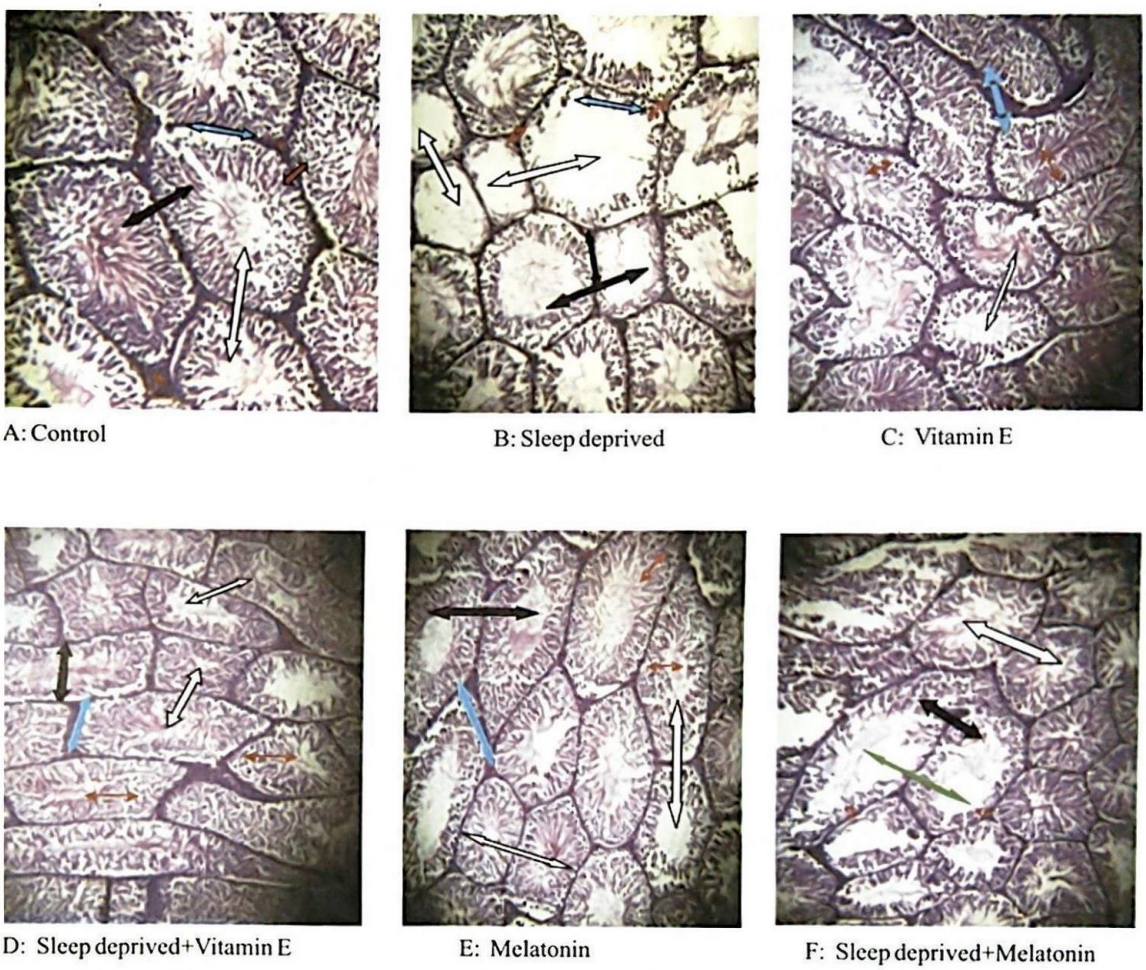

Fig. 2: Effects of Vitamin E and melatonin on testicular histology in sleep deprived wistar rats.
Mag: X400. H&E Staining technique

A:Photomicrograph of a testicular section of control rat showing normal seminiferous tubules (black arrow). The germinal epithelium appears normal (red arrow). There are normal lumen with strands of spermatozoa (white arrow). The interstitial cells appear normal (blue arrow).
B: Photomicrograph of a testicular section of sleep deprived rat showing seminiferous tubules with maturation arrest (black arrow). The height of germinal epithelium is reduced (red arrow). The lumen is wide and has few spermatozoa (white arrow).
C: Photomicrograph of a testicular section of vitamin E treated rats showing normal seminiferous tubules (black arrow). The germinal epithelium appears normal (red arrow). The lumen has strands of spermatozoa (white arrow). The interstitial cells appear normal (blue arrow).
D:Photomicrograph of a testicular section of vitamin E treated sleep deprived rats showing normal seminiferous tubules (black arrow). The germinal epithelium is intact (red arrow). The lumen appears normal with strands of spermatozoa (white arrow). The interstitial cells appear normal (blue arrow).
E: Photomicrograph of a testicular section of melatonin treated rats showing normal seminiferous tubules (black arrow). The germinal epithelium appears normal (red arrow). There are normal lumen with strands of spermatozoa (white arrow). The interstitial cells appear normal (blue arrow).
F: Photomicrograph of a testicular section of melatonin treated sleep deprived rat showing some normal seminiferous tubules (white arrow) and some tubules with maturation arrest (green arrow). The height of germinal epithelium is reduced in some of the seminiferous tubules (red arrow) and lumen is wide and has few spermatozoa (slender arrow) in some of the seminiferous tubules.

control. It was reduced in vitamin E only and increased in sleep deprived+vitamin E rats compared with sleep deprived rats. Superoxide dismutase was higher in the testes of sleep deprived, melatonin only

and sleep deprived+melatonin as compared with control and was reduced in vitamin E only and sleep deprived+vitamin E in comparison with sleep deprived group rats (Table 2). Catalase was increased in sleep deprived and reduced in sleep deprived+vitamin E compared with control. Vitamin E only and sleep deprived+vitamin E groups showed reduced testicular catalase concentration compared with sleep deprived group (Table 2).

Discussion

The present study demonstrated the effects of two anti-oxidants; vitamin E and melatonin on serum testosterone concentrations in sleep deprived Wistar rats. Several studies have reported a reduction in serum concentration of testosterone after sleep deprivation [6,7,33-36] and similar result was obtained in this study. Testosterone reduction in the sleep deprived group was associated with loss of body weight without affecting the relative testicular weight. The unaffected relative testicular weight after body weight loss suggests that absolute testicular weight was decreased along with the loss of fat. Ordinarily, a loss in the body weight of an animal would give rise to an increased relative organ weight provided there is no organ weight loss [37,38] as observed in the sleep deprived animals that also had vitamin E or melatonin. This implies that melatonin and vitamin E protected the testes from organ weight loss but not body weight loss caused by sleep deprivation. This was corroborated by testicular histology in which the pathologies present in the sleep deprived testes may be regarded as being severe but were considerably reduced to moderate in the sleep deprived+melatonin and mild in the sleep deprived +vitamin E. The weight loss observed in the sleep deprived group may have resulted from raised metabolic rate [39] and increased energy expenditure [40] and the reduction in organ weight may be attributed to decrease in circulating gonadotropin caused by sleep deprivation as been reported that sleep deprivation lead to reduction in circulating gonadotropin level [41].

Coupled with the ameliorative effect of vitamin E and melatonin on organ weight, testosterone level was restored to control value by vitamin E, albeit, administration of melatonin raised the level of testosterone significantly above the level in the sleep deprived group but not comparable with control value. The reduced testosterone in the sleep deprived also caused the arrest of spermatogenesis while the sleep deprived that took vitamin E or melatonin had evidence of spermatogenesis.

In males, one of the first fallouts of stress is reduced testosterone [42,43]. An explanation for this is that stress response involves an increase in serum glucocorticoid levels [43] and this is probably due to inhibition of hypothalamic–pituitary-gonadal axis by the activation of the hypothalamic-pituitary-adrenal axis [44,45]. However, the results of this study showed that stress response in the sleep deprived animals was comparable with that of the controls and more so, the sleep deprived animals that had vitamin E had increased corticosterone but normal levels of testosterone. This suggests that corticosterone may not be a factor involved in the reduction of testosterone levels caused by sleep deprivation in this study. It should be noted that the animals' responses to sleep deprivation with respect to corticosterone were really different as some animals exhibited higher level of corticosterone than others even within the same group.

The increase in testicular malondialdehyde (MDA) level in the sleep deprived group indicated that there was increased lipid peroxidation in the testes of the sleep deprived group. This report is consistent with the report of other studies that showed increased MDA after sleep deprivation in some organs [46,47]. Increased Oxidative stress decreases cell viability and destroys tissues. Testicular tissue contains poly unsaturated fatty acids that makes it highly susceptible to oxidative stress [48].This may be the cause of the pathologies seen in the histology of the testes of the sleep deprived rats. One possible explanation is that testosterone reduction was caused by the reduction in the viability of Leydig cells which are the sites of steroidogenesis.

The increase in reactive oxygen specie level in testicular tissue has been reported to reduce testosterone levels [48]. MDA levels in the sleep deprived+Vitamin E and sleep deprived+melatonin were comparable to that of control, thus, suggesting that vitamin E and melatonin were effective in ameliorating the effects of sleep deprivation on lipid peroxidation. However, why melatonin alone increased lipid peroxidation in control animals is not clear, although, melatonin has been termed a conditional pro-oxidant [20]. Since melatonin was able to reverse MDA level but not testosterone level to control value, it implies that other than lipid peroxidation, there may be other factors contributing to the reduction in testosterone level. This was also corroborated by histology results as the pathologies seen in the sleep deprived group have been ameliorated in the sleep deprived+Vitamin E and sleep deprived+melatonin, albeit , vitamin E

consistently showed more potent effect than melatonin. This also suggests that vitamin E may have more antioxidative influence than that of melatonin in the testes.

Along with the increased MDA, it was expected that anti-oxidant level in the testes would be reduced. However, superoxide dismutase and catalase levels in the sleep deprived rats were not reduced but were comparable with that of control. This was contrary to the report by Everson *et al.* [11] who observed reduction in catalase activity following five (5) days of total sleep deprivation. Catalase is known as a cellular antioxidant defense that functions to remove hydrogen peroxide. The observed increase in the levels of SOD and catalase showed that the antioxidants were available but it seemed that the reaction between the anti-oxidant and reactive oxygen species in the tissues was being inhibited or increased antioxidant was just tissue response to increased reactive oxygen species.

Results from the study suggests that oxidative stress is a major mechanism involved in the testosterone reduction during sleep deprivation in Wistar rats. Vitamin E demonstrated a more potent effect than melatonin in restoring testosterone concentration to control values. Further studies are required to elucidate the full role of melatonin and vitamin E and to explore other mechanisms affecting testosterone level during sleep deprivation.

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