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INDUCTION OF OXIDATIVE STRESS BY PARADOXICAL SLEEP DEPRIVATION IN DIFFERENT BODY ORGANS OF RATS

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ABSTRACT

Sleep deprivation is thought to be a risk factor for several diseases. The aim of the present study is to investigate the effect of paradoxical sleep deprivation for 48 h on different body organs in male albino rats. The data revealed a significant increase in lipid peroxidation levels in the heart, liver, and testis of sleep-deprived animals. Reduced glutathione content recorded a significant decrease in the cardiac and hepatic tissues after 48 hours of sleep deprivation, while non significant effects were obtained in both testicular and renal tissues. The hepatic catalase activity showed a significant decrease, while the changes in cardiac, testicular and renal catalase activities were non-significant. Sleep deprivation also induced a significant decrease in cardiac and renal superoxide dismutase activity. Conversely, a non significant increase in the enzyme activity was recorded in the hepatic and testicular tissues. Regarding nitric oxide level a significant decrease was recorded in the heart. However, a significant increase was obtained in the liver and testis. The renal nitric oxide level showed nearly no change from control. We concluded that sleep deprivation for 48 h could induce oxidative stress in the cardiac, hepatic and testicular tissues of male albino rats and it has no effect on renal tissue.

KEY WORDS: **paradoxical sleep deprivation, oxidative stress, different body organs, rat**

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INTRODUCTION

Sleep is a restorative process that has important functions for every organ in the body, and plays a crucial role in the balance of psychological and physical health[1]. Sleep ameliorates oxidative stress[2] as it removes oxidants produced during waking[3]. It also involves a process of repair and detoxification[4,5]. With the advent of industrialization, sleep disorders are becoming a major public health problem, affecting millions of people all over the world[6,7]. Sleep deprivation (SD) is a serious problem among adult [8,9]. It leads to disorders that cause irreparable damage[10]. It may be associated with deleterious effects such as obesity, type 2 diabetes, hypertension and cardiovascular disease[11,12]. Biochemical and physiological studies have shown that SD has the ability to change behavioral[13], hormonal[13,14] and neurochemical pathways[15,16]. Insufficient sleep was suggested to increase the risk of stress-induced brain remodeling in the regions involved in the regulation of memory, executive functions and anxiety[17,18,19]. It has been proposed that sleep restriction may elevate energy consumption and affect metabolic activity[20,21]. The increased cellular metabolism may disrupt the maintenance of homeostasis and induce the generation of large amounts of reactive oxygen species (ROS)[22,23]. It has been suggested that ROS accumulation and consequent allostatic overload may induce oxidative stress and the subsequent lipid peroxidation responsible for the pathophysiological progression of metabolic diseases associated with sleep disorders [24,25,26]. Oxidative stress has been implicated in the mechanisms of biologic aging as well as the pathogenesis of cancer, atherosclerosis, diabetes and neurodegenerative diseases[27]. It occurs whenever there is an imbalance between oxidant production and antioxidant defenses, either the former is increased, the latter are decreased or both. At the cellular level, such imbalance can lead to structural damage due to oxidative alterations of proteins, lipids and nucleic acids[28]. Although most ROS are produced in the electron transport chain, oxidants can be generated by extra mitochondrial sources such as NADPH oxidases and nitric oxide synthase [29,27]. It was reported that all macromolecules of the cell, including polyunsaturated fatty acids of the membrane are damaged by ROS thus causing disruption of cellular function[30]. The testis is highly vulnerable to oxidative stress as the testicular membranes are rich in polyunsaturated fatty acids[31]. Liu et al.[32] suggested that SD became an independent predictor of cardiovascular disease as it was correlated with increased morbidity and mortality due to myocardial infarction and heart failure. It has recently been reported that SD-induced oxidative stress could lead to atherosclerosis and myocardial apoptosis in ischemia and reperfusion[33]. Sleep deprivation also resulted in decreases in liver glutathione and catalase without compensatory increases in other enzymatic and non enzymatic antioxidants[34]. This incomplete antioxidant defense mechanism may result in injured cells that need repair or else they die[35]. Since most studies concentrated on the investigation of the effects of long periods of SD, the present work focused on the effect of 48 hours only of sleep deprivation on oxidative stress parameters in the heart, liver, testis and kidney of male albino rats.

MATERIALS AND METHODS

Experimental animals

This study was carried out on adult male albino rats, with average weight of 200–250 g. The animals were obtained from the animal house of the National Research Center, Egypt. They were maintained on stock diet and kept under fixed conditions of housing and handling with controlled light-dark cycle (12h light/ 12h dark) and temperature conditions (25 ± 2°C). All experiments were carried out in accordance with the research protocols established by the Animal Care Committee of the National Research Center, Egypt which followed the recommendations of the National Institutes of Health Guide for Care and Use of Laboratory Animals (CUFS No 41-15).

Design of experiment

At the beginning of the experiment, 16 animals were divided into 2 groups (n = 8). The first group was subjected to paradoxical sleep deprivation for 48 h using the platform method described by Zager et al.[36] This method consists of placing a group of rats (4) in a cage containing 5 circular platforms (3 cm in diameter) with water 1 cm below the upper surface of the platform. When the animal enters the paradoxical phase of sleep, it falls into the water as a result of muscle atonia and wakes up. Food and water were available through a grid on the top of the cage. The second group that acts as a control group was subjected to the same conditions of the first group, but the platform diameter was 15 cm, which permitted the animals to sleep on it.

Handling of tissue samples

After 48 h, both the sleep-deprived rats and the control group were sacrificed. The heart, liver, testis and kidney of each animal were quickly removed and rapidly weighed and frozen until analyzed. Each tissue was homogenized in 5 ml of ice cold phosphate buffer (50 mM pH 7.4, 0.1% triton X and 0.5 mM EDTA). The homogenates were centrifuged at 1753 g for 15 min at 4°C using a high speed cooling centrifuge (Type 3K-30, Sigma, Osterode-am-Harz, Germany). The clear supernatants were separated and used for analysis.

Determination of lipid peroxidation

Lipid peroxidation (LPO) was measured by determining the level of malondialdehyde (MDA) as indicated by the thiobarbituric reactive species (TBARS) in the tissues [37]. TBARS react with thiobarbituric acid giving a red colored complex whose peak absorbance was measured at 532 nm in a Helios Alpha Thermospectronic (UVA 111615, Cambridge, England) spectrophotometer.

Determination of reduced glutathione

Reduced glutathione (GSH) was determined in the different homogenates according to Ellman's method[38]. The reduction of Ellman's reagent by –SH groups of GSH forms 2-nitro-S-mercaptobenzoic acid, which has an intense yellow color that can be determined spectrophotometrically at 412 nm.

Determination of nitric oxide level

Nitric oxide (NO) level, measured as nitrite, was determined by Griess reagent as described by Moshage et al.[39], where nitrite, a stable end product of NO, is primarily used as an indicator for the production of NO. Nitrite is converted to a deep purple azo compound after the addition of Griess reagents. The purple/magenta color developed is read spectrophotometrically at 540 nm.

Determination of enzyme activities

Superoxide dismutase (SOD) activity was assayed by using Biodiagnostic kit No. SD 25 21 (Biodiagnostics Co., Giza, Egypt). This method relies on the ability of the enzyme to inhibit the phenazine metho sulphate-mediated reduction of nitro blue tetrazolium dye [40]. The change in absorbance was measured at 560 nm over 5 min.Catalase activity (CAT) was measured using Biodiagnostic Kit No. CA 25 17 (Biodiagnostics Co., Giza, Egypt) which is based on the spectrophotometric method described by Aebi[41]. Catalase reacts with a known quantity of hydrogen peroxide and the reaction is stopped after 1 min with catalase inhibitor. The remaining hydrogen peroxide reacts with 3,5-dichloro-2-hydroxybenzene sulfonic acid and 4-aminophenazone in the presence of peroxidase to form a chromophore whose color intensity is inversely proportional to the amount of catalase in the sample. The absorbance was read at 510 nm. Glutathione reductase activity (GR) was carried out using Biodiagnostic kit No. GR 25 23 (Biodiagnostic Co., Giza, Egypt), which is based on the spectrophotometric method of Goldberg and Spooner[42]. It depends on the reduction of glutathione (GSSG) in the presence of NADPH, which is oxidized to $NADPH^+$. The decrease in absorbance is measured at 340 nm.

Statistical analysis

The data were expressed as means ± standard error of mean (SEM). Data were analyzed by student t test. All analyses were performed using the Statistical Package for the Social Sciences software in a compatible computer. The difference between means was significant at $p < 0.05$. The percentage difference was calculated to evaluate the percentage of change from the control values and to compare between the degrees of oxidative stress in the different tissues.

$$\text{Percentage difference} = \frac{\text{Mean of sleep deprived rat - Mean of control} \times 100}{\text{Mean of control}}$$

RESULTS

The present study demonstrated the effect of SD for 48 h on the oxidative stress parameters in the heart, liver, testis and kidney of male albino rats (Tables 1, 2, 3 and 4). The data revealed a significant increase in MDA levels in the heart, liver, and testis of sleep-deprived animals, recording 120.15 %, 23.63 % and 46.52 % above the control levels as indicated in Tables 1, 2 and 3, respectively. However, there was a significant decrease in MDA level (-19.61 %) in the kidney (Table 4). GSH content recorded a significant decrease in the cardiac and hepatic tissues after 48 hours of SD (Tables 1 and 2). On the other hand, non significant effects were obtained in both testicular and renal tissues (Tables 3 and 4). In addition, SD resulted in a significant increase in GR activity in the heart, liver and testis of male rats as demonstrated in Tables (1, 2 and 3). The renal GR activity showed a non significant increase (Table 4).Table (2) showed a significant decrease in hepatic catalase activity (-31.77 %) of sleep-deprived rats, while the changes in cardiac, testicular and renal catalase activities were non significant (Tables 1, 3 and 4).Sleep deprivation also induced a significant decrease in cardiac and renal SOD activity by 26.59 % and 16.65 %, respectively (Tables 1and 4). Conversely, a non significant increase in the enzyme activity was recorded in the hepatic and testicular tissues (Tables 2 and 3). The data showed a significant decrease in NO level (-19.34 %) in the heart of sleep-deprived animals (Table 1). However, a significant increase was obtained in the liver and testis recording 15.73 % and 40.26 % above the control levels, respectively (Tables 2 and 3). The renal NO level showed nearly no change from control (Table 4).

Table 1
Effect of Sleep deprivation on the oxidative stress parameters in the cardiac tissue of male albino rats.

Parameters	Groups		% Changes
	Control	SD group	
MDA (nmol/g tissue)	3.17± 0.15	6.97± 0.21 *	120.15%
GSH (mg/g tissue)	1.48± 0.04	1.31± 0.05 *	-11.80%
GR (U/L)	11.35± 0.70	15.47± 1.49 *	36.33%
CAT (U/g tissue)	0.77± 0.03	0.68± 0.05 -	-11.86%
SOD (U/g tissue)	367.46± 12.79	269.77± 11.28 *	-26.59%
NO (µ mole/g tissue)	0.49± 0.02	0.39± 0.03 *	-19.34%

Data are expressed as Mean ± S.E, number of animals in each group is six, - p> 0.05 non significant, * p < 0.05Significant. % changes relative to the control

Table 2
Effect of sleep deprivation on the oxidative stress parameters in the hepatic tissue of male albino rats.

Parameters	Groups		% Changes
	Control	SD group	
MDA (nmol/g tissue)	4.18± 0.24	5.16± 0.38 *	23.63%
GSH (mg/g tissue)	1.30± 0.10	1.01± 0.02 *	-22.81%
GR (U/L)	26.80± 3.23	39.31± 3.38 *	46.69%
CAT(U/g tissue)	0.30± 0.03	0.20± 0.02 *	-31.77%
SOD(U/g tissue)	156.69± 9.89	178.31± 13.95 -	13.80%
NO(µ mole/ g tissue)	1.26± 0.06	1.46± 0.05 *	15.73%

Data are expressed as Mean ± S.E, number of animals in each group is six, - p> 0.05 non significant, * p < 0.05 Significant. % changes relative to the control.

Table 3
Effect of sleep deprivation on the oxidative stress parameters in the testicular tissue of male albino rats.

Parameters	Groups		% Changes
	Control	SD group	
MDA (nmol/g tissue)	1.48± 0.06	2.17± 0.17 *	46.52%
GSH (mg/g tissue)	1.99± 0.29	1.62± 0.04 -	-18.71%
GR (U/L)	23.54± 5.86	54.61± 6.73 *	132.05%
CAT(U/g tissue)	0.47± 0.04	0.43± 0.05 -	-8.51%
SOD (U/g tissue)	40.31± 5.34	58.01± 7.28 -	43.92%
NO (µ mole/ g tissue)	0.92± 0.09	1.29± 0.09 *	40.26%

Data are expressed as Mean ± S.E, number of animals in each group is six, - p> 0.05 non significant, * p < 0.05 Significant. % changes relative to the control

Table 4
Effect of sleep deprivation on the oxidative stress parameters in the renal tissue of male albino rats.

Parameters	Groups		% Changes
	Control	SD group	
MDA (nmol/g tissue)	8.24± 0.30	6.62± 0.18 *	-19.61%
GSH (mg/g tissue)	2.26± 0.06	2.11± 0.06 -	-6.65%
GR (U/L)	67.68± 1.80	73.06± 4.05 -	7.96%
CAT(U/g tissue)	0.72± 0.03	0.78± 0.04 -	7.74%
SOD (U/g tissue)	283.10± 12.67	236.71± 10.62 *	-16.65%
NO (µ mole/ g tissue)	2.01± 0.03	2.06± 0.05 -	2.29%

Data are expressed as Mean ± S.E, number of animals in each group is six, - p> 0.05 non significant, * p < 0.05 Significant. % changes relative to the control

DISCUSSION

Sleep deprivation is thought to be a risk factor for several diseases such as hypertension[43], coronary heart disease[44] and diabetes[45]. It also produces a moderate multi organ damage that occurs through oxidative stress and inflammation[1]. The present study revealed that 48 h of SD induced oxidative stress in the heart, liver, and testis of male albino rats as indicated from the significant increase in MDA levels (the product of lipid peroxidation). This was accompanied by a decrease in

GSH in the three organs being significant only in the heart and liver. Lipid peroxidation is an autocatalytic mechanism that leads to the oxidative damage of cellular membranes[46.] LPO damages polyunsaturated fatty acids and tends to reduce membrane fluidity which is critical for the proper functioning of the cell[30]. Thus, SD for 48 h could be considered as a risk factor for cellular damage through oxidative stress. Chang et al.[47] reported that total SD for 5 days resulted in a significant increase in hepatic MDA level and suggested that sleep deprivation may predispose the liver to considerable oxidative injury. Conversely, Gopalakrishnan et al.[28] found no evidence of oxidative damage at the lipid or protein level in peripheral tissues after short-term (8 hours) and long term (3 to 14 days) sleep deprivation. It is possible that the absence of oxidative stress in their study was due to the small number of animals used. Arjada et al.[48] stated that paradoxical SD (PSD) induced cell damage in the testis through the increase in MDA level and metabolic rate which in turn increased the generation of free radicals. The testicular membrane is extremely rich in polyunsaturated fatty acids; therefore, this organ is highly susceptible to oxidative stress[31]. The present results showed that SD for 48 hours induced a significant decrease in GSH content in both cardiac and hepatic tissues. However, a non significant decrease was recorded in the testicular tissue. In line with the present findings, Gopalakrishnan et al.[28] reported marked decreases in both liver glutathione and catalase activity in sleep-deprived animals, with no detectable changes in recycling activities, suggesting that the oxidative stress was not compensated. In addition, Everson et al.[34] found that 5 days of SD caused a decline in liver glutathione which was sustained or worsened by prolongation of SD. The main function of GSH is to remove H_2O_2 and organic peroxides so any decrease in the level of GSH indicates the increased production of free radicals[49]. Depletion of antioxidants is considered “disease-associated oxidative stress,” because reduced defenses have been correlated with increased susceptibility to disease[35]. Glutathione depletion of 20- 30% of normal can impair cellular defense against ROS, and may cause disruption in cell communication, aberrant protein degradation, and cell damage[50].The present decrease in GSH levels was accompanied by a significant increase in GR activity in the cardiac, hepatic and testicular tissues.Glutathione reductase (GR) catalyzes the reduction of glutathione disulfide (GSSG) to the sulfhydryl form GSH which is a pivotal molecule in the defense against oxidative stress. As GSH plays a fundamental role in scavenging deleterious ROS and maintenance of the protein thiol redox state, so, GR is critical to the cell's antioxidant defense mechanisms and maintenance of enzyme activities and protein functions.It may be concluded that the present reduction in GSH levels in the heart and liver may reflect the enhanced production of free radicals in both organs under the effect of 48 hours of SD. The increase in GR activity in the two organs represents a compensatory mechanism whereby GR enzyme attempts to replenish the reduced GSH levels[51]. This mechanism probably succeeds in regenerating and maintaining the total intracellular GSH in the testicular tissue which recorded the highest increase in GR activity regarding catalase activity, the present results recorded a significant decrease in catalase activity in the liver, while there were non-significant decreases in the heart and testis. Pigeolet et al.[52] suggested that SD results in the overproduction of H_2O_2. Thus, the present reduction in hepatic catalase activity may be due to the exhaustion of the enzyme in attempting to eliminate H_2O_2 generated by SD. Also, it may be due to the inactivation of the enzyme caused by the excessive ROS production in the mitochondria. Everson et al.[34] also recorded a decrease in catalase activity in the liver after 5 days of SD. The authors suggested that the antioxidant profile of the heart was different from that of the liver. They reported that, during SD, activation of the oxidative pentose phosphate pathway occurs in the heart as indicated by the significant increase in glucose 6- phosphate dehydrogenase (G6PD) and 6- phosphate glucose dehydrogenase (6PGD) and the maintenance of normal catalase activity. Also, the heart is deficient in catalase and antioxidant defense mechanisms compared to the liver[53]. Moreover, it has been shown that total SD is associated with increases in rat serum activities of aspartate transaminase (AST) and alanine transaminase (ALT)[34]. These increases have also been shown in humans deprived of sleep for 72 h[54]. The authors suggested that damaged hepatocytes release their contents, including ALT and AST, into the extracellular space; these contents ultimately reach the circulation thereby increasing the serum enzyme activities. This provides further evidence for the cellular damaging effects of SD that result from the overproduction of free radicals and the subsequent LPO as clear from the present data. On the other hand, Arjadi et al.[48] reported that in the testis, oxidative stress caused by SD induction reduces the damage of endogenous antioxidant mechanism by inhibition of G6PD and stimulation of a greater LPO reaction. This may explain the non significant changes in the antioxidant enzymes induced by SD in the testis in the present study. The present data showed a significant decrease in SOD activity in the cardiac tissue, while there were non-significant increases in SOD in both hepatic and testicular tissues. Superoxide dismutase constitutes an important basis in the biological defense mechanism through dismutation of endogenous cytotoxic superoxide radicals to H_2O_2 and O_2[55] which is then neutralized by catalase or glutathione peroxidase[56,57]. So, the decrease in SOD activity in SD-animals, in the present study, may reflect the inability of the cardiac tissue to counteract the increased free radicals resulting in the highest level of LPO among the studied organs. The non significant increases in SOD activities in the liver and testis of SD animals suggest that these organs have a more efficient antioxidant mechanism as compared to the heart and are thus able to maintain normal enzyme activities. In agreement with these findings, Gopalakrishnan et al.[28] did not detect changes in liver or muscle SOD activity in animals sleep deprived for 0 to 8 hs, or 3 to 14 days. On the other hand, the data recorded a significant decrease in MDA level in the renal tissue. This was accompanied by a significant decrease in SOD activity. This suggests that the kidney was more resistant to the effects of SD than the other organs. The decrease in SOD activity was utilized in scavenging the generated free radicals and thus LPO was reduced with no resultant oxidative stress. The present data recorded a significant increase in NO level in the hepatic and testicular tissues after 48

hours of SD. An increase in hepatic NO level was also reported by Lui et al.[1] after 24-72 h of SD. It has been postulated that NO has contradictory roles in cellular systems such as an antioxidant or sometimes a scavenger of superoxide anion[58,59]. Gulati et al.[60] reported opposite functions of NO from antioxidant at lower concentration to pro- oxidant at higher concentration which is correlated with a significant decrease in cardiac NO level. Adams et al.[61] found that inhibition of nitric oxide synthase (NOS) activity resulted in a significant enhancement of testosterone concentrations and concluded that NO exerted an inhibitory effect on testicular steroidogenesis. Human and animal studies have shown that SD is associated with reductions in circulating levels of androgens, including testosterone[62]. This was confirmed by the study of Wua et al.[63] who demonstrated that SD for 24 or 48 h caused significant decreases in serum testosterone concentrations. This is clinically important as decreased testosterone levels can disrupt gonadal and sexual functions, and eventually lead to reduced fertility[64]. Recently, Choi et al.[65] provided evidence that SD for 4 and 7 days may affect sperm quality, hormone levels, and histopathology of the testis in rats. Therefore, the increase in NO levels after 48 hours of SD may lead to suppression of gonadal steroidogenesis and hence impair reproductive functions. On the contrary, the present study revealed that SD for 48 hours resulted in a significant decrease in cardiac NO level. Liu et al.[1] postulated that myocardial oxidative stress was apparent from the increase in MDA level and decrease in NO level. It has been reported that, at high levels, NO reacts with superoxide anion to produce peroxynitrite, which is a highly toxic agent that may cause apoptosis in cardiac cells[66,67]. This may underlie the reduction in NO levels and increase in LPO levels observed in the heart of the present sleep deprived-animals. Increasing attention is directed to the concept that oxygen free radicals and nitric oxide (NO) play a major role in cardiovascular physiology and pathology[68,69]. Within the cardiovascular system, NO plays a role in the regulation of coronary blood flow and vessel wall tension[70,71]. Chronic NO-deficient hypertension and variations in the heart rate are associated with depletion of antioxidants and oxidative damage to the heart[72]. Although the underlying pathophysiological mechanisms linking sleep deprivation and cardiovascular disease have not been defined, one potential explanation was that sleep deprivation reduces the activity of antioxidant enzymes in rats and increases the markers of cell injury[34]. The present study confirms the deleterious effects of SD on the heart and provides further evidence that SD-induced oxidative stress may represent a risk factor for cardiovascular diseases.

CONCLUSION

In conclusion, the present results shed light on the antioxidative role of sleep. Sleep deprivation for 48 h could induce oxidative stress in the cardiac, hepatic and testicular tissues of male albino rats. However, it has no effect on renal tissue which may suggest that the renal tissue may be resistant to the effects of SD or may require a longer period to be affected by SD. The present data raise concerns about the adverse effects of SD on the vital functions of the major organs of the body and the possible pathological conditions that may develop from these effects.

CONFLICT OF INTEREST

Conflict of interest declared none.

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