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Full Length Research Article

Effect of Honey on Oxidative Stress in the Brain Tissue of Sleep Deprived Rats

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ABSTRACT

This study aimed to demonstrate the effect of honey on the oxidative stress in the brain of sleep-deprived rats. Twenty five 6 weeks old male wistar rats were randomly divided into five groups and subjected to paradoxical sleep deprivation and recovery for 5 days using the modified multiple platform (MMP) method. Group I: Normal control; Group II: sleep deprivation (SD); Group III: sleep deprivation and sleep recovery (SD+SR) – received 10 ml/kg distilled water orally each; while Group IV: sleep deprivation and honey (SD+Honey) and Group V: sleep deprivation, recovery with honey (SD+SR+Honey) received honey (1g/kg body weight) orally once daily. Brain tissue of the humanely sacrificed rats were excised and homogenized for assessment of oxidative stress markers. Results indicate a significant decrease ($p<0.05$) in the concentrations of malondialdehyde (MDA) in brain tissues of rats in Groups IV and V when compared with that from sleep deprived group (Group II). The Increase in reduced glutathione (GSH) concentration and catalase enzyme activity observed in homogenized brain tissue of rats in Groups III, IV, and V differ significantly ($p<0.05$) when compared with Group II. The results suggest that treatment with honey probably has ameliorative effects on oxidative stress in brain tissue of sleep deprived experimental rats.

Keywords: Sleep, Sleep deprivation, Honey, Oxidative stress

INTRODUCTION

Sleep deprivation (SD) refers to the condition of not been able to achieve sufficient and undisturbed periods of sleep because of environmental or personal reasons (Yongmei *et al.,* 2020). There are a lots of adverse effects associated with SD in humans. They include learning and memory impairment, psychological, physiological and immune dysfunctional effects (Ocalan *et al*, 2019; Besedovsky *et al*., 2019). Previous studies revealed that SD causes increased oxidative stress which eventually leads to a series of negative effects, such as cognitive impairment, altered immune status, and increased susceptibility to diseases in the cardiovascular, gastrointestinal and nervous systems (Periasamy *et al.,* 2015; Lungato *et al.,* 2016; Tobaldini *et al*., 2017). Valvassori *et al*., (2017) demonstrated that paradoxical sleep deprivation (PSD) in mice increased lipid peroxidation and oxidative damage to DNA and disrupted antioxidant enzymes in certain regions of the brain (frontal cortex, hippocampus).

Oxidative stress is a condition involving an increased rate of cellular damage induced by reactive oxygen species (ROS). ROS are reported to cause alterations in both cell membranes and constituents ending by cell mutation or damage (Yang and Linn, 2002; Juliet *et al.,* 2004). However, the cell defense mechanism against the formation of reactive oxygen species (ROS) involves production of antioxidant enzymes such as catalase (CAT), glutathione reductase (GR) and superoxide dismutase (SOD). The involvement of oxidative stress in the pathogenesis of cognitive impairment has also been reported (Mariani *et al*., 2005). The role of oxidative stress in many neurodegenerative diseases is not surprising because the brain is rich in fatty acids, consumes a lot of oxygen, and is deficient in endogenous antioxidants. These make it highly susceptible to

reactive oxygen species (ROS) (Uttara *et al*., 2009).

The brain is deficient in oxidative defense mechanisms and consequently, is at higher risk of damage mediated by (ROS), resulting in cellular dysfunction (Gupta *et al*., 2003). Oxidative stress has been implicated in the pathophysiological mechanisms involved in brain injury in several common neurodegenerative disorders such as Parkinson's, Alzheimer's, and Huntington's diseases. Several studies have shown polyphenolic compounds present in honey can quench biological reactive oxygen species, counter oxidative stress and restores the antioxidant defense system of cells (Aljadi and Kamaruddin, 2004).

Honey, an insect-derived natural product with curative, cosmetic, traditional and nutritional value (Mohammad *et al.,* 2014) has nootropic properties, such as memory-enhancing as well as neuropharmacological effects, such as antidepressant, anticonvulsant, anxiolytic and antinociceptive activities (Manyi-Loh *et al*., 2011). It is a good source of physiologically active polyphenols with antioxidant properties (Islam *et al.,* 2012). To the best of our knowledge, there is no study to date that has demonstrated the effect of honey on sleep deprivation and recovery in experimental animals. Hence, this study was designed to investigate ameliorative effects of honey on the induced oxidative stress status in the brain of sleep – deprived rats.

MATERIALS AND METHODS

Experimental Animals

Twenty-five (25) six weeks old Wistar rats weighing between 180 – 200 g were purchased from Ekiti State University, Ado –Ekiti, Ekiti-State, Nigeria, and used for the study. They 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.

Honey

Honey was purchased from the Department of Agriculture at Ekiti State University, Ado Ekiti, Ekiti State Nigeria. The honey was diluted with distilled water (1:1 v/v).

Experimental Design

The experimental protocol was approved by the Ekiti State University, with protocol number EKSU/A67/2019/02/008. Rats were acclimatized for seven days after which the rats were randomly assigned to one of the following experimental groups (n = 5 per group) and treated accordingly: Rats in Group I (Control) received distilled water (10 ml/kg, orally) daily. Rats in Group II received distilled water (10 ml/kg, orally) daily; designated as sleep deprived (SD) group. Rats in group III received distilled water (10 ml/kg, orally) daily; designated as sleep deprived and recovery (SD + SR) group. Group IV received honey (1 g/kg body weight, orally) daily; designated as sleep deprived with honey administration (SD + H). Group V received honey (1 g/kg body weight, orally) daily; designated as sleep deprived and sleep recovery with honey administration (SD + SR + H).

Sleep Deprivation Model

Except for the control group (Group I), all other experimental rats were subjected to paradoxical sleep deprivation for 20 hours (11:00 am - 7:00 am next morning) for 5 days with 4 hours (7:00 am-11:00 am) rest each day using the modified multiple platform (MMP) method as described by Oh *et al*. (2012). Briefly, the rats were placed in an acrylic water tank (123 X 44 X 44 cm) containing 14 circular platforms, 6.5 cm in diameter, with water up to 1 cm of their upper surface. Thus, the rats could move around inside the tank by jumping from one platform to another. When they reached the P- phase of sleep, muscle atonia set in, and they fell into the water and woke up. Rats were exposed to a 12±1 hour light-dark cycle to circadian rhythm throughout the experimental period and had unrestricted access to standard rat chow by placing chow pellets and water bottles on a grid located on top

of the tank. Daily change of water in the tank was also ensured. For the sleep recovery model, after 5 days of sleep deprivation, the animals in groups III and V were given a sleep recovery period of 5 days and they were transported to cages where they were allowed to sleep freely (Hipolide *et al.,* 2006) but received distilled water and honey supplement, respectively.

Determination of Biochemical Parameters

The rats were anaesthetized using a mixture of 25% (w/v) urethane and 1% (w/v) alpha chloralose (5 ml/kg; Intraperitoneal (i.p), BDH Chemicals Ltd., Poole, England). The animals were humanely sacrificed and brain tissues were quickly excised and weighed. Thereafter, they were washed in cooled 0.15 M NaCl and were then homogenized in 2 ml of ice-cold potassium phosphate buffer (0.1M, pH: 7.4) using an improvised homogenizer. Samples were centrifuged at 5000 rpm for 15 min to obtain the supernatant which was stored at - 20 ^{o}C and later used to assay for markers of oxidative stress.

Determination of Malondialdehyde Level

Malondialdehyde (MDA) was indirectly estimated by determining the accumulation of thiobarbituric acid reactive substances (TBARS) based on the method of Mihara and Uchiyama, (1978). Briefly, 3 ml of 1% H_3PO_4 and 1 ml of 0.6% TBA aqueous solution were added to 0.5 ml of 10% tissue homogenate. The mixture was stirred, then heated on a boiling water bath for 45 minutes and allowed to cool, after which 4 ml of n-butanol was added, shaken and the butanol layer was separated by centrifugation. The concentration of MDA (nmol/ml) was calculated by using the following formula:

$$\text{Concentration of the test} = \frac{\text{Absorbance (test)} - \text{Absorbance (blank)}}{\text{Extinction Coefficient (test)}} \times 1000000$$

Determination of Glutathione (GSH) Level

Reduced glutathione was assayed according to the method of Ellman, (1959). Exactly 0.5 g of tissue was rinsed with phosphate buffer solution. It was homogenized in 2.5 ml protein precipitation reagent and centrifuged at 3000 rpm for 10 minutes and the supernatant collected. To 100 µl of standard and samples in microcuvettes, 880 µl GSH dilution buffer and 20 µl GSH Chromogen were added then mixed. A standard curve was plotted using the absorbance values read at 415 nm against prepared concentrations of GSH standards. This was used to extrapolate GSH concentrations in the samples and expressed as µmole of GSH/g protein.

Determination of Catalase Activity

CAT activity was assayed by H_2O_2 consumption following the method of Aebi (1984). Briefly, 100 µl of tissue extract with an equal volume of absolute alcohol was incubated for 30 min in an ice bath for degradation of the inactive CAT–H_2O_2 complex II to release active CAT enzyme. After 30 min on ice, the tubes were brought back to room temperature and then 10 µl of Triton X-100 was added. In a cuvette containing 200 µl of phosphate buffer, 50 µl of tissue extract and 250 µl of 0.066 M H_2O_2 in phosphate buffer were added and the decrease in absorbance was read at 240 nm for 30 s. A molar absorptivity of 43.6 M cm^{-1} was used to determine CAT activity defined as one unit is equal to the µmoles of hydrogen peroxide degraded per minute per mg of protein. One catalase unit is equivalent to 0.01 decrease in absorbance at 240 nm/mg protein/min. Catalase activity is thus expressed as (Decrease in absorbance x 100/1) divided by protein amount in mg divided by time in min = unit/mg protein/min.

Statistical Analysis

Data are expressed as mean ± standard deviation (SD). Test of variance was done using ANOVA, followed by Newman-keuls multiple comparisons test. Statistically significant differences were compared at $p< 0.05$. Statistical analysis was performed using Graph - Pad (Prism 7) statistical software.

RESULTS

Result presented in Table 1 show an increase in brain weight ($p< 0.05$) among rats in groups I, III,

IV, and V (ranging between 1.82 ± 0.02 - 1.91 ± 0.02 g) when compared with group II.

Table 1: Effects of honey on brain weight in sleep-deprived wistar rat

GROUP	BRAIN WEIGHT (g)
Group I	1.79 ± 0.01
Group II	1.68 ± 0.03[a]
Group III	1.82 ± 0.02[b]
Group IV	1.88 ± 0.02[b]
Group V	1.91 ± 0.02[b]

Results are expressed as Mean ± SD of 5 rats per group. Values with different superscripts are significantly different ($p<0.05$). Group I: Normal control; Group II: Sleep deprived ;Group III : Sleep deprived and sleep recovery; Group IV : Sleep deprived and honey treated; Group V : Sleep deprived, sleep recovery and honey treated.

As shown in Table 2, the levels of GSH of group II and group III differ significantly ($p<0.05$) when compared with group I. Similarly, GSH concentrations in groups III, IV and V increased significantly ($p< 0.05$) when compared with group II. The result also shows a significant difference ($p< 0.05$) in the MDA concentrations of the rats in groups II and III when compared with the control. However, there was a significant difference ($p<0.05$) in the concentrations of MDA between group IV and V when compared with group II.

Table 2: Effects of honey on brain tissue Reduced glutathione levels in sleep-deprived wistar rat

GROUP	BRAIN TISSUE GSH (µmole/g protein)	Brain Tissue MDA (ng/mg protein)
Group I	1.41 ± 0.04	29.48 ± 1.57
Group II	1.08 ± 0.05[a]	40.71 ± 1.94[a]
Group III	1.21 ± 0.04[b]	35.76 ± 1.71[b]
Group IV	1.34 ± 0.04[c]	27.52 ± 1.54[c]
Group V	1.32 ± 0.03[c]	28.94 ± 0.93[c]

Results are expressed as Mean ± SD of 5 rats per group. Values with different superscripts are significantly different ($p<0.05$). Group I: Normal control; Group II : Sleep deprived ;Group III : Sleep deprived and sleep recovery; Group IV :Sleep deprived and honey treated; Group V : Sleep deprived, sleep recovery and honey treated.

The results of effects of honey on brain catalase activity in sleep – deprived wistar rats are presented in Table 3 shows no difference ($p>0.05$) in catalase activities of brain tissue homogenate obtained from animals in groups III, IV and V but the observed catalase activities in these groups were significantly different ($p<0.05$) compared with that observed for group II samples.

Table 3: Effects of honey on brain tissue catalase activity in sleep-deprived wistar rat

Group	BRAIN CATALASE (nmol of H_2O_2/min/mg protein)
Group I	42.11 ± 1.00
Group II	20.49 ± 2.04[a]
Group III	33.13 ± 2.26[b]
Group IV	36.07 ± 0.89[b]
Group V	36.87 ± 4.41[b]

Results are expressed as Mean ± SD of 5 rats per group. Values with different superscripts are significantly different ($p<0.05$). Group I :Normal control; Group II : Sleep deprived ;Group III : Sleep deprived and sleep recovery; Group IV : Sleep deprived and honey treated; Group V : Sleep deprived, sleep recovery and honey treated.

DISCUSSION

Sleep deprivation is a potent oxidative stressor that could cause alteration in behavior and cognitive performance. A bidirectional relationship between sleep deprivation and oxidative stress has been documented (Hill *et al.,* 2018). Findings in this study demonstrated a significant decrease in the GSH level and CAT activity and an elevated level of MDA among rats in the SD group when compared with rats in other groups. Karem *et al.* (2015) showed that the hippocampus is susceptible to free radical

damage following sleep deprivation as evidenced by a decrease in GSH levels in the region.

The decrease in GSH levels in the SD group confirms the fact that sleep deprivation induced the generation of free radicals and depleted the activities of antioxidant enzymes in the brain. GSH plays multiple roles as a cellular antioxidant defense because its main function is to remove hydrogen peroxide and organic peroxides (Antunes and Brito, 2017). Therefore, decline in the level of GSH may indicate the increased production of free radicals (Debnath and Mandal, 2000). In contrast, Singh *et al*., 2008 noticed that sleep deprivation did not affect oxidative stress parameters in the striatum; and the activity of glutathione peroxidase was not affected in any of the studied brain regions. The disparity between this report and ours may be attributed to differences in the age of animals and method of sleep deprivation used. Nermin *et al*. (2020) showed that sleep deprivation in an animal model is associated with the altered status of brain oxidative stress. Lipid peroxidation due to the reaction of free radicals with lipids is considered a hallmark of cellular oxidative damage. Once established, such damage can affect the membrane lipid bilayer and, specifically, the mitochondrial electron transport chain, thus becoming a major cause for a further increase in oxidant production. Studies reported that SD induced oxidative processes in several types of tissues, resulting in some cases, in cognitive impairment and behavioral changes (Lungato *et al*., 2013). For instance, Everson *et al.* (2005) also showed in their study a significant decrease in liver CAT activity in sleep-deprived rats.

The present study provides evidence that sleep recovery restores or promotes antioxidants and antioxidant activities in the brain. The significant increase in GSH level, CAT activity and significant decrease in the MDA level in honey treated groups compared to SD group may be due to the ability of honey to promote the activity of antioxidant enzymes (Abdulmajeed *et al.,* 2016). The phenolic acids and flavonoids are responsible for the well-established antioxidant activity of honey. The exact antioxidant mechanism is unknown, but the proposed mechanisms include free radical sequestration and metallic ion chelation (Mohammed *et al.,* 2015). Antioxidants protect brain cells from oxidative stress; thereby reducing brain damage and improved neuronal functions. Honey decreased the number of degenerated neuronal cells in the hippocampal CA1 region, a region that is known to be highly susceptible to oxidative insult (Cai *et al*., 2011). This may be as a result of the presence of ellagic acid in honey (Uzar *et al.,* 2012). Ellagic acid is a phenolic acid that is found in fruits, vegetables and also in honey. Ellagic acid has antioxidant activity and also shows chemo-preventive effects, as indicated by its antiproliferative activity (Seeram *et al.,* 2005). Interestingly, the chemo-preventive effects of ellagic acid are executed through the reduction of oxidative stress at the cellular level (Nurul *et al*., 2012); Treatment with ellagic acid, present in honey also restores the activities of catalase and the total antioxidant status of the brain to normal levels (Uzar *et al.,* 2012).

CONCLUSION

In conclusion, honey significantly improved brain oxidative status and has ameliorative effect on oxidative stress in the brain of sleep deprived rats.

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