PACIFIC JOURNAL OF MEDICAL SCIENCES

{Formerly: Medical Sciences Bulletin}

ISSN: 2072 – 1625

Pac. J. Med. Sci. (PJMS)

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LICORICE AMELIORATES IMBALANCE BETWEEN REACTIVE OXYGEN SPECIES AND ANTIOXIDANT ENZYMES IN THE BRAIN OF SLEEP DEPRIVED RATS.

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Running title: Effect of licorice on brain oxidative stress markers in sleep deprived rats.

Submitted July 2019; Accepted October 2019

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ABSTRACT

Sleep deprivation can be described as inadequate quantity or quality of sleep characterized by insufficient sleep duration, delayed sleep onset, and occurrence of napping episodes during the day. Sleep deprivation in animals and obstructive sleep apnea syndrome in human was reported to be associated with increased oxidative stress. *Glycyrrizha glabra* (licorice) is a medicinal plant known to be a highly efficacious medicinal herb with several pharmacological effects. Hence, the aim of this study was to demonstrate whether or not licorice root extract will regulate the imbalance between the reactive oxygen species and production of antioxidant enzymes in the brain of sleep deprived rats. Twenty - five 6-week-old male Wistar rats were randomly divided into five groups to undergo sleep deprivation and recovery for 5 days each. Group I (Control): Group II: sleep deprivation (SD); Group III: sleep deprivation and recovery (SD+SR) all received distill water (10ml/kg) orally; Group IV: sleep deprivation and licorice (SD+Lic), Group V: sleep deprivation, recovery with licorice (SD+SR+Lic) both received licorice (150mg/kg) orally once daily. MDA concentration among rats in Groups II (51%), III (46.7%) and IV (31.3%) were significantly higher when compared with control. Rats in Group III (20.5%), Group IV (24.6%) and Group V (30.8%) showed increased significant change in GSH concentration when compared with Group II. The concentration of CAT among rats in Group II was significantly lower than those rats in Group III (43.8%), Group IV (53.8%) and Group V (72.9%). These results clearly show that sleep deprivation significantly affects the oxidative status of rats. In conclusion, licorice root extract has ameliorative effect on the imbalance between the reactive oxygen species and production of antioxidant enzymes in the brain of sleep deprived rats.

Keywords: Sleep; Sleep deprivation; Licorice; Oxidative stress; Rats

INTRODUCTION:

Sleep is important for proper brain function, no less than air, water, and food [1]. However, sleep from this perspective could be defined on the basis of both the behavior of the person while asleep and related physiological changes that occur to the waking brain's electrical rhythms during sleep [2]. Despite the need by individuals to stay awake for long hours due to work or study, sleep remains an integral part of human health and is crucial for learning, performance, physical and mental health [2].

Sleep deprivation (SD) can be described as inadequate quantity or quality of sleep characterized by insufficient sleep duration, delayed sleep onset, and occurrence of napping episodes during the day [1]. It is an increasingly common occurrence in modern life which predisposes humans to many diseases as a result of immune system deficiency, endocrine deregulation and oxidative stress (OS) [3]. While sleep has important functions for every organ in the body sleep deprivation leads to disorders that cause irreparable damage [4]. Furthermore, sleep loss was also reported to be associated with adverse health effects such as obesity, type 2 diabetes, hypertension, and cardiovascular disease [5]. Sleep deprivation in humans and rats was also reported to promote increase in food intake [6]. In contrast, previous studies reported that sleep deprived animals showed intense catabolism [7] and energy expenditure, resulting in weight loss during the sleep deprivation period [8].

Sleep deprivation whether chronic or not can have huge impact on any economy globally leading to a great economic loss [9]. Pilcher and Huffcutt [10] reported that lack of sleep affects working memory, creativity, decision making, multitasking ability, response time, and focus. It was also reported that sleep deprivation prevents the brain from restoring its effectiveness, as it needs to work harder to accomplish the same amount of work [11].

Oxidative stress is known to be associated with several adverse outcomes such as cancers, neurological, cardiovascular and immunodeficiency diseases [12]. It was also reported to be involved in the mechanisms of aging, pathogenesis of cancer, atherosclerosis, diabetes, and neurodegenerative disorders [13]. Furthermore, SD in animals and obstructive sleep apnea syndrome in human was reported to be associated with increased oxidative stress [14].

Consequentially, free radicals been a product of oxidative stress were reported to accumulate during long wake period as a result of enhanced metabolic activity and are known to be responsible for the effects of sleep deprivation [15]. Thus, the free radical flux hypothesis proposed that the core function of sleep is to act as an antioxidant for the brain. Despite the appeal of this hypothesis, reported data to support it are conflicting. While some groups have reported decreased antioxidant capacity and oxidative damage in the brains of

sleep-deprived rats and mice [16], other reports have contradicted these findings [17].

An interesting consequence of the uncontrolled production of free radicals in the membrane of cell organelles was reported to be due to calcium (Ca^{2+}) leakage, which occurs concomitantly with the antioxidant enzyme release to cytosol. These events are believed to lead to cytotoxicity which may cause cell death by apoptosis or necrosis [4]. However, cells have a defense mechanism against the formation of ROS which includes production of antioxidant enzymes which include catalase (CAT), glutathione (GHS) and superoxide dismutase (SOD). Licorice (*Glycyrrizha glabra*) obtained from the dry roots and rhizomes of licorice plant have been demonstrated to be widely used in clinical prescriptions [18].

The pharmaceutical importance of licorice however lies in its capacity to yield a great variety of secondary substances, the most important bioactive compounds in licorice are triterpenes, flavonoids and polysaccharides [19]. These constituents have been shown to demonstrate biological activities including antitumor [20], antimicrobial [21], antiviral [22], anti-inflammatory [23], antidiabetic [24], immunoregulatory [25], antioxidant [26] hepatoprotective [27] and neuro-protective activities [28].

In a study to evaluate the antioxidant activity of licorice flavonoids, Liu et al [29] detected a significant change in the levels of oxidative stress markers in the colon of mouse, indicating that licorice flavonoids have strong antioxidant activities. However, there is no study till date that has investigated the effects of licorice on sleep deprivation and recovery in experimental animals.

Hence, the aim of this study was to demonstrate whether or not licorice root extract can regulate the imbalance between the reactive oxygen species (ROS) and production of antioxidant enzymes in the brain of sleep deprived rats.

METHODOLOGY:

Extract Preparation:

Licorice root powder was purchased from Amazon and was sold by Herbs and Crops Overseas, India with batch no: LRP-2017/02. To prepare the extract 50 g of the powder was mixed with 100 ml of sterile distilled water in a flask with occasional shaking for 10 minutes. The extract was then filtered through a muslin cloth for coarse residue and finally through Whatman No. 1 filters paper and kept in an airtight amber colored container. The dosage used in this present study was 150mg/kg body weight and was based on earlier study [30].

Animals:

Twenty - five 6-week-old male Wistar rats weighing averagely 200 ± 20 g purchased from Ekiti State University 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. The study was conducted in accordance with the standards established by the Guide for the Care and Use of Laboratory Animals [31]. Rats had unrestricted access to standard rat chow and tap water. After one week of acclimatization, the rats were randomly assigned to one of the following experimental groups (n = 5 per group) and treated accordingly. Rats Group I (Control) received distilled water (10ml/kg, orally) daily. Rats in Group II received distilled water (10ml/kg, orally) daily; designated as Sleep Deprived group (SD). Those in Group III received distilled water (10ml/kg, orally) daily; designated as Sleep Deprived, Sleep Recovery group (SD + SR). Those in Group IV received Licorice extract (150mg/kg, orally) daily; designated as Sleep Deprived with Licorice group (SD + Lic). Group V received Licorice extract (150mg/kg, orally) daily; designated as Sleep Deprived, Sleep Recovery with Licorice group (SD + SR + Lic).

Sleep models:

Sleep deprivation model was done in accordance with technique used by Oh et al [32]. The rats were sleep deprived for 5 days. The water in the tank was changed daily throughout the SD period.

For sleep recovery model, after 5 days of SD, the rats were given a SR period of 5 days. After the planned SR period, the rats were anaesthetized using a mixture of 25% (w/v) urethane and 1% (w/v) alpha chloralose (5ml/kg; Intra-peritoneal (i.p), BDH chemicals Ltd., Poole, England).

Determination of biochemical parameters:

Brain tissues were quickly excised and weighed. Thereafter, they were washed in cooled 0.15M 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. The supernatant obtained were stored at -200C prior to use and later used to determine the various biochemical parameters.

Malondialdehyde (MDA): Bio-diagnostic: Diagnostic and Research Reagents. Lipid Peroxide (Malondialdehyde): Colorimetric method; Cat No MD 25 29; info@bio-diagnostic.com; www.bio-diagnostic .com.

Glutathione (GSH):

Bio-diagnostic: Diagnostic and Research Reagents. Glutathione reduced: Colorimetric method; Cat No GR 25 11; info@bio-diagnostic.com; www.bio-diagnostic .com.

Catalase (CAT): Bio-diagnostic: Diagnostic and Research Reagents. Catalase assay: Colorimetric method; Cat No CA 25 17; info@bio-diagnostic.com;

Statistical analysis:

Data are expressed as means ± 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 Tukey's multiple comparisons test. Statistically significant differences were accepted at $p < 0.05$.

RESULTS:

The result of the study showed increased change in brain weight among rats in group I, Group IV and Group V when compared with Group II as shown in table 1.

Result in table 2 showed significant increased changes in concentration of MDA among the rats in Groups II (51%), III (46.7%) and IV (31.3%) when compared with control. However, there was significant decreased change in concentration of MDA between Group IV (28.6%) and V (41.1%) when compared with Group II. Moreover, when compared with Group III, there was significant decreased change in concentration between Groups IV (22.4%) and V (36%).

For GSH activity as shown in table 3, our result demonstrated that rats in Group II (30.3%) and Group III (12.4%) showed significant decreased change in concentration when compared with control group. In furtherance, rats in Group III (20.5%), Group IV (24.6%) and Group V (30.8%) showed increased significant change in GSH concentration when compared with Group II. The concentration of GSH among rats in Group V was significantly higher than those in Group III (13%) and those in group IV (8.2%). Lastly in table 4, the result from our study showed significant decrease in CAT concentration among rats in Group II (69.3%) in Group III (45.4%) and Group IV (33.5%) when compared with the control group. The CAT concentration increased by 11.6% among rats in Group V compared to the control. The concentration of CAT among rats in Group II was significantly lower than those rats in Group III (43.8%), Group IV (53.8%) and Group V (72.9%). The CAT concentration among rats in Group V was significantly higher than those in Group 3 (51.8%) and those in Group IV (41.2%). These results clearly demonstrate that SD significantly affects the oxidative status of rats.

Table 1: Effect of licorice on brain weight in sleep deprived Wistar rat

Groups	Brain Weight (g)
Group I (Control)	1.76 ± 0.03
Group II (SD)	1.67 ± 0.01
Group III (SD + SR)	1.70 ± 0.03
Group IV (SD + Lic)	1.78 ± 0.03
Group V (SD + SR + Lic)	1.79 ± 0.02

Results are expressed as means ± S.E.M. of 5 rats per group

Table 2: Effect of licorice on Brain MDA in sleep deprived Wistar rat

Groups	Brain MDA (ng/mg protein)
Group I (Control)	92.16 ± 6.35
Group II (SD)	187.9 ± 8.66[a]
Group III (SD + SR)	172.9 ± 7.62[a]
Group IV (SD + Lic)	134.2 ± 5.52[a,b,c]
Group V (SD + SR + Lic)	110.7 ± 8.72[b,c]

Results are expressed as means ± S.E.M. n= 5.
[abc] p<0.05 vs Control, Group II and Group III.

Table 3: Effect of licorice on Brain GSH in sleep deprived Wistar rat

Groups	Brain GSH (µmole/g protein)
Group I (Control)	1.45 ± 0.03
Group II (SD)	1.01 ± 0.02[a]
Group III (SD + SR)	1.27 ± 0.03[a,b]
Group IV (SD + Lic)	1.34 ± 0.02[b]
Group V (SD + SR + Lic)	1.46 ± 0.02[b,c,d]

Results are expressed as means ± S.E.M. n= 5.
[abcd] p<0.05 vs Control, Group II, Group III and Group IV

Table 4: Effect of licorice on Brain CAT in sleep deprived Wistar rat

Groups	Brain Catalase (nmol of H2O2/min/mg protein)
Group I (Control)	0.821 ± 0.06
Group II (SD)	0.252 ± 0.04[a]
Group III (SD + SR)	0.448 ± 0.02[a,b]
Group IV (SD + Lic)	0.546 ± 0.05[a,b]
Group V (SD + SR + Lic)	0.929 ± 0.04[b,c,d]

Results are expressed as means ± S.E.M. n= 5.
[abcd] p<0.05 vs Control, Group II, Group III, Group IV

DISCUSSION:

SD is known to enhance metabolic rate which in turn increase oxidative stress even though sleep seems to limit metabolic requirements [33] and this is in tandem with our study. For instance, our study demonstrated significant increase in MDA level among rats in SD group (Group II) when compared with rats in other groups which is in consonant with previous study by Halliwell and Gutteridge [34] where MDA level was reported to be related to an increased level of lipid peroxidation in cell membranes. However, our results suggest that lipid peroxidation in certain regions of the brain following sleep deprivation can generate free radicals and this free radical can cause neuronal damage thereby affecting neuronal transmission at the synapse leading to neurological disorders. Although previous study by Gopalakrishna and colleagues reported there was no significant difference in the concentration of lipid peroxidation after sleep deprivation in Wistar rats [17]. However, rats in group IV and V had significantly lower MDA level when compared with those in Group III. This could be attributed to antioxidant property of licorice and compensatory mechanism of sleep which helps prevent the brain from oxidative stress by reducing neuronal metabolic activity with less oxygen consumption.

Furthermore, our study showed significantly reduced level of GSH among rats in SD group (Group II) when compared to control and other groups and this goes with previous study by D'Almeida et al [35] where GSH level was also shown to be reduced in the brain of Wistar rats during sleep deprivation. One of the mechanisms by which GSH induced its antioxidant effect is by generating oxidized glutathione (GSSG) [34]. Furthermore, Honda et al [36] also demonstrated that GSSG has an inhibitory action on the exciting synaptic membrane of rat brain. They also speculated that GSSG can promote sleep-enhancing activity which is due to its physiological modulation on glutamatergic neurotransmission in the brain.

Lastly in our study, the concentration of CAT among rats in Group II (SD) was significantly lower than those rats in Group III (43.8%), Group IV (53.8%) and Group V (72.9%).However, a number of studies reported that SD induced oxidative processes in several types of tissues, resulting in some cases, in cognitive impairment and behavioral changes [4]. For instance, Everson et al also showed in their study a reduction of 23% and 36% in liver CAT activity in rats that were sleep deprived [37].

In conclusion, the results of our present study showed that licorice has ameliorative effect on the imbalance between reactive oxygen species and production of antioxidant enzymes in the brain of sleep deprived rats.

Conflict of interest:

The authors declare no financial or other conflicts of interests in the design and interpretation of study results.

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