REM SLEEP DEPRIVATION-INDUCED OXIDATIVE STRESS AND ITS ATTENUATION BY TEPHROSIA PURPUREA (L.) IN DISCRETE REGIONS OF RAT BRAIN

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INTRODUCTION

Sleep is essential for normal physiological functions in human and animals; it provides restorations of emotional and physical activity in a manner that is not well established [1]. The average sleep per 24 h has declined by 1.5 h over the past century [2]. Sleep occurs in two stages: Non-rapid eye movement (NREM) and REM [3]. Evidence from many studies suggests that an important function of sleep is a consolidation of new information into long-term memory. Multiple studies in both humans and rodents report memory impairments as a result of sleep deprivation (SD) [4]. Human studies show that the total SD for a single night disturbs memory function [5]. In addition, numerous animal studies also shown that 24-96 h of SD lead to impairment of memory and behavioral changes. Moreover, SD was shown to impair long-term potentiation of the hippocampus, which is one of the main centers for learning and memory function [6]. SD also elevates hippocampal oxidative stress, which reflects on neuronal excitability, cognitive functions, and molecular signaling [7]. SD reduces the expression of transcription and translation synaptic proteins in hippocampus and many regions of the brain [8]. During pregnancy, SD increases the risk of pre-eclampsia, gestational diabetes, intrauterine growth restriction, and the need for cesarean delivery [9]. Cytokines such as tumor necrosis factor-alpha and interleukin 1-beta gene expression increases in the hypothalamus during RSD [10]. SD leads to stroke, obesity, diabetes, osteoporosis, cardiovascular disease, cancer, and permanent cognitive deficits [11].

Tephrosia purpurea (TP) belongs to Fabaceae family, highly branched plant have been used in India as a traditional medicine for the treatment of inflammatory disorders [12], laxative, bleeding disorder, and diuretics [13]. It also has antioxidant activity, according to the Indian indigenous system of medicine. Hence, this present study was focused to evaluate physiological antioxidant defense elements such as lipid peroxidation (LPO), superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), reduced glutathione (GSH), Vitamin C and Vitamin E in different regions of the rat brain, and plasma corticosterone level.

METHODS

Animals

Wistar strain male albino rats (200-250 g) were maintained under standard laboratory conditions with water and food; the animals were handled according to the principles of laboratory care framed by the committee for the purpose of control and supervision of experiments on animals, Government of India. Animal experiments were carried out after getting clearance from the Institutional Animal Ethical Committee (IAEC NO: 54/12/2015).

Experimental protocol

Animals were divided into five equal groups; each group consists of six animals. The ethanolic extract of TP was used for this study.

- Group - I: Control animals were used for studying the baseline values
- Group - II: Animals were administered 2% of Tween 80 as a vehicle
- Group - III: Animals were exposed to 72 h RSD
- Group - IV: Animals were treated with only an ethanolic extract of TP
- Group - V: Animals pretreated with ethanolic extract of TP for 15 days +72 h RSD with the same treatment.

Plant collection and extract preparation

The dried leaves of TP L., were purchased from Government Irula Tribe Women's Welfare Society, Tandarai, Chengalpattu, Tamil Nadu, India-603001. Bill No: 122/05/2016. The plant material was powdered
and passed through the sieve (coarse 10/44). About 200 g of plant powder was extracted with 1000 mL of 95% ethanol under reflux by heating over a water bath at 60°C. The extract was then vacuum dried. The yield of ethanolic extract of TP was 31.23% (w/w). The suspension of the extracted drug was prepared by dissolving in 2% Tween 80 before administration to animals [14].

RSD

RSD for 72 h was induced using the multiple platform models [15]. It was started and ended at the beginning of the light phase, and the room was maintained at the controlled temperature (23±1°C) and light-dark cycle (lights on between 07.00 and 19.00 h). In this experiment 6 rats from same group placed in a water tank (120 cm × 70 cm × 50 cm) containing 10 round platforms (each round platform made up of 7 cm diameter and 10 cm height, it was raised 2 cm above the water level) arranged in two lines and 20 cm away from each other (edge to edge), in which the rats can move around freely from one platform to another one. Loss of muscle tone at the beginning of each REM (paradoxical) sleep episode causes rats to fall in the water, thus being awakened. During the SD period, the animals had free access to water bottles and chow pellets attaching from a grill located on the top of the chamber. As the animals can move freely within the multiplatform chamber, it has been reported that it has less immobilization stress compared to the single version of platform technique [16].

Biochemical analysis

The activity of LPO was indirectly estimated by determining the accumulation of thiobarbituric acid reactive substances in the tissue homogenate by the method of Okhawa et al. [17]. According to Marklund and Marklund [18], the SOD activity was measured as the degree of inhibition of auto-oxidation of pyrogalol at alkaline pH. The activity of CAT was measured as the amount of hydrogen peroxide consumed per minute per milligram of protein by the method of Sinha [19]. GPx level was estimated by measuring the amount of reduced GSH consumed in the reaction mixture according to the method of Rotruck et al. [20]. The reduced GSH level was measured based on the development of relatively stable yellow color, when 200 mM 5, 5’ – dithiobis- (2- nitrobenzoic acid) solution was added, according to the method of Moron et al. [21], Vitamin C was estimated by the method of Omaye et al. [22], and Vitamin E was measured by the method of Desai [23]. The total protein was estimated by the method of Lowry et al. [24], using Bovine serum albumin as standard. The corticosterone level was measured using Elisa kit. (Cusabio, biotech Co Limited, China. CNo.CSB-B07014r).

Statistical analysis

All data were expressed as the mean ± standard deviation. The statistical significance was evaluated by one-way analysis of variance using SPSS statistical package version 20.0 (SPSS, Cary, NC, USA). When there is a significant difference, Tukey’s multiple comparison tests were performed by fixing the significance at level p<0.05.

RESULT

Plasma corticosterone

The plasma corticosterone (d.f.=4, F=44.132) levels (Fig. 1) was increased in Group-III compared to Group-I control. However, corticosterone levels in Group-V animals were significantly decreased when compared to Group-III after 72 h RSD exposure.

LPO

The levels of LPO (Fig. 2) were significantly increased in Group-III after 72 h RSD in discrete regions of the brain [hypothalamus (d.f.=4, F=466.323), hippocampus (d.f.=4, F=659.728), brainstem (d.f.=4, F=688.601), and prefrontal cortex (d.f.=4, F=1047.254)] when compared to Group-I control (animals not exposed to RSD). The LPO levels were lower in the drug-treated group (Group-V) compared to the Group-III animals. The values of decreased LPO level in Group-V were not statistically different from Group-I animals.

SOD

The SOD is not evenly distributed in the regions studied as indicated by the control values. The activities of SOD (Fig. 3) were significantly increased in Group-III after 72 h RSD in discrete regions of the brain [hypothalamus (d.f.=4, F=109.285), hippocampus (d.f.=4, F=40.393), brainstem (d.f.=4, F=86.777), and prefrontal cortex (d.f.=4, F=18.375)] when compared to Group-I. The SOD activities were lower in the drug-treated group (Group-V) compared to the Group-III animals. The symbols represent statistical significance: *, #p<0.05.

![Fig. 1: Data are expressed as mean ± standard deviation for six rats in each group. The values are expressed ng/mL. *Compared with control, # compared with 72 h REM sleep deprivation. The symbols represent statistical significance: *, #p<0.05](image1)

![Fig. 2: Data are expressed as mean ± standard deviation for six rats in each group. The values are expressed µmol of MDA formed mg protein−1. *Compared with control, # compared with 72 h RSD. The symbols represent statistical significance: *, #p<0.05](image2)

![Fig. 3: Data are expressed as mean ± standard deviation for six rats in each group. The values are expressed U/min mg protein−1. *Compared with control, # compared with 72 h REM sleep deprivation. The symbols represent statistical significance: *, #p<0.05](image3)
activities of SOD decreased in Group-V were not statistically different from Group-I animals.

CAT
The results are summarized in Fig. 4. The CAT levels were increased in Group-III animals after 72 h RSD in discrete regions of the brain (hypothalamus [d.f.=4, F=34.737], hippocampus [d.f.=4, F=51.935], brainstem [d.f.=4, F=26.988], and prefrontal cortex [d.f.=4, F=53.782]) when compared to Group-I. The CAT levels were lower in the drug-treated group (Group-V) compared to the Group-III animals.

GPx
The results are summarized in Fig. 5. The GPx also showed regional variations among the brain region studied. The GPx levels increased in Group-III after 72 h RSD in discrete regions of the brain (hypothalamus [d.f.=4, F=70.217], hippocampus [d.f.=4, F=152.491], brainstem [d.f.=4, F=8.067], and prefrontal cortex [d.f.=4, F=67.038]) when compared to Group-I. The GPx levels were lower in the drug-treated group (Group-V) compared to the Group-III animals. The values of decreased GPx in Group-V were not statistically different from Group-I animals.

Reduced GSH
The results are summarized in Fig. 6. The GSH levels were significantly decreased in Group-III animals after 72 h of RSD in discrete regions of the brain (hypothalamus [d.f.=4, F=14.617], hippocampus [d.f.=4, F=31.587], brainstem [d.f.=4, F=52.122], and prefrontal cortex [d.f.=4, F=28.979]) when compared to Group-I animals. The GSH levels were higher in the drug-treated group (Group-V) compared to the Group-III animals. The values of increased GSH in Group-V were not statistically different from Group-I animals.

Vitamin C and E
Vitamin C (Table 1) and Vitamin E (Table 2) levels were significantly decreased in Group-III animals after 72 h RSD when compared to Group-I control animals. On the other hand, Vitamin C and Vitamin E levels were higher in the drug-treated group (Group-V), compared to Group-III animals. The values of increased Vitamin C and Vitamin E in Group-V were not statistically different from Group-I animals.

• Vitamin C level after 72 h REM sleep deprivation and T. purpurea treatment.
• Vitamin E level after 72 h REM sleep deprivation and T. purpurea treatment.

In Group-II and IV animals, all the parameters in all the regions of the brain were not significantly different from Group-I animals.

DISCUSSION
Many studies have shown that SD-induced oxidative damage in several types of tissues [25]. Further, Lungato et al., stated that SD alters gene expression and antioxidant enzyme activity in mice [26]. Oxidative stress had linked with cognitive impairments [27].

Antioxidants and free radicals scavenging system present in the cell protect against the damaging effects of free radicals [28]. In normal cells, there is a balance exists between oxidative products and antioxidants, such as SOD, CAT, GPx, GSH, and Vitamin C and E. Brain is more vulnerable to oxidative damage due to its high oxygen consumption [29]. For the transmission of impulses between the neuronal cells, it requires energy in the form of ATP; during the production of ATP using oxygen, a small percentage (<3%) of oxygen in mitochondria is mindfully converted to superoxide radical this explains the reason for the increased production of free radicals in the brain. Furthermore, stress response results in the creation of some other reactive oxygen species such as hydrogen peroxide (H$_2$O$_2$), hydroxyl radicals (.OH), and superoxide anion radicals (O$_2^-$) that cause LPO, especially in cell membrane leads to tissue damage [30]. The platform technique used for SD raises the possibility that long-term impairment of synaptic plasticity [31].

In the present study, 72 h RSD are producing marked and increased LPO, SOD, CAT, GPx activities and decreased in the levels of GSH, Vitamin C, and Vitamin E in discrete regions of the brain that were...
tested (hypothalamus, hippocampus, brainstem, and prefrontal cortex). Further, it also significantly increases the corticosterone level in plasma.

Acute exposure to stress leads to an excessive generation of oxygen free radicals this might be the reason for the production of the excess amount of LPO [32]. SOD is a major intracellular enzyme, which protects the cell against oxygen free radicals by speeding up its dissmutation of the superoxide anion (O$_2^−$). In recent study states that the redox-sensitive transcription factor NF-E2-related factor 2 (Nrf2) plays an important role in activating antioxidant enzymes, the increased SOD activity could be due to the activation of Nrf2 induced by SD [33]. CAT was responsible for the detoxification of significant amounts of H$_2$O$_2$, CAT level was increased in 72 h RSD.

GPx is a major antioxidant enzyme in many tissues, especially in the brain, which metabolizes peroxides, such as H$_2$O$_2$, and protects cell membranes from LPO [34]. GPx activity in the brain is more important than CAT for the destruction of H$_2$O$_2$ because this enzyme was more in mitochondria and cytosol. The GSH levels were significantly reduced in SD animals; this might be due to its multiple activities in scavenging the free radicals produced by SD, which leads to its greater consumption [35]. In the present study also the GSH level was significantly reduced in the 72 h RSD.

Vitamin C acts as a powerful scavenger of superoxide-induced LPO. Endogenous Vitamin C and E levels have been reported to decline under stress conditions [36]. Plants have both enzymatic and non-enzymatic systems to scavenge active oxygen species. Vitamin C and E are non-enzymatic compounds that protect the human body from free radicals [37].

Vitamin E is a powerful antioxidant that inhibits the free radical reaction and prevents oxidative stress [38]. Further, Vitamin E administration to an animal model of diabetes normalizes the GSH level and activities of GPx, CAT, and SOD [39].

In the present study, TP treated sleep-deprived animals showed a marked decrease in SOD, GPx, and CAT activity when compared with sleep-deprived animals. The further LPO level was also significantly decreased in the TP treated sleep-deprived animals. GSH, Vitamin C, and Vitamin E level were significantly increased in the TP treated sleep-deprived animals when compared with the sleep-deprived animals.

The leaves of TP contain the flavonoids, (+)-tephrorins A and B, and (+)-tephrosine [40]. Furthermore, it has anti-inflammatory activity [41], antimicrobial activity [42], an antidiabetic activity [43], cytotoxic activity [44], antiviral activity [45], a nephroprotective activity [46], and hepatoprotective activity [47]. The anticancer activity of TP was evaluated using MCF 7 cell lines [48].

CONCLUSION

The observed data confirmed the presence of oxidative damage in the discrete regions of the brain when exposed to 72 h RSD. The significant variations in SOD, LPO, CAT, GPx, GSH, Vitamin C, Vitamin E, and corticosterone were normalized by an ethanolic extract of TP in the rat. Further, there was no adverse effect found in the rat model of treatment with TP alone. In future, it is necessary to conduct more extensive studies with TP to explicate their molecular mechanism in antioxidant activity.

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AUTHORS CONTRIBUTIONS

All the authors have contributed equally.

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest concerning this research article.

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