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Quercetin Modulates Behavioural and Biochemical Alterations in Stressed Mice

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Disruption of the active phase of sleep alters the physiological homeostasis of the body and results in oxidative breakdown which may trigger a wide array of defects. The central nervous system and the metabolic system are some of the most affected systems as described in several literatures. Some plant based compounds with antioxidant property have been previously described in the abrogation of the deleterious effects of active sleep disruption. One of such compounds is quercetin. This study was premeditated to expatiate on the probable neuroprotective effect of quercetin on mice exposed to 72hr active sleep disruption. Mice were allotted into five treatment groups (n = 6): group 1 served as control, group 2 received 10 mL/kg vehicle, groups 3 and 4 received 25 and 50 mg/kg quercetin respectively, and group 5 received 50 mg/kg astaxanthin. Treatment lasted for 7 days while groups 2-5 were exposed to the sleep deprivation protocol starting from day 4. Behavioural tests followed by biochemical assays and histopathological changes in the prefrontal cortex were evaluated. Data were analysed by ANOVA set at $p<0.05$ significance. The results revealed that quercetin, in both doses, significantly amplified memory performance, attenuated depression-like behaviour, replenished catalase and superoxide dismutase, attenuated nitric oxide levels in brain and liver of mice when compared to control group and protected against loss of prefrontal cortex neurons. In conclusion, quercetin possesses protective effects against sleep deprivation-induced brain damage.

Keywords: Antioxidants; Hepatoprotective; Liver; Neuroprotective; Oxidative stress; Quercetin.

Acute stress such as insufficient sleep is an infamous challenge in modern society, affecting a significant number of people at various points in their lives. Although occasional sleep disruptions are usually no more than a nuisance, persistent lack of sleep can lead to various alterations in bodily functions that may alter the quality of life[1]. Meanwhile, there are reports that acute stress increase oxidations and diminishes antioxidant protection particularly in the liver[2].A similar ripple effect had previously been noted in the brain due to the relationship between the functional status of the liver and the brain[3].

On the other hand, there is literary evidence on the neuroprotective effect of quercetin[4].Several scientific literatures have shown that quercetin can bestow neuroprotection and antagonize oxidative stress-mediated disorders in vivo. For example,

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oral quercetin was revealed to protect laboratory animals from oxidative stress and neurotoxicity which were induced by various insults[5,6]. Also, Bona et al.[7], reported that quercetin reversed the increase in serum levels of liver enzymes in rats caused by inhaled chloroform, supporting the belief that quercetin possesses antioxidant, hepatoprotective and neuroprotective ability. However, there is paucity of information on the role of quercetin in sleep deprivation-induced stress. Therefore, this study is justified in that it contributes to the body of literature on the probable modulatory effect of quercetin on behavioural and biochemical alterations in the brain and liver of sleep deprived mice.

MATERIAL AND METHODS

Animals and Housing

Male Albino Swiss mice (n = 30; 22.0±2.0 g) used in this study were procured from the central animal facility, Faculty of BMS, Delta State University, Abraka. Mice were housed in plastic cages in groups of six and maintained under room temperature with a 12h light–dark cycle (lights on from 07:00 to 19:00 hours). They were allowed access to water and rodent chow *ad libitum*. Note that mice were acclimatized for about one week before the experiment. The experimental protocols were performed according to the NIH guideline for laboratory animals with due approval from the ethical committee of the institution (REF/FBMS/DELSU/21/105).

Drugs and Chemicals

Quercetin, astaxanthin and DTNB were obtained from Aldrich, Germany. Acetic acid was gotten from Sigma-Aldrich, Inc., St Louis, USA. TCA was obtained from Burgoyne Burbidge's& Co., Mumbai, India.TBA and DMSO were obtained from Guanghua Chemical Factory Co. Ltd., China.Tris-buffer was obtained from Hopkins& Williams Company, USA. $NaHCO_3$,NaH_2PO_4. H_2O, K_2HPO_4, $K_2Cr_2O_7$, KCl and $Na_2HPO_4.H_2O$ were obtained from BDH Chemicals Ltd, Poole, England. Sodium Carbonate was obtained from Fisons, Loughborough Leics, England. NaOH was obtained from J.T Baker Chemicals Co., Phillips burg, N.J., USA.

Drug Preparation and Treatment Groups

The concentrations of quercetin used in this study were obtained following a serial dilution of 100 mg quercetin in 20 mL of 0.5% DMSO. The mice were indiscriminately distributed into five (5) treatment groups (n = 6) based on the drug they received: group 1 received vehicle (10 mL/kg 0.5%DMSO, p.o), group 2 received vehicle in addition to being sleep-deprived, while group 3 received low-dose quercetin and group 4 received high-dose quercetin(25 mg/kg and 50 mg/kg p.o, respectively) in addition to being sleep-deprived, group 5 received astaxanthin (50 mg/kg) in addition to being sleep-deprived. Total treatment duration was seven (7) days. From the fourth day, mice in groups 2-5 were subjected to 72 hr sleep deprivation.

Experimental Design

Deprivation of the active stage of sleep was carried out in line with the method of Shinomiya and colleagues[8] with moderate modifications.

At the end of the 72 hr sleep deprivation duration, 1 hr after the last treatment, the effect of active sleep deprivation on behaviour, hepatic/brain oxidative stress parameters and lipid profile was assessed. Also, animals were euthanized on the seventh day. Liver and brain tissues were harvested and specific tissues were kept aside for histopathological evaluation.

Behavioural Tests

Open field test (OFT)

The OFT was used to determine the spontaneous motor activity (SMA) of mice following the method described[9,10]. Number of square lines crossed and duration of ambulation of each mouse was recorded within a10 min period.

Tail suspension test(TST)

The TST was carried out in accordance with the procedure described[11] with slight changes. A mouse was adjudged to be non-mobile if it made no movements with its head above water level.

Novel object recognition test

The novel object recognition memory test explores the animal's preference for novelty. In this study, the method described[12] was followed. The percentage preference, which was used as an index of recognition memory, was calculated as the total time spent by a mouse in exploring the novel object divided by the summation of total time spent exploring both the familiar and novel objects multiplied by 100%.

Assessment of lipid profile

Serum triglyceride levels

Serum triglyceride level in mouse serum was determined by enzymatic colorimetric method according to the protocol earlier described[13]. Concentration of triglyceride (mg/dL) in the sample was obtained from the equation:

$C_X = [K(A_X-A_b) + C_b] \times IF$

Where:

C_x = Concentration of sample

K = Concentration factor

A_x = Mean of absorbance of Sample

A_b = Mean of absorbance of blank

C_b = concentration of blank

IF = instrument factor (or dilution correction)

High-density lipoproteins

High-density lipoproteins (HDL)level was determined in mouse serum by enzymatic colorimetric method according to the protocol described[13]. Concentration of HDL(mg/dL) in the sample is obtained from the equation:

$C_X = [K(A_X-A_b) + C_b] \times IF$

Where:

C_x = Concentration of sample

K = Concentration factor

A_x = Mean of absorbance of Sample

A_b = Mean of absorbance of blank

C_b = concentration of blank

IF = instrument factor (or dilution correction)

Biochemical Assays

Superoxide dismutase (SOD) activity approximation

The level of SOD activity in the brain and liver was approximated according to the method modified by Umukoro and his colleagues[14] which involves the use of adrenaline. This activity was expressed in units of adrenaline consumed per minute per mg protein.

Estimation of catalase (CAT) activity

Brain and liver catalase activity was determined according to the Hadwan method[15]. The catalase activity was expressed in µmol.

Estimation of nitric oxide

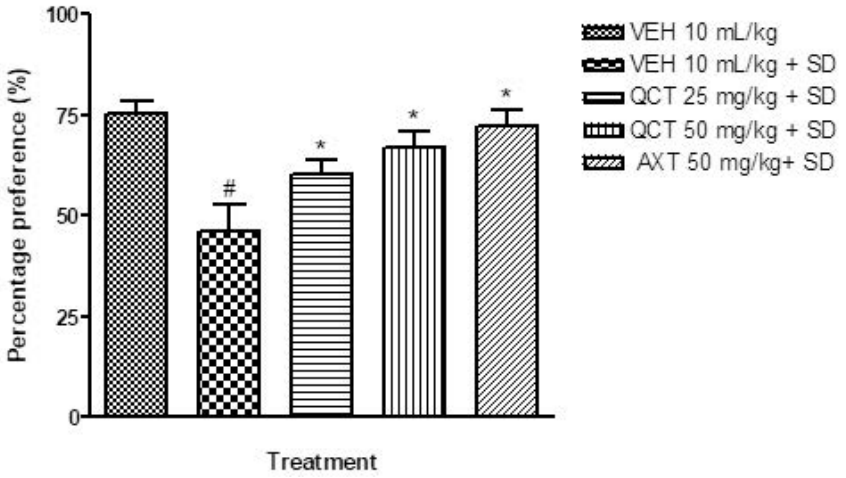
Brain and liver nitrite concentration was estimated following the method of Green et al.[16], which involves the use of Greiss reagent.

Statistical Analysis

Data sets were presented as Mean ± S.E.M. The results were analysed using the one-way ANOVA technique, and a specific post hoc test (Student's Newman–Keuls) was carried out to determine the criterion of significance using Graph Pad Biostatistics software. Significance for all tests was then established at $p<0.05$.

RESULTS AND DISCUSSION

This study was undertaken to assess the effect of active sleep deprivation alone and

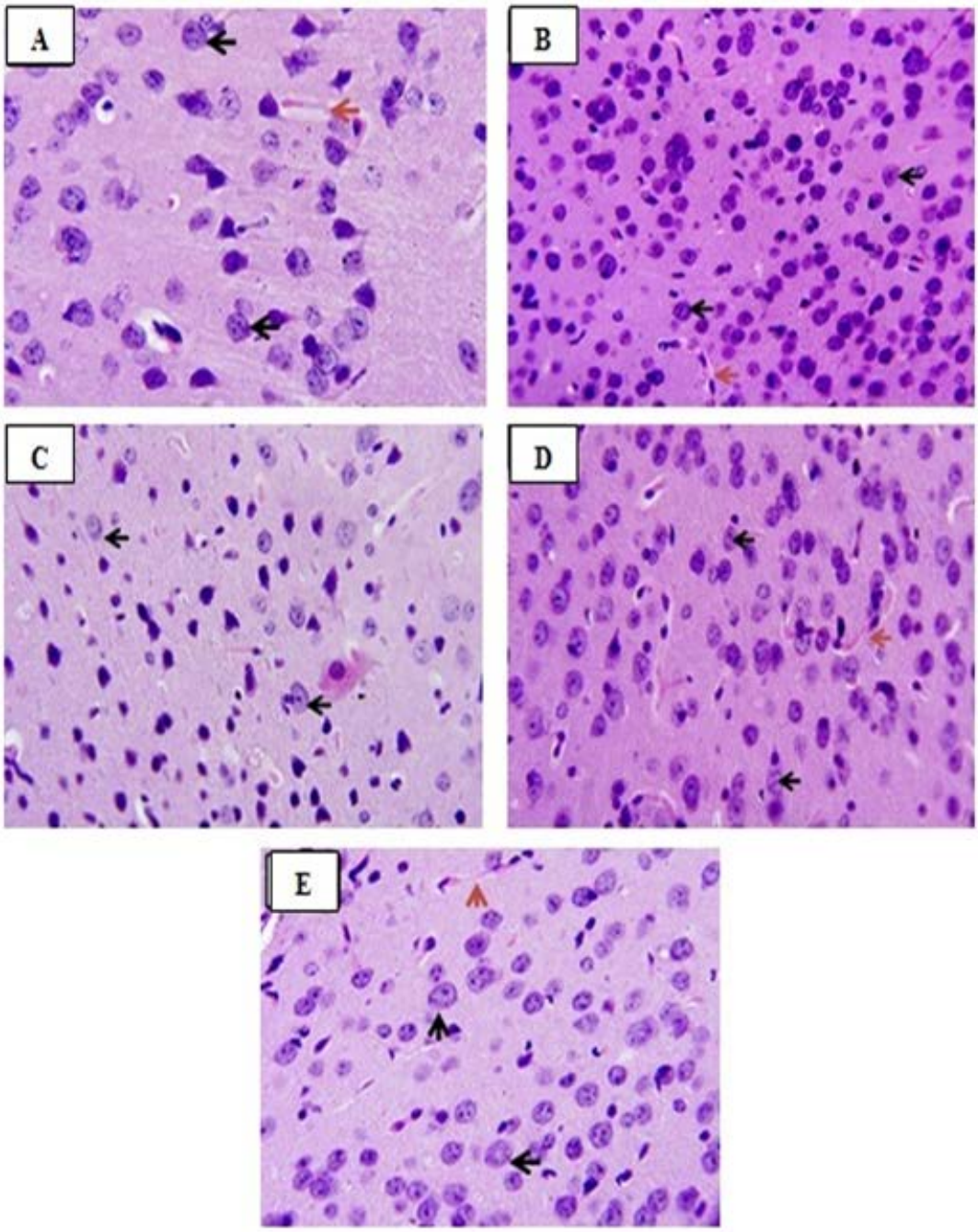


depicts significance ($p<0.05$) compared to non sleep-deprived group.

* depicts significance ($p<0.05$) compared to vehicle + SD group.

VEH: Vehicle. AXT: Astaxanthin. QCT: Quercetin. SD: Sleep deprivation.

Fig. 1. Effect of quercetin on recognition memory in sleep deprived mice



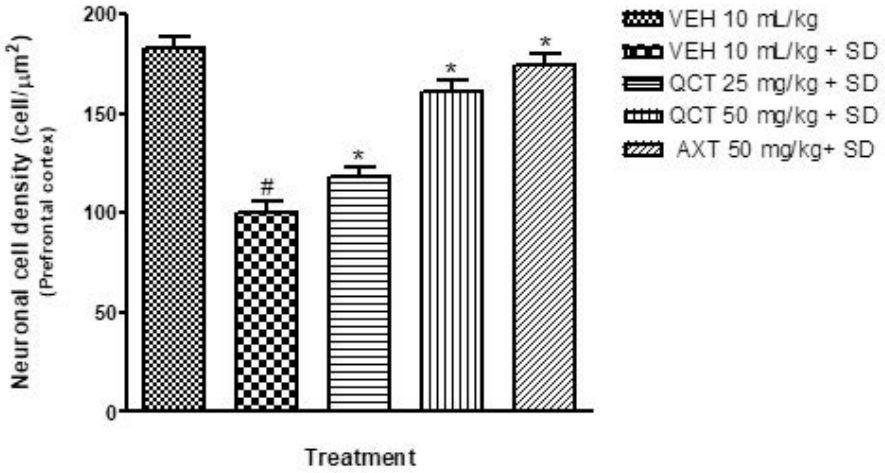
Slide A: Non-sleep deprived group, VEH 10 mL/kg.
Slide B: Sleep deprived group, VEH 10 mL/kg +SD.
Slide C: Low-dose quercetin group, QCT 25 mg/kg +SD.
Slide D: High-dose quercetin group, QCT 50 mg/kg +SD.
Slide E: Astaxanthin group, AXT 50 mg/kg +SD.
Black arrows: Normal neuronal cells.
Red arrows: Neuronal cells undergoing necrosis.
VEH: Vehicle. AXT: Astaxanthin. QCT: Quercetin. SD: Sleep deprivation.

Fig. 2. Photomicrograph of the prefrontal cortex of sleep deprived mice

in combination with quercetin supplementation on brain and liver oxidative stress status and behavioural phenotypes in mice. There are many mechanisms via which sleep deprivation can be responsible for oxidative consequences and liver toxicity, but these effects are probably not attributable to a single night of sleep deprivation. In this study, we measured the effects of 72 hr sleep deprivation on lipid profile parameters, hepatic and brain oxidative stress biomarkers, anxiety-like symptoms, depression-like behaviour and recognition memory consolidation in mice.

The results of this study showed that sleep deprivation significantly diminished recognition memory, as it decreased the percentage preference for the familiar object in mice (Figure 1). Recognition memory was impaired in the sleep deprived group with a significant reduction in percentage preference in the novel object recognition paradigm when compared to the non-sleep-deprived group. Traditionally, the usefulness of this test in memory assessment is based on the innate inclination of animals (particularly rodents) for unfamiliar objects[12]. The preference of mice for a novel object as compared to a familiar one indicates the existence of the object's familiarity in the animals' memory. Thus, the duration of exploration depends on the degree of residual memory of the object. In this test, quercetin increased the duration of exploration of the novel object, which suggests memory improvement[12].

Quercetin also protects neuronal cells. This was substantiated in this study by histomorphometric analysis of the prefrontal cortex



depicts significance ($p<0.05$) compared to non sleep-deprived group.
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VEH: Vehicle. AXT: Astaxanthin. QCT: Quercetin. SD: Sleep deprivation.

Fig. 3. Effect of quercetin on viable prefrontal cortex neurons in sleep-deprived mice

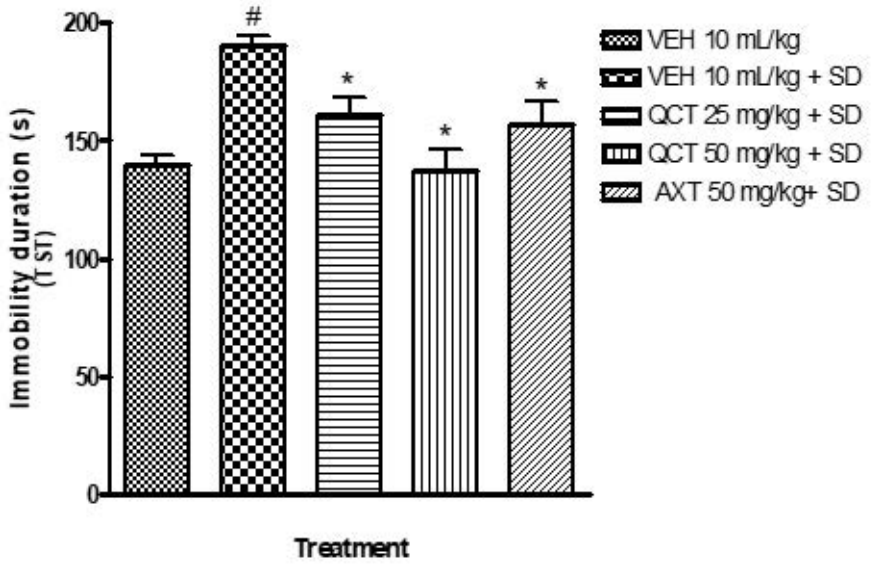
Table 1. Effect of quercetin on brain oxidative stress parameters in sleep deprived mice

Treatment	CAT (units/mg protein)	SOD (units/mg protein)	NO (μ M)
VEH 10 mL/kg	33.47±1.93	16.91±0.62	47.37±4.55
VEH 10 mL/kg +SD	15.87±1.94#	10.32±0.95#	76.59±6.37#
QCT 25 mg/kg +SD	28.88±3.81*	16.93±0.86*	51.65±3.01*
QCT 50 mg/kg +SD	32.60±3.71*	20.67±0.94*	39.77±4.45*
AXT 50 mg/kg +SD	27.10±1.88*	15.61±1.07*	42.34±5.14*

depicts significance ($p<0.05$) compared to non sleep-deprived group.
* depicts significance ($p<0.05$) compared to vehicle + SD group.
VEH: Vehicle. AXT: Astaxanthin. QCT: Quercetin. SD: Sleep deprivation.

of mice[17]. The data revealed that sleep deprived mice had significant necrosis of brain cells (slide B) when compared with the non-sleep deprived group (slide A). This was similar to the observed changes in neuronal densitities of the prefrontal cortex of mice in all groups[18]. This was reversed by quercetin supplementation (slides C and D; Figure 2 and 3) as effectively as astaxanthin (slide E). This could also play a role in the memory impairment observed since a study by Umukoro and Eduviere[19]had implicated the prefrontal cortex as one of the notable brain regions with a role in recognition memory. Although more preclinical studies are necessary before commenting on how quercetin improves memory in mice, the present data suggest modulatory effect of quercetin on sleep deprivation-induced recognition memory impairment.

Also from this study, recognition memory impairment caused by sleep deprivation was accompanied by increased brain oxidative stress, as indicated by elevated levels of nitrite and decreased antioxidant defence systems in the brain (Table 1). In the brain tissue of the sleep deprived group, SOD and CAT activity were significantly ($p<0.05$) lower, whereas nitrite levels was significantly increased ($p<0.05$) when compared to the control group. This effect was however attenuated by quercetin pre-



depicts significance ($p<0.05$) compared to non sleep-deprived group.
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VEH: Vehicle. AXT: Astaxanthin. QCT: Quercetin. SD: Sleep deprivation.

Fig. 4. Effect of quercetin on depression-like behaviour in sleep-deprived mice

Table 2. Effect of quercetin on spontaneous motor activity in sleep deprived mice

Treatment	Number of lines crossed	Ambulation (min)
VEH 10 mL/kg	143.70±8.19	3.52±0.36
VEH 10 mL/kg + SD	213.50±10.22#	6.88±0.39#
QCT 25 mg/kg + SD	175.50±13.18*	4.80±0.38*
QCT 50 mg/kg + SD	165.70±7.66*	4.34±0.46*
AXT 50 mg/kg+ SD	185.50±4.87*	5.09±0.28*

depicts significance ($p<0.05$) compared to non sleep-deprived group.
* depicts significance ($p<0.05$) compared to vehicle + SD group.
VEH: Vehicle. AXT: Astaxanthin. QCT: Quercetin. SD: Sleep deprivation.

treatment as effectively as astaxanthin. This is in line with previous studies[19, 20]. Thus, the capability of quercetin to reverse sleep deprivation-induced memory impairment in mice suggests an action that may involve the inhibition of central oxidative stress. This action possibly resulted from its ability to replenish antioxidant defence systems (SOD and catalase) and decrease NO levels. .

Furthermore, other mood-related behaviours (depression and anxiety) were assessed in this study. Sleep deprived mice were more active in the open field test (Table 2), signalling anxiety; and increased duration of immobile state in the TST (Figure 4), signalling depression. Sleep deprivation significantly ($p < 0.05$) diminished the motor activity of mice as shown in less number of lines crossed and duration of ambulation and significantly ($p<0.05$) increased the immobility time of mice in the TST when compared to the non-sleep deprived group. However, quercetin-treated groups exhibited improved mood recorded as increased number of lines crossed in the open field test and decreased duration of immobility in the TST. This agrees with previous studies which have outlined the beneficial role of quercetin on anxiety- and depressive-like symptoms[22].

Also in this study, triglyceride level was significantly ($p<0.05$) elevated while high-density lipoprotein was significantly ($p<0.05$) diminished in the sleep deprived group (Table 3). Literary evidence has associated sleep disorders with serious complications like type 2 diabetes mellitus[23] and glucose intolerance or insulin resistance[24].The liver which is the site of metabolism is particularly vulnerable to oxidative stress thus predisposing the organism to further damage[25, 26]. Results from this study also support this evidence with sleep deprived mice showing a significant ($p<0.05$) elevation in nitrite levels and decrease in antioxidant levels (Table 4). The administration of quercetin in sleep deprived mice was able to attenuate these deleterious effects of sleep deprivation.

Table 3. Effect of quercetin on triglyceride and high-density lipoprotein levels in sleep deprived mice

Treatment	HDL (mg/dL)	Triglycerides(mg/dL)
VEH 10 mL/kg	118.3±2.10	110.0±2.68
VEH 10 mL/kg +SD	88.00±4.02#	166.3±5.76#
QCT 25 mg/kg +SD	106.8±2.48*	141.4±6.41*
QCT 50 mg/kg +SD	113.3±3.45*	134.9±3.42*
AXT 50 mg/kg +SD	104.0±2.36*	149.9±3.40*

depicts significance ($p<0.05$) compared to non sleep-deprived group.
* depicts significance ($p<0.05$) compared to vehicle + SD group.
VEH: Vehicle. AXT: Astaxanthin. QCT: Quercetin. SD: Sleep deprivation

Table 4. Effect of quercetin on liver oxidative stress parameters in sleep deprived mice

Treatment	CAT (units/mg protein)	SOD (units/mg protein)	NO (μ M)
VEH 10 mL/kg	65.80±5.15	58.14±3.71	34.62±5.71
VEH 10 mL/kg +SD	26.28±3.21#	27.64±2.91#	81.13±4.85#
QCT 25 mg/kg +SD	49.60±5.49*	43.10±4.70*	51.81±6.87*
QCT 50 mg/kg +SD	58.03±5.42*	52.44±3.62*	40.28±3.00*
AXT 50 mg/kg +SD	48.23±4.31*	45.35±3.90*	49.41±5.15*

depicts significance ($p<0.05$) compared to non sleep-deprived group.
* depicts significance ($p<0.05$) compared to vehicle + SD group.
VEH: Vehicle. AXT: Astaxanthin. QCT: Quercetin. SD: Sleep deprivation.

CONCLUSIONS

Based on the antioxidant and protective effects of quercetin on the brain and liver of mice in this study, we believe that this compound stands a chance of being a potential therapeutic agent for the treatment of neuronal degeneration resulting from sleep deprivation. Nevertheless, more reliable methods such as immunocytochemistry are recommended for further investigation of the neuroprotective effect of quercetin.

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Conflict of Interest

The authors declare the absence of conflicts of interest

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