

Acute Hepatic Dysfunction Induced by Sleep Deprivation: The Role of Quercetin Supplementation

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ABSTRACT

Background: Quercetin, a bioflavonoid found in fruits and vegetables, is widely distributed as a secondary metabolite in the plant kingdom. Its use as a food supplement has become popular. The liver is the main organ for metabolism of foods and drugs thus making it one of the organs mostly at risk of cellular damage. This study was therefore designed to evaluate the effect of quercetin supplement on blood glucose levels, liver enzyme activity and oxidative stress status in sleep deprived mice.

Methodology: Thirty mice were assigned into 5 groups (n=6): control group, model control group, two quercetin groups and positive control group. The mice were treated for 7 days and all groups except control were sleep deprived on day 5 through day 7. Following euthanization, blood samples were obtained for determination of blood glucose levels, liver function tests and biochemical assays. Histological analysis of liver tissues was carried out. Liver and adrenal glands were also harvested for weighing.

Results: The results obtained showed that sleep deprivation significantly increased blood glucose level and liver function parameters in mice with a concomitant reduction in antioxidant levels. However, quercetin significantly abrogated these effects and also normalized the relative organ weights of mice after sleep deprivation.

Conclusion: Quercetin showed a protective role against sleep deprivation induced alterations in liver function. The capability of quercetin to ameliorate the potential adverse effects of sleep deprivation in this study, suggest that quercetin might be a useful agent against hyperglycaemia and liver function impairment.

Keywords: Sleep Deprivation, Liver, Quercetin, Food supplement, Diabetes

INTRODUCTION

Stress is an unavoidable aspect in today's world which is commonly encountered by people



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of different age groups. Stress is defined as an obstruction in the normal homeostatic functioning of an organism which is usually caused by a stressor which can either be physiological or psychological^[1]. One of the most common physiological stressors is sleep deprivation or insufficient sleep. Several physiological disturbances such as aging, heart diseases, diabetes, neurocognitive disorders, etc were observed after animals were subjected to sleep deprivation; however, these disturbances were slowly mitigated once the animals were allowed to sleep *ad libitum*^[2]. Stress has also been shown to inhibit metabolic function, such as lipid elimination and glycometabolism^[3]. Since the liver is the main organ responsible for metabolism, acute stress may therefore inhibit liver function. Also, there are reports that acute stress increase oxidation and diminishes the liver's antioxidant protection^[4]. Furthermore, it was suggested that stress influences hepatic blood flow by inducing vasospasm and centrilobular hypoxia, leading to liver damage^[5]. In more recent years, as the understanding of stressors has improved the need to attenuate the deleterious effects of stress on the liver has gained a new dimension^[6].

Quercetin, 3,3',4',5,7-pentahydroxyflavanone (or its synonym 3,3',4',5,7-pentahydroxy-2-phenylchromen-4-one), is the most abundant of the dietary flavonoids (the name comes from the Latin quercetum, meaning oak forest, after the oak genus *Quercus*), with an average daily consumption of 25-50mg^[7]. It is used as an ingredient in dietary supplements, beverages, and foods. An extensive amount of *in vivo* and *in vitro* animal research has focused on the antioxidant potential of quercetin^[8]. Animal evidence suggests that quercetin's antioxidant ability confers protection on the heart, brain, and other tissues against factors such as

ischemia-reperfusion injury, or toxic compounds, etc that can cause oxidative stress^[9-10]. An antioxidant effect of quercetin was also observed in rats that were exposed to varying doses of ultraviolet A light^[11]. These preliminary animal results are the reason why this study was designed to outline the potential benefit of quercetin supplement on hepatic dysfunction induced by sleep deprivation.

MATERIALS AND METHODS

Procurement of experimental animals

Thirty (30) Albino Swiss mice (male; weighing 22.0±2.0 g) used in the study were procured from the animal house, faculty of Basic Medical Sciences of the affiliated institution and were housed in plastic cages at room temperature with equal exposure to light and dark. Also, all procedures received validation from the institutional ethics committee (REF/FBMS/DELSU/21/105).

Treatment groups

Mice received oral doses (p.o) of Quercetin (Sigma, Germany), astaxanthin or distilled water. Thus, the animals were randomly allotted into five treatment groups of six animals each (n = 6) viz: control group received distilled water (10 mL/kg); model control group also received distilled water (10 mL/kg); Q₂₅ group received low-dose quercetin (25 mg/kg); Q₅₀ group received high-dose quercetin (50mg/kg) and positive control group received astaxanthin (50 mg/kg). The mice were treated for 7 days. All groups apart from the control group were further subjected to the sleep deprivation protocol starting from day 4 of treatment.

Experimental design

Mice were sleep deprived by placing them on

individual stands of a grid suspended on water as modified by Eduviere *et al.*^[12]. This model is considered viable because mice, at the outset of paradoxical sleep, are expected to lose muscle tone and then fall into the water. This spurs them out of sleep in a bid to escape the water and get back on the stand.

Measurement of blood glucose

Blood glucose level was measured using a digital glucometer (Accu-Check Actives, Roche Diagnostics India).

Assessment of liver function

Mice in the respective groups were euthanized by anaesthesia and blood was obtained via ocular puncture afterwards. Thereafter, the blood was centrifuged in order to obtain serum used for liver function analysis.

Alanine aminotransferase (ALT) activity in serum was determined and alterations (if any) were recorded^[13]. Aspartate aminotransferase (AST) activity in serum was determined and alterations (if any) were recorded^[13]. Alkaline phosphatase (ALP) activity in serum was determined and alterations (if any) were recorded^[13].

Determination of weights of adrenal gland and liver

Also following euthanization, adrenal glands and livers of the mice in the respective treatment groups were excised and weighed. The relative organ weights were then calculated as organ weight per 100 g/body wt.

Biochemical assay

Level of MDA was determined as an indicator of lipid peroxidation in the liver according to the method described by Umukoro and colleagues^[14]. To measure the GSH activity, equal volumes of

liver homogenate and 20% TCA were mixed and then centrifuged using a cold centrifuge^[14].

Histological examination of the liver

Following organ weight determination, tissue slices of the mice livers were obtained with a microtome and subsequently subjected to Hematoxylin and Eosin staining and photographed at X400 microscopic magnification

Statistical analysis

All data are presented as Mean \pm Standard error of mean (SEM). The results were analyzed by one way analysis of variance and Student's Newman-Keuls post-hoc tests. Main significance was determined using Graph Pad InStat[®] Biostatistics software. The level of significance for all tests was set at $p < 0.05$

RESULTS

Blood glucose levels

Here, sleep deprivation significantly ($p < 0.05$) increased blood glucose level in model control

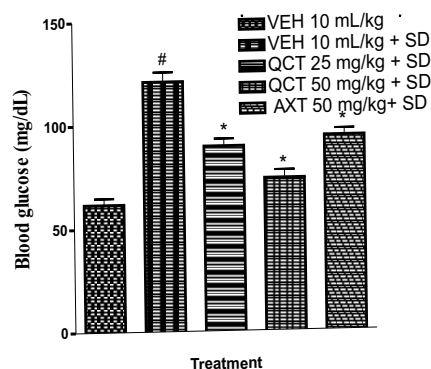


Fig 1: # Indicates significant difference ($p < 0.05$) compared to the control group.

*** indicates significant difference ($p < 0.05$) compared to the model control group.**

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

mice when compared to control mice. However, supplementation with quercetin significantly suppressed elevated blood glucose level when compared to model control mice

Organ weights

Here, sleep deprivation significantly ($p < 0.05$) increased relative weights of liver and adrenal glands in model control mice when compared to control mice. However, supplementation with quercetin significantly reduced relative weights of liver and adrenal glands when compared to model control mice (Table1).

Table 1:

Treatment	Relative organ weight (g per 100 g body weight)	
	Adrenal gland (mg)	Liver ($\times 10^3$ mg)
VEH 10 mL/kg		0.89±0.02
VEH 10 mL/kg + SD	7.23±0.57 [#]	1.12±0.04 [#]
QCT25 mg/kg + SD	4.33±0.34 [*]	0.99±0.03 [*]
QCT 50 mg/kg + SD	3.55±0.62 [*]	0.91±0.05 [*]
AXT 50 mg/kg+ SD	3.67±0.53 [*]	0.96±0.04 [*]

indicates significant difference ($p < 0.05$) compared to the control group.

* indicates significant difference ($p < 0.05$) compared to the model control group.

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

Liver function enzymes

The effect of quercetin on liver function (measured by activity of ALT, AST and ALP) in mice subjected to sleep deprivation are shown in Figures2-4respectively. Here, sleep deprivation significantly ($p < 0.05$) increased activity of liver enzymes in model control mice when compared to control mice. However, supplementation with quercetin significantly ($p < 0.05$) reduced activity of liver enzymes when compared to model control mice.

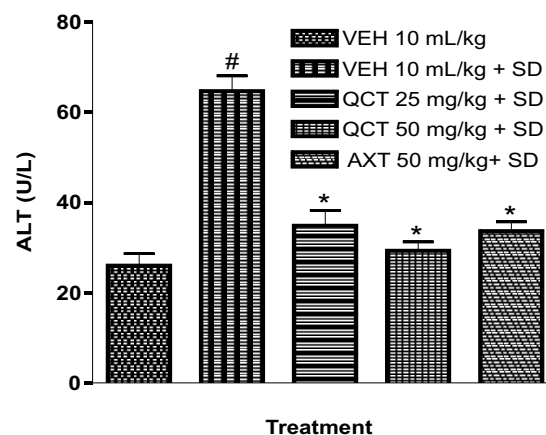


Figure 2

indicates significant difference ($p < 0.05$) compared to the control group.

* indicates significant difference ($p < 0.05$) compared to the model control group.

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

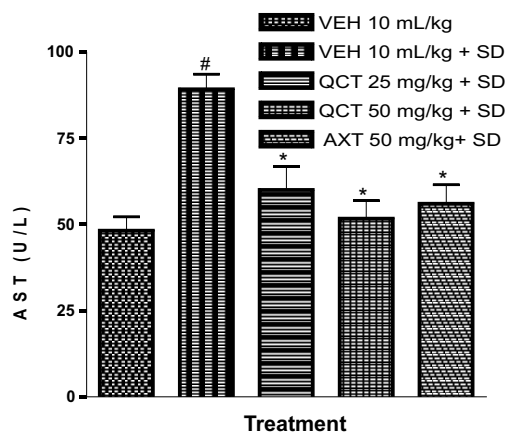


Figure 3

indicates significant difference ($p < 0.05$) compared to the control group.

* indicates significant difference ($p < 0.05$) compared to the model control group.

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

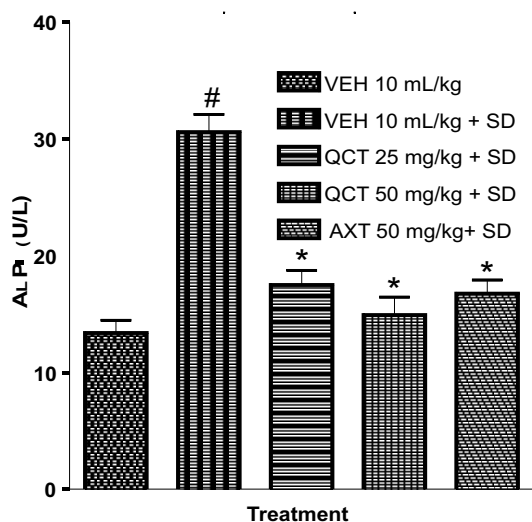


Figure 4 indicates significant difference ($p < 0.05$) compared to the control group. # indicates significant difference ($p < 0.05$) compared to the model control group. * indicates significant difference ($p < 0.05$) compared to the model control group. VEH: Vehicle, AXT: Astaxanthin, QCT: Quercetin, SD: Sleep deprivation.

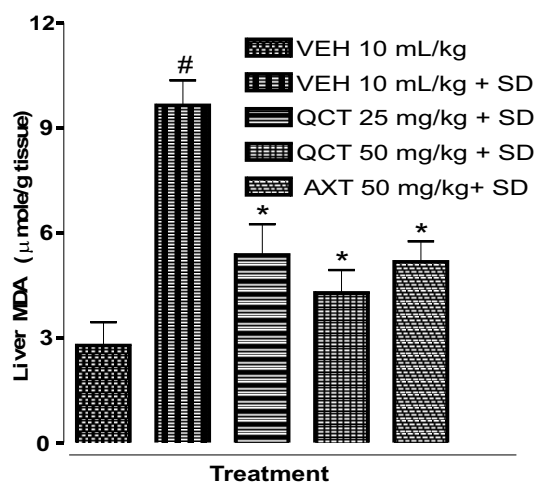


Figure 5 # indicates significant difference ($p < 0.05$) compared to the control group. * indicates significant difference ($p < 0.05$) compared to the model control group. VEH: Vehicle, AXT: Astaxanthin, QCT: Quercetin, SD: Sleep deprivation.

Change in liver MDA content

Liver MDA levels in the model control group significantly ($p < 0.05$) exceeded that of the control group. However, administration of quercetin resulted in a significant ($p < 0.05$) decrease in MDA levels. This reduction was significant for both high- and low-dose quercetin groups (Figure 5).

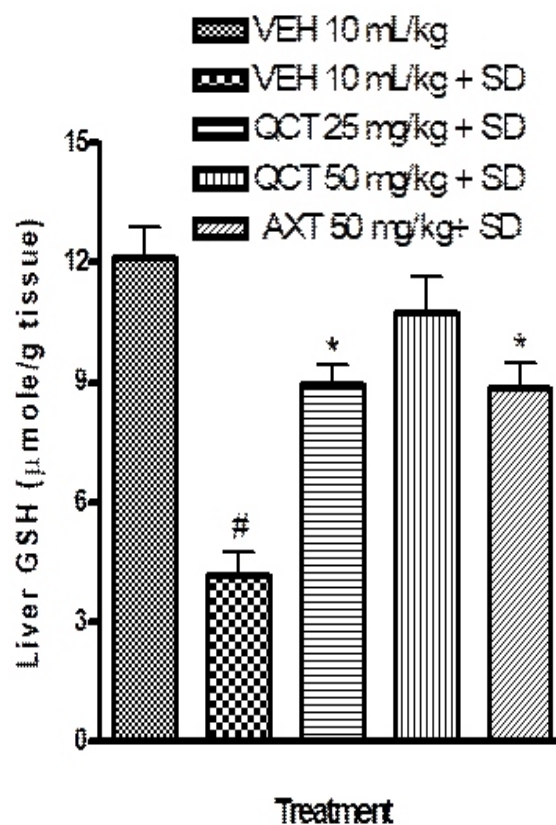


Figure 6 # indicates significant difference ($p < 0.05$) compared to the control group. * indicates significant difference ($p < 0.05$) compared to the model control group. VEH: Vehicle, AXT: Astaxanthin, QCT: Quercetin, SD: Sleep deprivation.

Change in liver GSH level

Liver GSH level was decreased significantly ($p < 0.05$) in liver tissue of model control group when compared to the control group. However, administration of quercetin significantly ($p < 0.05$) increased GSH activity when compared to the model control group (Figure 6).

Histology of hepatocytes

Figure 7 depicts the photomicrograph of the liver cells of mice after sleep deprivation. As shown below, quercetin supplementation protected the liver from the adverse morphometric changes caused by sleep deprivation on mice hepatocytes.

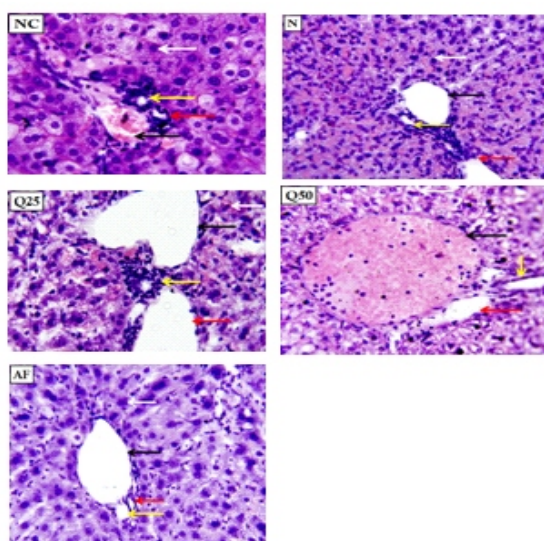


Figure 7

Slide NC: Control group. Slide N: Model control group. Slide Q25: Q₂₅ group. Slide Q50: Q₅₀. Slide AF: Positive control group. Key: White arrow= hepatocytes. Black arrow= portal vein. Red arrow= hepatic artery. Yellow arrow= Bile duct

DISCUSSION

The results of this study showed that quercetin significantly attenuated increases in blood glucose levels, suppressed hypertrophy of adrenal glands and liver, reversed histomorphological liver cell alterations, and decreased the activity of liver function enzymes in mice that were exposed to 72 hours sleep deprivation using a physical model.

Sleep is probably the most vital lifestyle component of all living organisms. The quality and quantity of sleep correlate with physical and mental health and general body homeostasis [15]. However, sleep disorders are closely correlated with co-morbid complications, such as type 2 diabetes mellitus and hypertension [16]. In this regard, numerous experimental studies have also demonstrated the relationship between sleep/sleep disorders and glucose intolerance/insulin resistance [17-19]. The results from the current study align with these literature by revealing that sleep deprived mice (i.e., model control group) had significantly higher blood glucose levels than the control group. This increase could be linked to the recorded significant increase in MDA content of sleep deprived mice than the control since previous studies have demonstrated that the liver which is the site of metabolism is particularly vulnerable to oxidative stress and excessive as well as prolonged stress has been shown to exert deleterious effect on the liver [14].

Nevertheless, response to any form of stress essentially involves activation of the HPA axis resulting in the release of adrenaline and steroids from adrenal glands [15,20]. The release of these chemical substances is meant to provide the needed energy for the organism to adapt or cope with the aversive stressful situations [21]. However, when the stress persists and becomes uncontrollable, there is breakdown in adaptive mechanisms resulting in

damage to various organs of the body^[14,22]. In the current study, exposure of mice to sleep deprivation resulted in increase in the weight of adrenal glands and liver, which suggest enlargement of these glands. This adrenal hypertrophy occurs in response to increased corticosterone secretion from cortical cells of the adrenal glands during persistent stress^[23]. The prevention of sleep deprivation-induced enlargement of the adrenal glands by quercetin further supports its potential activity as a compound that could be beneficial for the mitigation of sleep loss related complications. Also, sleep deprivation can injure the liver, and alter many enzymatic (e.g. ALT, AST, ALP) and non-enzymatic biochemical markers which are used as indices for liver damage. Serum ALT activity is one of the most commonly used markers for detecting liver damage^[24]. In the current study, sleep deprivation elevated serum ALT, AST, ALP levels in the sleep deprived mice, signalling liver damage. Additionally, the observed histomorphological alterations from the current study align with the changes described here.

Summary

Oxidative stress has been mixed up in the pathophysiology of various disorders. In spite of this, naturally acquired or artificially synthesized antioxidant compounds such as quercetin may prevent these disorders and thereby promote health. Taken together, food supplements such as quercetin possess the ability to attenuate liver damage in individuals usually exposed to insufficient sleep-induced stress. However, further studies are required to clarify the dose(s) of quercetin that will exert these beneficial effects.

Conflicting Interests

The authors declare no conflicting interests

Source of funding

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