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Original Research Article

Verapamil ameliorates sleep deprivation-induced hepatotoxicity in mice

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ABSTRACT

Background: Sleep deprivation has drastically increased among varying populations with current sleep polls showing that people are sleeping for fewer hours compared to a century ago. Existing literature have shown that sleep deprivation induces multi-organ injuries through oxidative stress. In view of this, present research was designed to investigate potential benefit of verapamil against liver dysfunction induced by paradoxical sleep deprivation in mice.
Methods: Thirty adult male Albino Swiss mice were divided into 5 groups, each consisted of six mice: group 1 was considered the vehicle group; group 2 was considered the model control which was sleep-deprived. Group 3 and 4 received verapamil (25 and 50 mg/kg; PO) and group 5 was given Astaxanthin (50 mg/kg; PO) in addition to being sleep-deprived. Samples of the liver were subjected to histopathological examinations after the restricted times (72 hours sleep deprivation). Serum biochemical parameters and relative weights of some organs were also assessed. One-way ANOVA followed by Newman-Keuls tests were used for statistical evaluation.
Results: The results showed that mice subjected to only sleep deprivation showed signs of liver injury indicated by: increased activity of liver enzymes, alteration of lipid profile parameters as well as an increase in pro-oxidants and a reduction in antioxidant biomarkers. However, verapamil caused a reduction in hepatocytes degeneration and delayed the occurrence of oxidative stress.
Conclusions: The present findings suggest that treatment with verapamil significantly reversed sleep deprivation-induced hepatic dysfunctions via its antioxidant mechanism.

Keywords: Sleep deprivation, Oxidative stress, Verapamil, Hepatotoxicity, Liver damage

INTRODUCTION

Good sleep is important for health and for staying active. Sleep is considered a cyclic, physiologic state of decreased responsiveness from which individuals arise spontaneously. Although formerly known as a negative phenomenon where watchfulness is switched off, it has now been recognized that it is an estate of forceful brain neural actions with substantial physiologic activity, to the degree that the brain is therefore considered more active during sleep than wakefulness.[1] Sleep is necessary for survival just as food, but the major function of sleep currently remains elusive. However, studies have consistently shown that sleep remains a vital process of life which is crucial for the maintenance of the body's homeostasis.[2,3]

Sleep deprivation is referred to as sleep loss or sleep time less than the average basal level of about 9 hours per night. A recent animal study has shown that rat life spans are decreased from 2 or 3 years to only 5 weeks if deprived of rapid eye movement; REM (also known as Paradoxical) sleep and to 2 or 3 weeks if deprived of total sleep.[4] The most common causes of sleep deprivation are often related to contemporary lifestyle and work-related factors as seen in shift workers; hence the condition affects a significant number of people.[5] Sleep deprivation leads to ineffectiveness in attention, learning, emotional regulation, and decision-making.[3-5] It is not only

debilitating in itself but has been linked with development of secondary neurological disorders.[6-8]

Different studies have shown that sleep deprivation is harmful to the health of an individual and can affect the consolidation of memory, alertness and mood, cardiovascular system, immune system, hormone, temperature, and glucose regulation.[9-11] Furthermore, research conducted by Buckley and Schatzberg revealed that hyperactivity of the hypothalamic-pituitary-adrenal (HPA) axis, which is the over-activation of hormonal interaction between a part of the brain and the adrenal gland, can impact sleep function-perhaps in response to stress-and subsequently increase secretion of cortisol and norepinephrine and further trigger the release of oxidative stress markers causing impairment of various physiological functions.[12-14] Consequently, a rise in generation of oxidative free radicals and impaired anti-oxidative defence system leads to the energy deficits and cell death in multiple organs. This imbalance in anti-oxidative parameters which could be direct consequence of sleep deprivation gives way to development of endocrine related disorders such as diabetes.[15,16]

Diabetes is a specific disease that affects the endocrine system's ability to produce the hormone insulin and has been reported to be affected by sleep.[16] According to the institute of medicine (IOM) committee on sleep medicine and research as reported by Colten and Altevogt, people who slept 6 hours per night were 1.7 times more prone to developing diabetes than their peers who slept longer.[9] Further investigative experiments have demonstrated that diabetes involves oxidative stress characterized by a significant excess of oxidant agents over antioxidants. Since calcium influx has been shown to activate protein kinase C (PKC) which plays a crucial role in many cellular functions and signal transduction pathways, PKC has also been implicated in the pathophysiology of diabetes via its induction of oxidative stress.[17] Therefore, calcium antagonists, such as verapamil, inhibit the action of angiotensin II, which is an enzyme responsible for the activation of PKC, thereby reducing oxidative stress.[17]

Verapamil which is a non-dihydropyridine calcium antagonist has been reported to show beneficial effects on the indices of oxidative stress in streptozotocin-induced diabetes.[17] The nephroprotective action of calcium antagonists is contributed by various factors, such as a diminished general hemodynamic load leading to a reduced hemodynamic load of the glomeruli, a direct effect of the drugs on glomerular basement membrane (GBM) resulting in decreased proteinuria and direct anti-proliferative and cytoprotective action. Experimental studies revealed that these drugs protect cell membranes from the attack of reactive oxygen species (ROS) and therefore inhibit lipid peroxidation in the membranes. The study also showed that verapamil reduced oxidative stress in the kidneys as depicted by a decreased malondialdehyde (MDA) level.[17]

Also, results from a histopathological study by Waer and colleagues suggested that treatment with verapamil significantly reversed hepatotoxic dysfunctions induced by carbon tetrachloride (CCl_4)and ethanol combination.[18] When hepatocytes were impaired by chemical poisons such as CCl_4 and ethanol, calcium ions (Ca^{2+}) increased causing cell depolarization, augmentation of excitability, and release of large amounts of inflammatory factor, thus resulting in activation of Kupffer cells and hepatic stellate cells (HSCs). Hepatocytes of plasma membrane contain Ca^{2+} channels which admit Ca^{2+} in response to receptor-operated channel agonists. The receptor-operated Ca^{2+} channels allow various divalent cations to enter the hepatocyte. Verapamil therefore inhibits Ca^{2+}entry into liver cells through its effect on receptor-operated channels. However, evidences exist in literature which shows that Verapamil blocks the initiating agent of hepatic fibrosis by inhibiting the activation of HSCs.[18,19] This drug also acts as a vasodilator and has effect on hepatic blood vessels as it prevents hypertension and hypoxia in liver cells; and as an angiotenic, it can increase the local blood circulation and oxygen supply. This in-turn results in inhibition of lipid peroxidation which is a major cellular mechanism involved in CCl_4 hepatotoxicity, thus limiting hepatonecrosis and fibrogenesis.[19] Also, a study conducted in 2010 by Bognár et al posited that verapamil has the ability to scavenge free radicals.[20] Therefore, this study was designed to evaluate the ameliorative potential of verapamil; a calcium channel blocker, on liver functionality in sleep deprived mice.

METHODS

Animals

Albino Swiss mice weighing 24.0±2.0 gm were utilized in the study. They were retrieved from the Animal House of the College of Health Sciences, Delta State University, Abraka and kept in transparent cages at ambient temperature with 12:12 h exposure to light and darkness. Rodent pellet and portable water were provided ad libitum throughout the course of the investigation, that is, from January-February 2020. The procedures adopted was in accordance with established guidelines of National Institute of Health.[21]

Equipment and apparatus

Spectrophotometer (Inesa, 752N), centrifuge (ATKE), pH meter (EDT instruments), water bath (Equitron), weighing balance (Ohaus), Eppendorf tubes, test tubes, tube racks, syringes, dissection kits and boards, oral cannula, blood glucose strips (Accu-Check Actives), and blood glucose meter (Roche Diagnostics India Pvt., Ltd).

Drugs/chemicals and sources

The following drugs and chemicals were used in this study: verapamil, astaxanthin, 5, 5′-dithio-bis(2-

nitrobenzoicacid) (DTNB; Aldrich, Germany), trichloroacetic acid (TCA; Burgoyne Burbidges and Co. Mumbai, India), thiobabituric acid (TBA; Guanghua chemical factory Co. Ltd., China), Tris-buffer (Hopkin and Williams company, USA), $NaHCO_3$ (BDH chemicals Ltd, Poole, England), acetic acid (Sigma-Aldrich, Inc., St Louis, USA), sodium $Na_2HPO_4.H_2O$ (BDH chemical Ltd, Poole, England), K_2HPO_4 (BDH Chemical Ltd, Poole, England), $NaH_2PO_4.H_2O$ (BDH Chemical Ltd, Poole, England), $K_2Cr_2O_7$ (BDH chemical Ltd, Poole, England), Carbonate (Fisons, Loughborough Leics, England), KCl (BDH chemical ltd, Poole, England) as well as the NaOH (J. T Baker chemicals co., Phillipsburg, N. J., USA).

Treatment groups

Briefly, 120 mg of verapamil was dissolved in 12mL of distilled water and the resultant stock solution was diluted further to obtain the desired concentration used. Also, astaxanthin was dissolved in olive oil. Experimental mice received oral (i.e., PO) doses of either verapamil (25 and 50 mg/kg), distilled water (10 ml/kg) or astaxanthin (50 mg/kg). Both doses of verapamil were selected based on pilot studies we performed.

The mice were randomly allotted into groups of 6 animals each: distilled water (10 ml/kg) was administered to groups 1 and 2 while both groups 3 and 4 received verapamil (25 and 50 mg/kg) and group 5; astaxanthin (50 mg/kg). Drug administration was from 9:00 to 10:00 am each day. The animals were treated for 7 successive days, only mice in groups 2 to 5 were exposed to a 3-day sleep deprivation starting from treatment day 4.

Sleep deprivation

The multiple platform-over-water model of sleep deprivation was used in this study. Multiple stands were attached to a platform that was suspended in water inside a plastic container. The container was filled with water, approximately 1 cm beneath the platform surface. The stands were 2 cm apart and each mouse was placed on the stands. According to Shinomiya et al mice, at the onset of REM sleep would fall into the water due to muscle relaxation and after falling; they struggle to locate the platform.[22] Although this procedure principally targets REM sleep, a significant amount of stress is unavoidable.[23] Pre-treated mice in each group were sleep deprived except for animals in group 1. After the 3-day sleep deprivation period, mice from each group were euthanized and prepared for further analysis.

Measurement of blood glucose level

A blood glucose strip was slot into the blood glucose meter and blood (obtained from the tail of each mouse) was dropped on the strip. Instantly, the value of blood glucose level (in mmHg) was displayed as well as recorded.

Estimation of organ weights

Liver and adrenal gland of each mouse was excised and measured using a weighing scale. Organ weights were estimated as weight/100 g per body weight.

Liver function

Each mouse blood was obtained via ocular puncture. To obtain serum for liver function assay, blood was centrifuged for 15 min with the aid of a cold centrifuge set at 10,000 rpm and 4^{o}C.

Total bilirubin estimation

Bilirubin level was estimated by colorimetry using Randox assay kit according to prescribed protocol by the manufacturer. The Lo and Wu assay protocol was also adopted with slight modifications.[24] Approximately 0.2 ml of sample was mixed with 3 ml of working solution (made up of 0.7 mmol/l dilute hydrochloric acid, pH 0.7; containing 2.5 mmol of sodium nitrite, 10 mmol of sulfanilic acid, 1.0 mol of citric acid, 0.5 mol of caffeine, 3.0 mol of urea, and 0.5 g of surfactant) for the test and the sample blank respectively. Afterwards, the mixture was incubated at a wavelength of 578 nm for 5 min at 25 ^{o} C set against sample blank.

Alanine aminotransferase test

Alanine aminotransferase (ALT) activity was estimated using a spectrophotometer. About 0.1 ml of serum from each mouse was added to 0.5 ml solution comprising L-alanine (200 mmol/l), α-oxoglutarate (2 mmol/l), and 100 mmol/l of sodium phosphate buffer (pH-7.4). The mixture was made to stand at 37˚C for 30 min and 0.5 ml 2,4-dinitrophenylhydrazine (2 mmol/l) was added and incubated at 25˚C for 20 min. Afterwards, 5.0 ml sodium hydroxide (0.4 mmol/l) was added. Absorbance was set against the blank at 546 nm for a 5 min duration.[25] The calculated value of ALT was expressed in U/l.

Aspartate aminotransferase test

Aspartate aminotransferase (AST) activity was determined using a spectrophotometer. About 0.1 ml of serum from each mouse was added to 0.5 ml solution comprising L-alanine (200 mmol/l), α-oxoglutarate (2 mmol/l), and sodium phosphate buffer (100 mmol/l, pH 7.4). The mixture was kept at 37˚C for 30 min and 0.5 ml 2, 4-dinitrophenylhydrazine (2 mmol/l) was added and incubated at 25˚C for 20 min. Afterwards, 5.0 ml sodium hydroxide (0.4 mmol/l) was added. Absorbance was set against the blank at 546 nm for a 5 min duration.[25] The AST estimated value was expressed in U/l.

Alkaline phosphatase determination

Alkaline phosphatase (ALP) activity was determined using a spectrophotometer. Approximately 0.05 ml of each mouse serum was added to 0.5 ml solution containing magnesium chloride (0.5 mmol/l) and di-

ethanolamine buffer in a test tube. Thereafter, 3.0 ml of 10 mmol/l p-nitrophenylphosphate was added to the mixture and incubated at 25˚C. After that, absorbance was read at different times (0, 1, 2 and 3 min) respectively at 405 nm.[25] The value of ALP was expressed in U/l.

Lipid profile

Briefly, blood was obtained from each mouse through ocular puncture. Serum for lipid profile test was gotten from blood via centrifuging for 15 min with the aid of a cold centrifuge set at 10,000 rpm and 4ºC.

Evaluation of serum cholesterol level

Evaluation of serum cholesterol level was carried out by enzymatic colorimetric method using the Spinreact assay kit according to the prescribed protocol by the manufacturer. In line with the method of Young and Friedman, the reagent (comprising peroxidase, cholesterol esterase, 4-aminophenazone and cholesterol oxidase) was mixed with the buffer to obtain the working reagent.[26] Next, 1.0 ml of this working reagent was added serum (10 µL) or cholesterol standard, and the mixture was incubated for 10 min at ambient room temperature. Afterwards, absorbance (Abs) of the samples and standard was read versus the blank at 500 nm. The concentration (in mg/dl) of cholesterol in the sample was calculated using the following equation: [(Abs) sample-(Abs) blank/(Abs) standard-(Abs) blank]×200 (Standard conc.)

Serum triglyceride

Triglyceride level was assessed by enzymatic colorimetry with the aid of Randox assay kit according to the manufacturer's protocol. In line with Bucolo and David's method,[27] the reagent (1 ml) was mixed with sample or standard (10 µL) and the resulting mixture was incubated at 37ºC for 5 min. Absorbance (Abs) was measured at 500 nm. The equation below was used to estimate total serum triglyceride concentration (mg/dl): (Abs) sample/ (Abs) standard×200 (Standard conc.)

High-density lipoproteins

Enzymatic colorimetry using the Randox assay kit was also employed in the determination of total serum level of high-density lipoprotein (HDL). About 1.0 ml of the reagent was added to 10 µL of the sample/standard and the mixture was incubated at 37ºC for 10 min.[27] thereafter, absorbance (Abs) was measured at 500 nm. The following equation was used to estimate HDL concentration (mg/dl) in the sample: (Abs) sample/ (Abs) standard×200 (Standard conc.).

Biochemical assay

Also, mice livers were excised, weighed and homogenized. The supernatants were utilized for the determination of oxidative stress parameters.

Glutathione

Glutathione (GSH) concentration was determined by the method described by Pratibha and his colleagues with slight modifications.[28] Liver supernatants (0.25 ml) were mixed with 2.0 ml of 0.6 mM DTNB and the final volume was made up to 3 ml with phosphate buffer, respectively. The absorbance was then read at 412 nm against a blank reagent using a spectrophotometer. The estimated value of GSH concentration in the liver tissues was expressed in µmol/g tissue.

Malondialdehyde (MDA)

Malondialdehyde is a biomarker of lipid peroxidation, which is a notable mechanism of oxidative stress. Its concentration was determined according to the method outlined by Adam-Vizi and Seregi.[29] About 0.5 ml of 30% TCA was added to 0.4 ml of the supernatant and 1.6 ml Tris-KCl buffer mixture. Also, 0.5 ml TBA was added and the resultant mixture was placed in a water bath of 80°C for 45 min. After cooling in ice, the mixture was centrifuged at 3,000 rpm for 15 min. Finally, the absorbance of the clear supernatant was measured against a reference blank of distilled water at 532 nm. The molar extinction coefficient of 1.56×10^5 M^{-1} cm^{-1} was used to calculate MDA concentration and values were in µmol/g tissue.

Superoxide dismutase (SOD)

About 0.1 ml of liver supernatant was added to 2.6 ml of 0.05 M carbonate buffer. Then, 0.3 ml of freshly prepared adrenaline (0.3 mM) was added to the mixture. Absorbance was determined using a spectrophotometer at 480 nm and monitored for 3 min (at 60s intervals). The SOD activity was expressed as units of adrenaline per min/mg protein.[30]

Catalase (CAT)

About 0.1 ml of liver supernatant was added to 2.0 ml sodium phosphate buffer and 0.9 ml H_2O_2 (800 µmol). Then, 1.0 ml of this mixture was added to 2.0 ml dichromate/acetic acid reagent. Afterwards, absorbance was read at 570 nm. The catalase activity was expressed as µmol of H_2O_2 decomposed/min/mg protein.[31]

Nitric oxide (NO)

Greiss reagent, which was used in this study, is an indicator of NO production. About, 100 µL of Greiss reagent was mixed with 100 µL of the liver supernatant and absorbance was read at 540 nm.[32] Nitrite level was assessed from a standard curve obtained from sodium nitrite (0-100 uM).

Histology

The excised livers of mice in the respective groups were fixed with 10% phosphate buffered formaldehyde and

then subjected to the routine method for paraffin wax embedment to obtain paraffin wax embedded tissue blocks. Transverse sections (5-6 µm thick) of the liver were obtained with the aid of a microtome (Leica, Germany). Finally, these sections were fixed on glass slides, examined and photographed.

Statistical analysis

All statistical data are presented as mean ± SEM. One-way ANOVA and post-hoc tests (Student's Newman-Keuls) were carried out to obtain the level of significance using Graph Pad InStat® Biostatistics software. The level of significance was set at $\alpha_{0.05}$.

RESULTS

Verapamil on blood glucose levels

As shown in Figure 1, glucose concentration in blood serum of sleep-deprived animals was considerably higher than in the vehicle group. Verapamil significantly affected the elevated glucose concentration in a dose-dependent pattern.

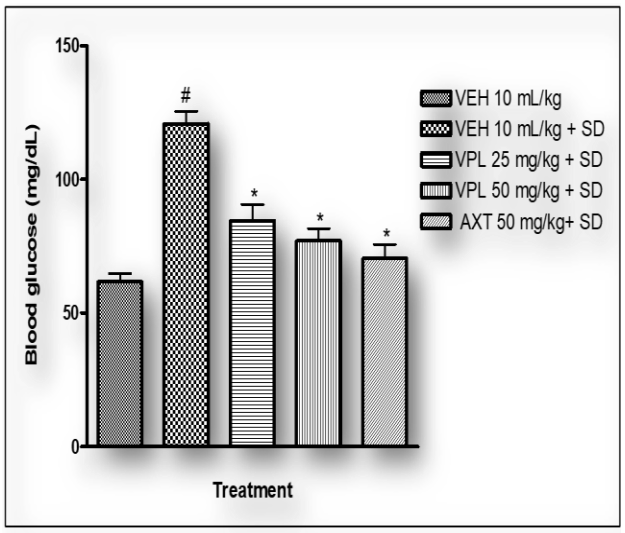

Figure 1: Effect of verapamil on blood glucose levels in mice subjected to sleep deprivation.

#Indicates statistical difference (p<0.05) compared to the vehicle (not sleep deprived) group. *Indicates statistical difference (p<0.05) compared to the vehicle + SD group. VEH: Vehicle VPL: Verapamil SD: Sleep deprivation AXT: Astaxanthin.

Verapamil on relative organ weights

As shown in Table 1 the liver and adrenal gland weights were significantly higher in the sleep-deprived group than in the vehicle group after experiment. In verapamil treated group, liver weight was significantly lower.

Table 1: Effect of verapamil on organ weights of sleep deprived mice.

Treatment	Organ wt (g/100 g body wt)	
	Adrenal gland (mg)	Liver ($X10^3$mg)
VEH 10 ml/kg	3.53±0.64	0.89±0.05
VEH 10 ml/kg ± SD	7.23±0.56#	1.12±0.03#
VPL25 mg/kg ± SD	4.68±0.39*	0.98±0.02*
VPL 50 mg/kg ± SD	4.08±0.27*	0.93±0.05*
AXT 50 mg/kg ± SD	3.67±0.53*	0.96±0.04*

#Indicates statistical difference (p<0.05) compared to the vehicle (not sleep deprived) group. *Indicates statistical difference (p<0.05) compared to the vehicle + SD group. VEH: Vehicle VPL: Verapamil SD: Sleep deprivation AXT: Astaxanthin.

Verapamil on serum lipid profile

Serum cholesterol and high-density lipoproteins level of sleep-deprived mice was significantly lower with respect to the vehicle group. Treatment of sleep-deprived mice with verapamil resulted in a significant increase of cholesterol and high-density lipoproteins with respect to the sleep-deprived mice.

Also, a significant increase of serum triglycerides level was found in the sleep-deprived group compared to the control group. However, after being treated with verapamil, a significant decrease of the serum triglycerides level compared to the sleep deprived group, was noted. These effects are shown in Table 2.

Table 2: Effect of verapamil on serum lipid profile in mice subjected to sleep deprivation-induced stress.

Treatment	Cholesterol (mg/dl)	Triglyceride (mg/dl)	High density lipoprotein (mg/dl)
VEH 10 ml/kg	119.40±6.26	110.00±2.68	118.30±2.11
VEH 10 ml/kg±SD	115.00±2.80#	166.30±5.76#	88.00±4.03#
VPL 25 mg/kg±SD	116.90±4.55*	151.40±3.95*	97.50±3.55*
VPL 50 mg/kg±SD	118.50±2.14*	141.60±4.01*	102.70±3.34*
AXT 50 mg/kg±SD	117.90±4.54*	149.90±3.41*	104.00±2.37*

#Indicates statistical difference (p<0.05) compared to the vehicle (not sleep deprived) group. *Indicates statistical difference (p<0.05) compared to the vehicle + SD group. VEH: Vehicle VPL: Verapamil SD: Sleep deprivation AXT: Astaxanthin.

Verapamil on liver oxidative parameters

Malondialdehyde (MDA) content of the liver of sleep-deprived group was significantly higher compared to the vehicle group. Treatment of sleep-deprived mice with verapamil resulted in a significant decrease of MDA content when compared to the sleep-deprived group.

Superoxide dismutase (SOD) activity in the liver of sleep-deprived group was significantly diminished compared to the vehicle group. After treatment, the mice given verapamil showed a significant increase in the activity of this enzyme.

In the sleep-deprived group, a significant decrease of hepatic catalase (CAT) activity was found when compared to the vehicle group. In the groups treated with verapamil, a significant increase of CAT activity in the liver, in comparison with the sleep deprived group, was noted after the experiment.

Glutathione (GSH) concentration in the liver was significantly lower in sleep-deprived mice with respect to the vehicle group. Verapamil treatment resulted in a significant increase in the concentration of this antioxidant, with respect to sleep-deprived mice.

Liver nitrite level in sleep deprived mice was significantly higher than in the vehicle group. Verapamil treatment resulted in a significant decrease in nitrite levels when compared to the sleep deprived group. These are outlined in Table 3 below.

Table 3: Effect of verapamil on liver oxidative parameters in mice subjected to sleep deprivation-induced stress.

Treatments	GSH (µmol/g tissue)	CAT (units/mg protein)	SOD (units/ mg protein)	MDA (µmol/g tissue)	Nitrite (µM)
VEH 10 ml/kg	12.10±0.79	65.80±5.15	58.14±3.72	2.78±0.66	34.62±5.71
VEH 10 ml/kg ± SD	4.15±0.59$^{\#}$	26.28±3.21$^{\#}$	27.64±2.9$^{\#}$	9.64±0.72$^{\#}$	81.13±4.85$^{\#}$
VPL 25 mg/kg ± SD	6.80±0.45*	41.69±2.93*	37.65±2.43*	6.54±0.38*	62.34±4.25*
VPL 50 mg/kg ± SD	8.04±0.38*	46.28±3.95*	43.67±3.82*	5.60±0.22*	53.79±4.09*
AXT 50 mg/kg ± SD	8.83±0.66*	48.23±4.31*	45.35±3.90*	5.17±0.59*	49.41±5.15*

#Indicates statistical difference ($p<0.05$) compared to the vehicle (not sleep deprived) group. *Indicates statistical difference ($p<0.05$) compared to the vehicle + SD group. VEH: Vehicle VPL: Verapamil SD: Sleep deprivation AXT: Astaxanthin.

Verapamil on alanine aminotransferase (ALT)

As shown in Figure 2, sleep deprivation caused a very significant increase of serum ALT in sleep-deprived mice when compared to the vehicle group. However, administration of verapamil significantly diminished ALT activity in a dose-dependent pattern when compared to the sleep-deprived group.

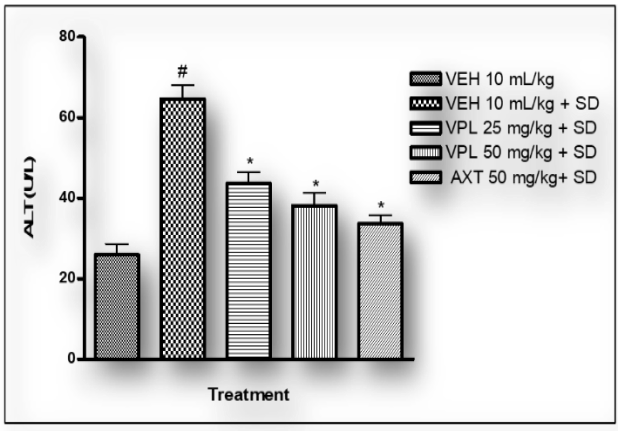

Figure 2: Effect of verapamil on alanine aminotransferase in mice subjected to sleep deprivation.

#Indicates statistical difference ($p<0.05$) compared to the vehicle (not sleep deprived) group. *Indicates statistical difference ($p<0.05$) compared to the vehicle + SD group. VEH: Vehicle VPL: Verapamil SD: Sleep deprivation AXT: Astaxanthin.

Verapamil on aspartate aminotransferase (AST)

As shown in Figure 3, sleep deprivation caused a significant increase of serum AST in sleep-deprived mice when compared to the vehicle group. However, administration of verapamil significantly diminished AST activity when compared to the sleep-deprived group.

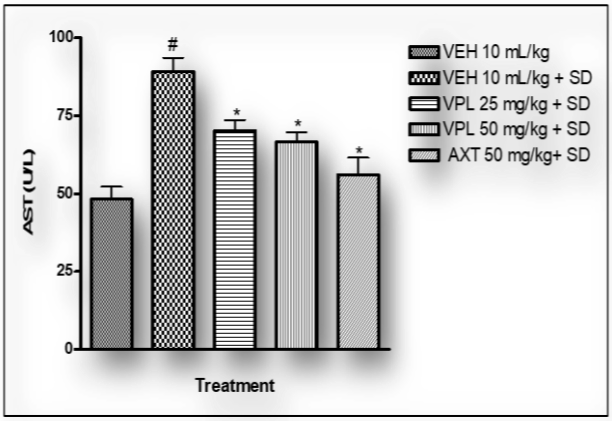

Figure 3: Effect of verapamil on aspartate aminotransferase in mice subjected to sleep deprivation.

#Indicates statistical difference ($p<0.05$) compared to the vehicle (not sleep deprived) group. * Indicates statistical difference ($p<0.05$) compared to the vehicle + SD group. VEH: Vehicle VPL: Verapamil SD: Sleep deprivation AXT: Astaxanthin.

Verapamil on alkaline phosphatase

As shown in Figure 4, sleep deprivation caused a highly significant increase of serum ALP in sleep-deprived mice when compared to the vehicle group. However, administration of verapamil significantly diminished ALP activity in a dose-dependent pattern when compared to the sleep-deprived group.

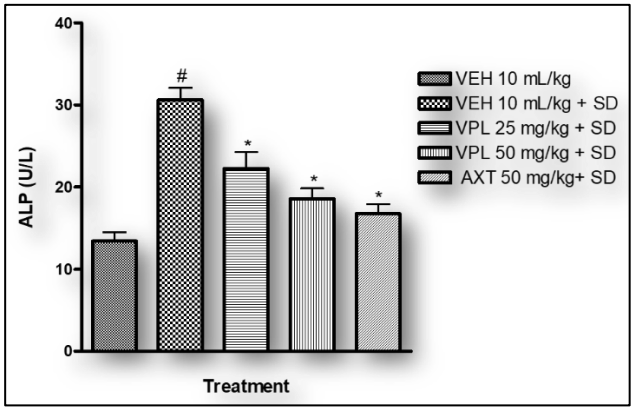

Figure 4: Effect of verapamil on alkaline phosphatase in mice subjected to sleep deprivation.

#Indicates statistical difference ($p<0.05$) compared to the vehicle (not sleep deprived) group. *Indicates statistical difference ($p<0.05$) compared to the vehicle + SD group. VEH: Vehicle VPL: Verapamil SD: Sleep deprivation AXT: Astaxanthin.

Verapamil on total bilirubin content

As shown in Figure 5, hepatic bilirubin content in mice was significantly increased in the sleep deprivation group compared to the vehicle only group. Administration of verapamil caused a significant decrease in bilirubin content of the liver in a dose-dependent pattern when compared to the sleep-deprived group.

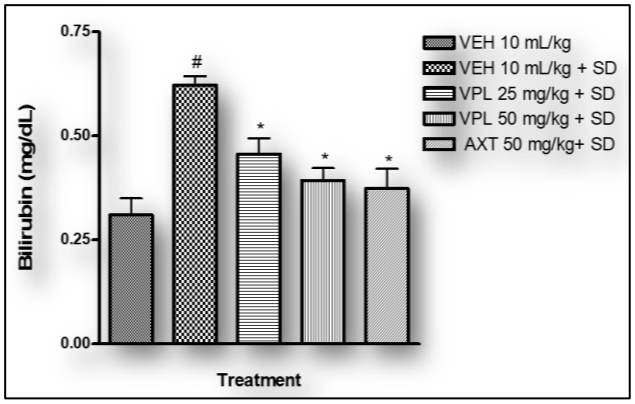

Figure 5: Effect of verapamil on total bilirubin in mice subjected to sleep deprivation.

#Indicates statistical difference ($p<0.05$) compared to the vehicle (not sleep deprived) group. *Indicates statistical difference ($p<0.05$) compared to the vehicle + SD group. VEH: Vehicle VPL: Verapamil SD: Sleep deprivation AXT: Astaxanthin.

Photomicrograph of the liver cells of mice after sleep deprivation

Hematoxylin and Eosin (H and E) staining of liver cells was used in this histological study. As shown in Figure 6, livers from vehicle group mice indicated a normal architecture. In contrast, the sleep-deprived group showed remarkable structural changes such as thickness of portal vein and shrinking of hepatocytes. Finally, it can be observed that a higher dose of verapamil (50 mg) proved to be more effective than 25 mg in reversing these detrimental effects of sleep deprivation on the liver.

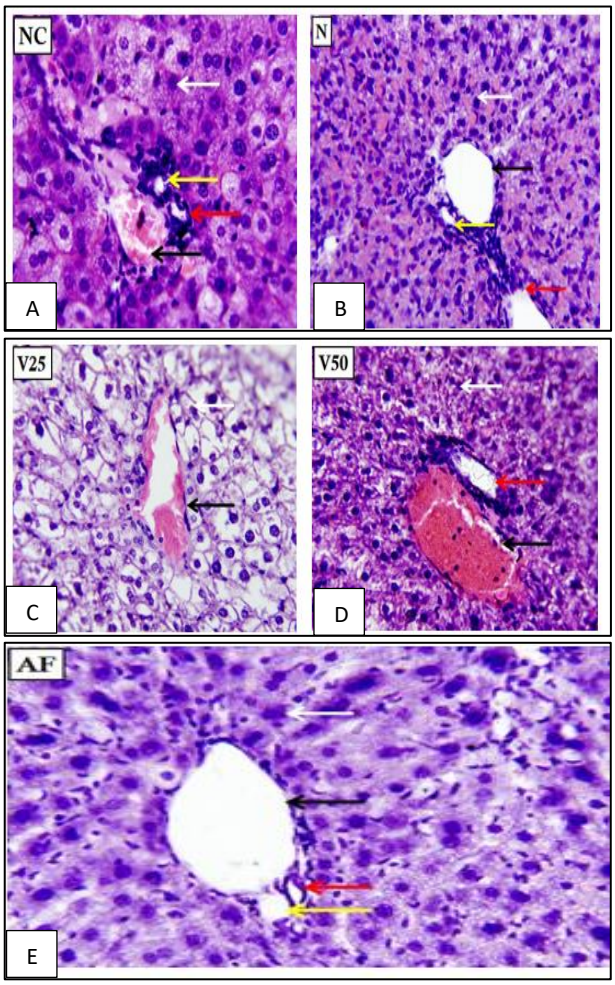

Figure 6 (A-E): Photomicrograph of liver cells of mice after sleep deprivation.

NC: Vehicle only. N: Vehicle+ SD. V25: Verapamil 25 mg/kg+ SD. V50: Verapamil 50 mg/kg + SD AF: Astaxanthin 50 mg/kg + SD. Magnification: (X400). White arrow= hepatocytes; black arrow=portal vein; red arrow=hepatic artery; yellow arrow=bile duct.

DISCUSSION

Several rodent sleep deprivation models have previously been described. Our model was selected based on evidence from literature that gentle stimulation with physical contact in mice is a preferred technique of sleep deprivation rather than genetic modification.[33,34]

The result from the present experiment indicates that sleep deprivation induced moderate hepatotoxicity in mice via significant alterations in liver enzyme activity and function. This is similar to the results of a study by Periasamy et al in which sleep deprivation was found to affect mice liver via mechanisms related to oxidative stress and inflammation.[35]

In that same study, melatonin (the sleep hormone) reduced hepatic injury and inflammation by lowering serum aspartate aminotransferase (AST), alanine aminotransferase (ALT), liver total cholesterol, and triglycerides in high-fat diet fed rats. So, a disturbance in the levels of melatonin in blood, as observed in sleep deprivation, would affect its functions and aggravate oxidative stress hence causing hepatic dysfunction.[15] Increased oxidative stress and insufficient antioxidant activities may therefore result in liver cell injury. The generation of free radicals can hence be considered as one of the mechanisms of toxicity caused by sleep deprivation.

In the present study, 3 days of sleep deprivation was associated with the development of increased hepatic glucose production, indicative of hepatic insulin resistance. Sleep deprivation was associated with significant increase in blood glucose level compared with the vehicle group. This is in line with a study by Shigiyama et al.[34] Also, previous studies reported that sleep deprivation leads to insulin resistance in rodents and humans.[36-39] In addition, a study by Hsieh et al reported the association of short sleep duration with fatty liver in humans.[40] In general, sleep deprivation seems to induce increased hepatic glucose production.

Interestingly, the 3-day sleep deprivation used in the present study significantly increased hepatic triglyceride content when compared with the vehicle group. There seems to be an increase in lipid oxidation in our acute sleep deprivation model. In this regard, a previous review suggested that patients with fatty liver presented with both increased triglyceride synthesis and increased lipid oxidation.[41]

In addition, Bugianesi et al earlier reported that hepatic lipid content, assessed in liver biopsies, was significantly related with lipid oxidation in patients with non-alcoholic fatty liver disease.[42] Therefore, lipid accumulation in the liver seems to be associated with increased hepatic lipid oxidation. According to Vatner and his colleagues, this could result from excessive delivery of circulating fatty acids to the liver; part of which would be oxidized, and the remainder esterified to triglycerides in the liver.[43]

Biochemical assays of the present study reveal that the serum activity of AST and ALT was significantly increased in sleep-deprived mice. Both enzymes are considered potent indicators of hepatotoxicity. Usually, AST levels fluctuate in response to the extent of cell death; therefore, it may be slightly elevated early in liver-related disease processes, and extremely elevated during the most acute phase of such disease.[43] Despite the obvious indication of hepatotoxicity, verapamil was found to significantly reduce the activity of both enzymes thereby attenuating hepatotoxicity.

Although no significant changes in cholesterol levels across treatment groups was observed in the present study, a former study by Adeneye and his colleagues reported that the ability of verapamil to chelate minerals has some protective effect such as decreasing iron-mediated free radical formation, lowering serum cholesterol and lipid peroxides in the experimental animals.[44]

In the present study, liver histomorphometric presentations showed that verapamil decreased the toxic effect of sleep deprivation on hepatocytes of mice. Both doses of verapamil reduced the collagen fibres, infiltrations, steatosis; improved the general architecture of hepatocytes and shape of nucleus.

The results are in accordance with earlier observations which found that verapamil reduced hepatocytes degeneration, necrosis and delayed the formation of liver fibrosis in rats and also confirmed that complex factors (such as insufficient sleep, ethanol, and high-fat diet) caused severe histopathological changes such as steatosis, macrophage infiltration and liver fibrosis which were evident in the disruption of tissue architecture, extension of fibres and accumulation of collagen.[45]

CONCLUSION

It is well known that calcium has an important role in a number of cell functions, such as differentiation, proliferation, contraction, migration, apoptosis and protein synthesis. The present study demonstrated that verapamil probably suppressed hepatotoxicity by inhibiting calcium ion (Ca^{2+}) influx into hepatocytes through its effect on receptor-mediated channels and scavenging free radicals or inhibiting lipid peroxidation, which is a major cellular mechanism involved in hepatotoxicity. In conclusion, this study found that verapamil has an ameliorating effect on liver dysfunction induced by sleep deprivation.

However, more in-depth investigations are required to ascertain the pharmacology of verapamil in various sleep deprivation models since this study could not investigate further due to insufficient funding.

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REFERENCES

1. El Shakankiry HM. Sleep physiology and sleep disorders in childhood. Nature Sci Sleep. 2011;3:101-14.
2. Kumar VM. Sleep and sleep disorders. Indian J Chest Dis Allied Sci. 2008;50:129-36.
3. Davis KF, Parker KP, Montgomery GL. Sleep in infants and young children. Part one: normal sleep. J Pediatr Health Care. 2004;18(2 Pt 1):65-71.
4. Bathory E, Tomopoulos S. Sleep regulation, physiology and development, sleep duration and patterns, and sleep hygiene in infants, toddlers, and preschool-age children. Curr Probl Pediatr Adolesc Health Care. 2016;4(3):1-14.
5. Orzel-Gryglewska J. Consequences of sleep deprivation. Int J Occupational Med Environmental Health. 2010;23(1):95-114.
6. Mathangi DC, Shyamala R, Subhashini AS. Effect of REM sleep deprivation on the antioxidant status in the brain of Wistar rats. Ann Neurosci. 2012;19:161.
7. Martins PJF, Nobrega JN, Tufik S, D'Almeida V. Sleep deprivation-induced gnawing-relationship to changes in feeding behavior in rats. Physiol Behav. 2008;93:229-34.
8. Ward CP, McCarley RW, Strecker RE. Experimental sleep fragmentation impairs spatial reference but not working memory in Fischer/Brown Norway rats. J. Sleep Res. 2009;18:238-44.
9. Harvey CR, Bruce AM. [edi] Sleep Disorders and Sleep Deprivation: An Unmet Public Health Problem. Institute of Medicine, Committee on Sleep Medicine and Research. Washington, DC: National Academy of Sciences Press. 2006.
10. Lund HG, Reider BD, Whiting AB, Prichard JR. Sleep patterns and predictors of disturbed sleep in a large population of college students. J Adolescent Health. 2010;46(2):124-32.
11. Plaford GE. Sleep and learning: The magic that makes us healthy and smart. Maryland: Lanham: Rowman and Littlefield. 2009.
12. Buckley TM, Schatzberg AF. On the interactions of the hypothalamic-pituitary-adrenal (HPA) axis and sleep: normal HPA axis activity and circadian rhythm, exemplary sleep disorders. J Clin Endocrinol Metab. 2005;90:3106-14.
13. Shansky RM, Lipps J. Stress-induced cognitive dysfunction: hormone-neurotransmitter interactions in the prefrontal cortex. Front Hum Neurosci. 2013;7:123.
14. Wright KP Jr., Drake AL, Frey DJ, Fleshner M, Desouza CA. Influence of sleep deprivation and circadian misalignment on cortisol, inflammatory markers, and cytokine balance. Brain Behav Immun. 2015;47:24-34.
15. Taha M, Rady HY, Olama NK. Effect of sleep deprivation on the liver, kidney and heart: histological and immunohistochemical study. Int J Sci Rep. 2018;4(7):172-81.
16. Chanana P, Kumar A. Further investigations on the neuroprotective potential of centella asiatica against sleep deprivation induced anxiety like behaviour: possible implications of mitoprotective and anti-stress pathways. J Sleep Disor: Treat Care. 2017;6(2):1-9.
17. Kedziora-Kornatowska K, Szram S, Kornatowski T, Szadujkis-Szadurski L, Kedziora J, Bartosz G. The effect of verapamil on the antioxidant defence system in diabetic kidney. Clinica Chimica Acta. 2002;322:105-12.
18. Waer HF, Nomani NA, Elbealy ER. Ameliorated effects of verapamil on hepatotoxicity induced by ethanol and carbon tetrachloride. J Cytol Histol. 2012;3:142.
19. Shafik AN, Khodeir MM, Gouda NA, Mahmoud ME. Improved antifibrotic effect of a combination of verapamil and silymarin in rat-induced liver fibrosis. Arab J Gastroenterol. 2011;12:143-9.
20. Bognár B, Ahmed S, Kuppusamy ML, Selvendiran K, Khan M et al. Synthesis and study of new paramagnetic and diamagnetic verapamil derivatives. Bioorg Med Chem. 2010;18:2954-63.
21. Ward JW, Elsea JR. Animal case and use in drug fate and metabolism. In: Edward, R. Garrette Hirtz, Jean L. Hirtz, Marcel Dekker, Eds. Methods and Techniques, vol 1, New York. 1997;372-90.
22. Shinomiya K, Shigemoto Y, Okuma C, Mio M, Kamei C. Effects of short-acting hypnotics on sleep latency in rats placed om grid suspended over water. Eur J Pharmacol. 2003;460:139-44.
23. Machado RB, Hipolide DC, Benedito-Silva AA, Tufik S. Sleep deprivation induced by the modified multiple platform technique: Quantification of sleep loss and recovery. Brain Res. 2004;1004:45-51.
24. Lo DH, Wu TW. Assessment of the fundamental accuracy of the Jendrassik-Grof total and direct bilirubin assays. Clin Chem. 1983;29(1):31-6.
25. Steven RB. Q Methodology and Qualitative Research. Qualitative Health Res. 1996.
26. Young DS, Friedman RB. Effects of disease on clinical laboratory tests, AACC PRESS, Washington, DC, USA. 1995.
27. Bucolo G, David H. Quantitative determination of serum triglycerides by use of enzymes. Clin Chem. 1973;19:476-82.
28. Pratibha R, Sameer R, Rataboli PV, Bhiwgade DA, Dhume CY. Enzymatic studies of cisplatin induced oxidative stress in hepatic tissue of rats. Eur J Pharmacol. 2006;532(3):290-3.
29. Adam-Vizi V, Seregi M. Receptor dependent stimulatory effect of noradrenaline on Na+/K+ ATPase in rat brain homogenate. Role of lipid peroxidation. Biochem Pharmacol. 1982;31:2231-6.
30. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem. 1972;247(10):3170-5.
31. Sinha AK. Colorimetric assay of catalase. Analytical Biochem. 1972;47:389-94.

32. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR. Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal Biochem. 1982;126(1):131-8.
33. Ferrell JM, Chiang JY. Short-term circadian disruption impairs bile acid and lipid homeostasis in mice. Cell Mol Gastroenterol Hepatol. 2015;1:664-77.
34. Shigiyama F, Kumashiro N, Tsuneoka Y, Igarashi H, Yoshikawa F, Kakehi S et al. Mechanisms of sleep deprivation-induced hepatic steatosis and insulin resistance in mice. Am J Physiol Endocrinol Metab 2018;315:E848-58.
35. Periasamy S, Hsu D, Fu Y, Liu M. Sleep deprivation-induced multi-organ injury: role of oxidative stress and inflammation. XCLI J. 2015;14:672-83.
36. Baud MO, Magistretti PJ, Petit JM. Sustained sleep fragmentation affects brain temperature, food intake and glucose tolerance in mice. J Sleep Res. 2013;22:3-12.
37. De Oliveira EM, Visniauskas B, Sandri S, Migliorini S, Andersen ML, Tufik S et al. Late effects of sleep restriction: potentiating weight gain and insulin resistance arising from a high-fat diet in mice. Obesity (Silver Spring). 2015;23:391-8.
38. De Bernardi Rodrigues AM, Da Silva CC, Vasques AC, Camilo DF, Barreiro F, Cassani RS et al. Brazilian Metabolic Syndrome Study (BRAMS) Investigators: Association of sleep deprivation with reduction in insulin sensitivity as assessed by the hyperglycemic clamp technique in adolescents. JAMA Pediatr. 2016;170:487-94.
39. Rao MN, Neylan TC, Grunfeld C, Mulligan K, Schambelan M, Schwarz JM. Subchronic sleep restriction causes tissue-specific insulin resistance. J Clin Endocrinol Metab. 2015;100:1664-71.
40. Hsieh SD, Muto T, Murase T, Tsuji H, Arase Y. Association of short sleep duration with obesity, diabetes, fatty liver and behavioural factors in Japanese men. Intern Med. 2011;50:2499-502.
41. Saponaro C, Gaggini M, Carli F, Gastaldelli A. The subtle balance between lipolysis and lipogenesis: a critical point in metabolic homeostasis. Nutrients. 2015;7:9453-74.
42. Bugianesi E, Gastaldelli A, Vanni E, Gambino R, Cassader M, Baldi S et al. Insulin resistance in non-diabetic patients with non-alcoholic fatty liver disease: sites and mechanisms. Diabetologia. 2005;48:634-42
43. Vatner DF, Majumdar SK, Kumashiro N, Petersen MC, Rahimi Y, Gattu AK et al. Insulin-independent regulation of hepatic triglyceride synthesis by fatty acids. Proc Natl Acad Sci USA. 2015;112:1143-8.
44. Adeneye AA, Benebo AS, Agbaje EO. Protective effect of the aqueous leaf and seed extract of Phyllanthusamaruson alcohol-induced hepatotoxity in rats. West Afr J Pharmacol Drug Res. 2007;22:42-50.
45. Malgorzata T, Szelag A. The role of calcium channel-blocking drugs in preserving rat liver for transplantation. Adv Clin Exp Med. 2007;5:609-18.

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