MINOCYCLINE ATTENUATED RAPID EYE MOVEMENT SLEEP DEPRIVATION-INDUCED LIVER DYSFUNCTION IN MICE

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ABSTRACT

Context: Sleep deprivation (SD) is a stressor that has been shown to increase the generation of free radicals therefore inducing mild organ injury through oxidative stress. Minocycline is a known antibiotic with anti-inflammatory and anti-apoptotic effects.

Aim: This study evaluated the effect of minocycline on lipid profile parameters and oxidative stress markers caused by rapid eye movement (REM) sleep deprivation in mice.

Settings and Design: A total of 30 mice weighing 22+2g were allotted into five groups of six animals each. Group 1 served as control, Group 2 was sleep deprived, mice in groups 3-5 in addition to being sleep deprived for 72 hrs, received minocycline (25 mg/kg), minocycline (50 mg/kg), astaxanthin (50 mg/kg) respectively. Glucose levels, organ weights, liver enzymes, lipid profile, oxidative stress parameters were assessed thereafter.

Methods and Material: REM sleep deprivation was induced using the multiple platforms over water model.

Statistical analysis used: All data were analysed using one-way ANOVA followed by post-hoc tests. The Graph Pad InStat® Biostatistics software was used to determine the criterion for significance in all tests.

Results: The results showed that sleep deprivation increased levels of pro-oxidants, decreased levels of antioxidants but increased glucose and triglyceride levels. It also decreased high-density lipoproteins, liver enzyme activity and bilirubin. Conversely, these effects were significantly attenuated by minocycline as well as astaxanthin.

Conclusions: In summary, REM sleep deprivation produced hepatotoxic alterations in sleep deprived mice and these alterations were reversed by minocycline.

Key-words: Sleep deprivation, Minocycline, Oxidative stress, Hepatic function

INTRODUCTION

Sleep is an important lifestyle component which is necessary for maintenance of normal physiology. Sleep deprivation (SD) represents a common type of stress that has physiological consequences, possibly leading to oxidative stress.[1] Studies have indicated that SD can significantly impair hepatic energy metabolism and ultimately contribute to alterations in hepatic lipogenesis and development of metabolic deficiencies.[2,3]

Minocycline, a broad-spectrum tetracycline antibiotic, has been shown to offer neuroprotection through its anti-inflammatory and antioxidant effects.[4,5] This study therefore aimed to investigate the hepatoprotective potential of minocycline on hepatic oxidative parameters, lipid profile and blood glucose level in mice subjected to sleep deprivation.

MATERIALS AND METHODS

Subjects and Methods:

Experimental Animals

Male Swiss mice weighing 22.0±2.0 g used in the study were obtained from the Central Animal House, Faculty of Basic Medical Sciences, Delta State University, Abraka and were housed in plastic cages at room temperature with 12:12 h light–dark cycle. They were fed with balanced rodent pellet diet and water ad libitum. Mice were acclimatized for at least one week before commencement of the experiment. The experimental procedures were performed in accordance with the National Institute of Health (NIH) Guideline for the Care and Use of Laboratory Animals.[6]

Equipment and apparatus

Centrifuge (ATKE), water bath (Equitron), spectrophotometer (Inesa, 752N), glucometer (Accu-Check Actives, Roche Diagnostics India Pvt., Ltd), pH meter (EDT instruments), weighing balance (Ohaus), test tubes, eppendorf tubes, test tube racks, oral cannula, dissection kits and boards, syringes (1mL, 2mL and 5mL).

Drugs and Chemicals

Minocycline, Astaxanthin, thiobabituric acid (TBA) (Guanghua Chemical Factory Co. Ltd., China), 5,5'-dithio-bis(2-nitrobenzoic acid)-DTNB (Aldrich, Germany), KCl (BDH Chemical Ltd, Poole, England), NaOH (J.T Baker Chemicals Co., Phillipsburg, N.J., USA), Tris (hydroxymethyl)-amino-methane (Tris-buffer) (Hopkin & Williams Company, USA), Acetic acid (Sigma-Aldrich, Inc., St Louis, USA), NaHCO3 (BDH Chemicals Ltd, Poole, England), Sodium Carbonate (fisons, Loughborough Leics, England), Na2HPO4.H2O (BDH Chemical Ltd, Poole, England), trichloroacetic acid-TCA (Burgoyne Burbidges & Co., Mumbai, India), NaH2PO4.H2O (BDH Chemical Ltd, Poole, England), K2HPO4 (BDH Chemical Ltd, Poole, England), K2Cr2O7 (BDH Chemical Ltd, Poole, England) were used in the study.

Drug Preparation and Treatment

Briefly, 100 mg of minocycline was dissolved in 20 mL of distilled water to obtain the stock solution. The stock solution was further diluted with distilled water to obtain the concentration used in the study. Also, astaxanthin used in the study was dissolved in olive oil before use. Male mice received oral (p.o) doses of minocycline (25 and 50 mg/kg), astaxanthin (50 mg/kg) or distilled water (10 mL/kg), via the use of oral cannula. The doses of minocycline used in the study were selected based on the results obtained from pilot

studies performed in our laboratory. Thus, the animals were allotted into five (5) treatment groups (n = 6): groups 1 and 2 received distilled water (10 mL/kg) while groups 3–4 received minocycline (25 and 50 mg/kg) respectively and group 5 received Astaxanthin (50 mg/kg). All animals in the respective treatment groups (group 2-5) were subjected to sleep deprivation except for mice in group 1. The time of drug administration was between the hours of 8 am to 10 am each day.

Experimental protocol

Sleep deprivation of the REM phase was carried out according to the method developed by Shinomiya et al.[7] Mice were sleep deprived by placing them individually on multiple stands suspended on water inside a plastic cage. The cage was filled with water to 1 cm below the stands. The stands were set 2 cm apart from each other, and food and water were provided ad libitum. This method is based on the principle that at the onset of rapid eye movement (REM) sleep, the mice fall into water due to atonia and after falling, they wake up quickly and get back on the stands. It is worthy of note however, that although this procedure primarily targets REM sleep, a considerable loss of non-rapid eye movement (NREM) sleep, accompanied by a non-negligible amount of stress is inevitable.[8] The animals in groups 2-5 were treated for 7 days, and subjected to 72 hours sleep deprivation beginning from the 4th day of treatment. At the end of the sleep deprivation period, 1 hr after the last treatment, the effect of REM sleep deprivation on hepatic oxidative parameters, lipid profile and blood glucose levels were determined.

Assessment of lipid profile

Mice in the respective groups were euthanized and blood was obtained via ocular puncture. Thereafter, the blood was centrifuged for 10,000 rpm at 4oC for 15 min to obtain serum which was used for lipid profile assay.

Determination of total serum cholesterol

Serum cholesterol level was determined with assay kit (Spinreact, Spain) in accordance with the method described by Song et al.[9] Briefly, the reagent (containing the enzymes: cholesterol esterase, cholesterol oxidase, peroxidase, and 4-aminophenazone) was dissolved with the buffer to give the working reagent. The mixture was gently mixed to dissolve the contents. About 1 mL of the working reagent was mixed with 10 µL of serum or cholesterol standard (200 mg/dL) and the mixture was incubated for 10 min. at room temperature. Afterwards, the absorbance (A) of the samples and standard was read against the blank at 500 nm. The concentration (mg/dL) of cholesterol in the sample was obtained using the following equation:

[(A) Sample – (A) Blank / (A) Standard – (A) Blank] × 200 (Standard Conc.)

Determination of serum triglyceride levels

Serum triglyceride level was determined using the assay kit (Randox, United Kingdom) following the method described by Bucolo and David.[10] One mL of the reagent was mixed with 10 µL of the sample or standard and the mixture incubated for 5 min at 37oC. Absorbance (A) was read at 500 nm. Concentration of triglyceride (mg/dL) was obtained using the equation:

(A) Sample / (A) Standard × 200 (Standard Conc.)

Determination of high-density lipoprotein

High-density lipoprotein (HDL) level was determined in mouse serum using the assay kit (Randox, United Kingdom) following the method described by Bucolo and

David.10 One mL of the reagent was mixed with 10 µL of the sample or standard and the mixture incubated for 10 min at 37oC. Absorbance (A) was read at 500 nm. Concentration of HDL (mg/dL) in the sample was obtained using the equation:

(A) Sample / (A) Standard × 200 (Standard Conc.)

Measurement of blood glucose

Blood glucose level was measured with the aid of a blood glucose monitoring meter (AccuCheck®) and blood glucose strip (AccuCheck®). A strip was placed into the meter and a drop of blood was obtained from each mouse and drawn up into the meter. Immediately the blood glucose reading was displayed on the screen of the glucose monitoring meter and this value was recorded.

Preparation of liver tissues for biochemical assays

Mice in the respective groups were euthanized and the livers were removed. Thereafter, each mouse liver obtained was weighed, homogenized with 10% w/v phosphate buffer (0.1M, pH 7.4) and centrifuged for 10,000 rpm at 4oC for 15 min. Then, the supernatants were used for the biochemical assays.

Determination of glutathione (GSH) concentration

Aliquots of liver supernatant of each mouse in the respective treatment groups were taken and GSH concentration was determined using the method of Moron and his colleagues described by Pratibha et al.[11] Equal volume (0.4 mL) of liver homogenate and 20% TCA (0.4 mL) was mixed and then centrifuged using a cold centrifuge at 10,000 rpm at 4°C for 20 min. The supernatant (0.25 mL) was added to 2 mL of 0.6 mM DTNB and the final volume was made up to 3 mL with phosphate buffer (0.2M, pH 8.0). The absorbance was then read at 412 nm against blank reagent using a spectrophotometer. The concentrations of GSH in the liver tissues were expressed as micromoles per gram tissue (µmol/g tissue).

Estimation of liver level of malondialdehyde (MDA)

The liver level of MDA, a biomarker of lipid peroxidation, was estimated according to the method of Adam-Vizi and Seregi.[12] An aliquot of 0.4 mL of the supernatant was mixed with 1.6 mL of Tris–KCl buffer to which 0.5 mL of 30% TCA was added. Then, 0.5 mL of 0.75% TBA was added and placed in a water bath for 45 min at 80°C. This was then cooled in ice and centrifuged at 3,000 rpm for 15 min. The clear supernatant was collected and absorbance measured against a reference blank of distilled water at 532 nm using a spectrophotometer. The MDA concentration was calculated using a molar extinction coefficient of 1.56×105 M-1 cm -1 and values were expressed as µmoles of MDA per gram tissue.

Determination of superoxide dismutase (SOD) activity

The level of SOD activity in the liver was determined by the method of Misra and Fridovich.[13] Briefly, 0.1 mL of liver supernatant was added to 2.6 mL of 0.05 M carbonate buffer (pH 10.2) to equilibrate in the spectrophotometer and the reaction started by the addition of 0.3 mL of freshly prepared 0.3 mM adrenaline to the mixture, which quickly mixed by inversion. The reference cuvette contained 2.6 mL buffer, 0.3 mL of adrenaline and 0.1 mL of distilled water. Then, increase in absorbance at 480 nm was monitored at 60 s intervals for 3 min. Superoxide dismutase (SOD) activity was expressed as units of adrenaline consumed per minute per mg protein.

Estimation of catalase (CAT) activity

Catalase activity was determined according to the method previously described by Sinha.[14] Aliquots of mouse liver supernatant (0.1 mL) was added to 2 mL of sodium phosphate buffer (0.05 M; pH 7.4) and 0.9 mL of H2O2 (800 µmoles). The reaction mixture was mixed by a gentle swirling motion at room temperature and 1 mL of this mixture was added to 2 mL dichromate/acetic acid reagent. The absorbance was read using a spectrophotometer at a wavelength of 570 nm and change in absorbance at 60 s intervals. The catalase activity was expressed as µmol of H2O2 decomposed per minute per mg protein.

Estimation of protein

This was done in accordance with previously described method for CAT and SOD[13,14].

Estimation of liver level of nitrite oxide (NO)

Liver nitrite concentration was estimated using Greiss reagent, which serves as an indicator of nitric oxide production. 100 µl of Greiss reagent (1:1 solution of 1% sulfanilamide in 5% phosphoric acid and 0.1% of N-1-naphthyl ethylenediaminedihydrochloride) was added to 100 µL of the supernatant and absorbance was measured at 540 nm.[15] The liver nitrite concentration was estimated from a standard curve obtained from sodium nitrite (0-100 µM).

Assessment of liver function

Mice in the respective groups were euthanized and blood was obtained via ocular puncture. Thereafter, the blood was centrifuged for 10,000 rpm at 4oC for 15 min to obtain serum which was used for liver function tests.

Assessment of total bilirubin

Total bilirubin level was determined by colorimetric method using assay kit (Randox, United Kingdom) according to the manufacturer's protocol following the method of Jendrassik and Grof earlier described by Lo and Wu.[16] About 0.2 mL of sample was added to 3.0 mL of working solution (dilute hydrochloric acid; 0.7 mmol/L, 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. Thereafter, the mixture was incubated at room temperature (25 o C) for 5 min, and read at 578 nm against the sample blank.

Assessment of alanine aminotransferase (ALT) activity

Alanine aminotransferase (ALT) activity in serum was determined spectrophotometrically. Blood was collected from each mouse through ocular puncture with the aid of heparinised capillary tube into a lithium heparinised sample bottle and centrifuged at 10,000 rpm for 15 min using cold centrifuge. The serum was collected separately for each animal and 0.1 mL of each sample was mixed with 0.5mL solution consisting of sodium phosphate buffer (100 mmol/L, pH 7.4), L-alanine (200 mmol/L), and α-oxoglutarate (2 mmol/L). Thereafter, the mixture was incubated for exactly 30 min at 37˚C. Then, 0.5 mL of 2, 4 dinitrophenylhydrazine (2 mmol/L) was added to the reaction mixture and allowed to stand for exactly 20 min at 25˚C. Thereafter, 5.0 mL of sodium hydroxide (0.4 mol/L) was added and the absorbance was read against the reagent blank after 5 min at 546 nm. The units of ALT were expressed as U/L.

Assessment of aspartate aminotransferase (AST) activity

Aspartate aminotransferase (AST) activity in serum was determined spectrophotometrically. Blood was collected from each mouse through ocular puncture with the aid of heparinised capillary tube into a lithium heparinised sample bottle and centrifuged at 10,000 rpm for 15 min using cold centrifuge. The serum was collected separately for each animal and 0.1 mL of each sample was mixed with 0.5 mL solution consisting of sodium phosphate buffer (100 mmol/L, pH 7.4), L-aspartate (100 mmol/L), and α-oxoglutarate (2 mmol/L). Thereafter, the mixture was incubated for exactly 30 min at 37˚C. Then, 0.5 mL of 2, 4 dinitrophenylhydrazine (2 mmol/L) was added to the reaction mixture and allowed to stand for exactly 20 min at 25˚C. Thereafter, 5.0 mL of sodium hydroxide (0.4 mol/L) was added and the absorbance was read against the reagent blank after 5 min at 546 nm. The units of AST were expressed as U/L.

Assessment of alkaline phosphatase (ALP) activity

Alkaline phosphatase (ALP) activity in serum was determined spectrophotometrically. Blood was collected from each mouse through ocular puncture with the aid of heparinised capillary tube into a lithium heparinised sample bottle and centrifuged at 10000 rpm for 15 min using cold centrifuge. The serum was collected separately for each animal and 0.05 mL of each sample was mixed with 0.5 mL solution consisting of diethanolamine buffer (1 mol/L, pH 9.8) and Magnesium Chloride (0.5 mmol/L) in a test tube. Then, 3 mL of p-nitrophenylphosphate (10 mmol/L) was added to the reaction mixture and allowed to stand at 25˚C. Thereafter, absorbance was read at time 0, 1, 2 and 3 min, respectively at 405 nm. The units of ALP were expressed as U/L.

Determination of organ weights

The adrenal gland and liver of each of the animals in the respective treatment groups which were euthanized was excised and weighed. Thus, the relative organ weights were calculated as organ weight/100 g of body weight.

Statistical analysis

All data are presented as Mean ± SEM. The results were analysed by one-way analysis of variance (ANOVA) and post-hoc tests (Student's Newman–Keuls) were carried out to determine the source of significance using Graph Pad InStat® Biostatistics software. The level of significance for all tests was set at α0.05.

RESULTS

Effect of Minocycline on blood glucose levels in mice subjected to sleep deprivation

As shown in Figure 1, blood glucose level was significantly increased in sleep deprivation-only group compared to the vehicle group. However, administration of minocycline significantly reduced blood glucose level.

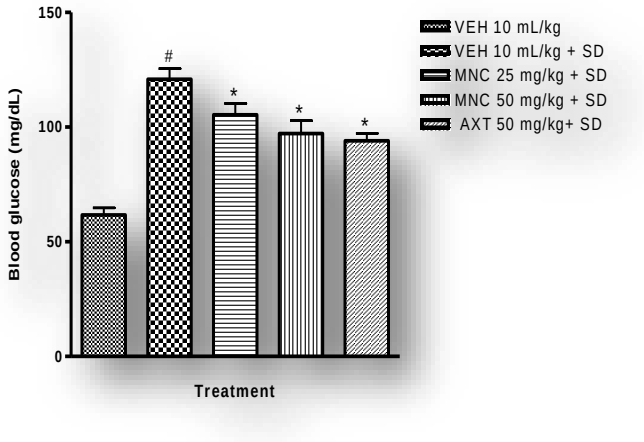

Figure 1: Effect of minocycline on blood glucose levels in mice subjected to sleep deprivation

Each result is expressed as mean ± S.E.M of grouped mice (n=6).

# indicates significant difference ($p < 0.05$) compared to the vehicle (not sleep deprived) group.

* indicates significant difference ($p < 0.05$) compared to the vehicle + SD group. (One-way ANOVA followed by Student-Newman-Keuls post-hoc test).

VEH: Vehicle, AXT: Astaxanthin, MNC: Minocycline, SD: Sleep deprivation.

Effect of Minocycline on serum cholesterol levels in mice subjected to sleep deprivation

As indicated in Figure 2, serum cholesterol showed no significant ($p > 0.05$) changes across all treatment groups.

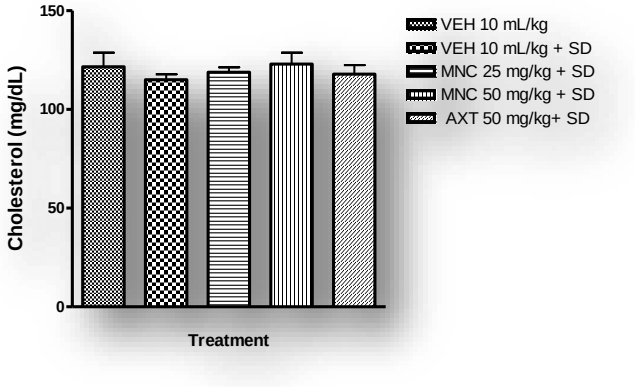

Figure 2: Effect of minocycline on serum cholesterol levels in mice subjected to sleep deprivation

Each result is expressed as mean ± S.E.M of grouped mice (n=6).

There is no significant difference ($p > 0.05$) between groups. (One-way ANOVA followed by Student-Newman-Keuls post-hoc test).

VEH: Vehicle, AXT: Astaxanthin, MNC: Minocycline, SD: Sleep deprivation

Effect of Minocycline on serum triglyceride levels in mice subjected to sleep deprivation

The result revealed that serum triglyceride was significantly ($p < 0.05$) elevated in sleep deprived mice, whereas minocycline (25 and 50 mg/kg) significantly ($p < 0.05$) reduced the levels of triglyceride in mice serum (Figure 3).

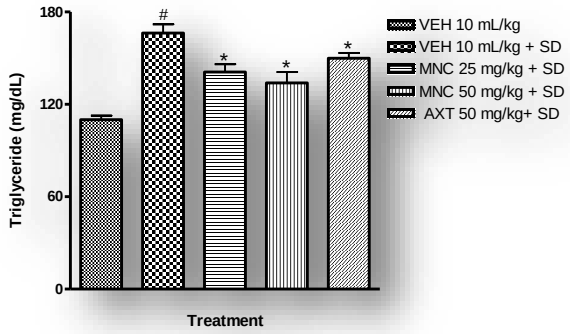

Figure 3: Effect of minocycline on serum triglyceride levels in mice subjected to sleep deprivation

Each result is expressed as mean ± S.E.M of grouped mice (n=6).

# indicates significant difference ($p < 0.05$) compared to the vehicle (not sleep deprived) group.

* indicates significant difference ($p < 0.05$) compared to the vehicle + SD group. (One-way ANOVA followed by Student-Newman-Keuls post-hoc test).

VEH: Vehicle, AXT: Astaxanthin, MNC: Minocycline, SD: Sleep deprivation.

Effect of Minocycline on serum levels of HDL in mice subjected to sleep deprivation

Figure 4 revealed that serum high-density lipoprotein was significantly ($p < 0.05$) reduced in sleep

deprived mice, whereas minocycline (25 and 50 mg/kg) significantly ($p < 0.05$) raised the levels of high density lipoprotein serum in mice as significantly as astaxanthin.

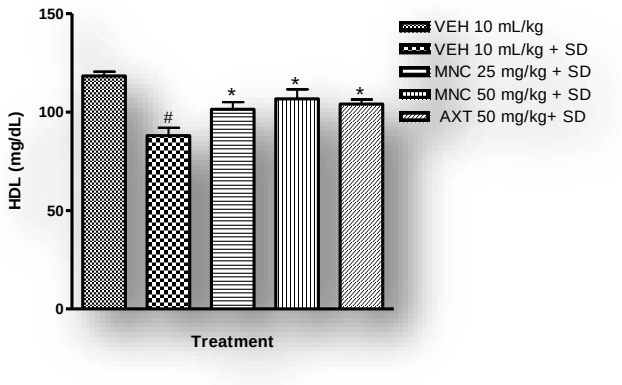

Figure 4: Effect of minocycline on serum levels of high density lipoprotein in mice subjected to sleep deprivation

Each result is expressed as mean ± S.E.M of grouped mice (n=6).

# indicates significant difference ($p < 0.05$) compared to the vehicle (not sleep deprived) group.

* indicates significant difference ($p < 0.05$) compared to the vehicle + SD group. (One-way ANOVA followed by Student-Newman-Keuls post-hoc test).

VEH: Vehicle, AXT: Astaxanthin, MNC: Minocycline, SD: Sleep deprivation.

Effect of Minocycline on liver nitrite levels in mice subjected to sleep deprivation

Sleep deprivation significantly increased level of liver nitrite in mice as against the non-sleep-deprived group. However, administration of minocycline significantly attenuated the liver nitrite increase caused by 72 hours REM sleep deprivation (Figure 5).

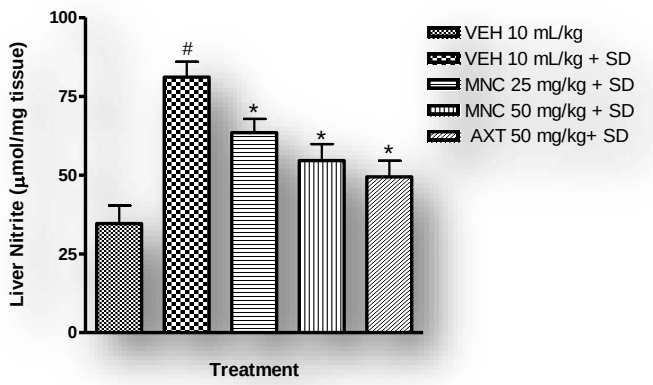

Figure 5: Effect of minocycline on liver nitrite levels in mice subjected to sleep deprivation

Each result is expressed as mean ± S.E.M of grouped mice (n=6).

# indicates significant difference ($p < 0.05$) compared to the vehicle (not sleep deprived) group.

* indicates significant difference ($p < 0.05$) compared to the vehicle + SD group. (One-way ANOVA followed by Student-Newman-Keuls post-hoc test).

VEH: Vehicle, AXT: Astaxanthin, MNC: Minocycline, SD: Sleep deprivation.

Effect of Minocycline on liver catalase activity in mice subjected to sleep deprivation

As indicated in Figure 6, sleep deprivation significantly decreased liver catalase activity in mice. However, administration of minocycline, in both doses, significantly ($p < 0.05$) reversed the decrease in liver catalase produced by sleep deprivation.

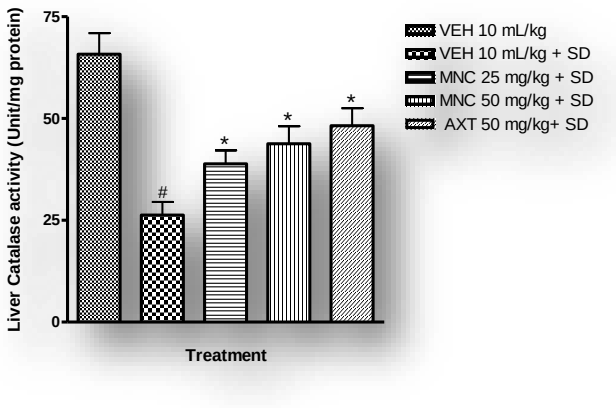

Figure 6: Effect of minocycline on liver catalase activity in mice subjected to sleep deprivation

Each result is expressed as mean ± S.E.M of grouped mice (n=6).

# indicates significant difference ($p < 0.05$) compared to the vehicle (not sleep deprived) group.

* indicates significant difference ($p < 0.05$) compared to the vehicle + SD group. (One-way ANOVA followed by Student-Newman-Keuls post-hoc test).

VEH: Vehicle, AXT: Astaxanthin, MNC: Minocycline SD: Sleep deprivation.

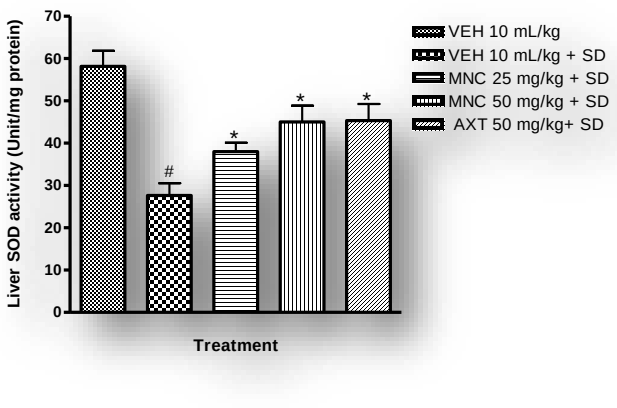

Figure 7: Effect of minocycline on liver superoxide dismutase activity in mice subjected to sleep deprivation

Each result is expressed as mean ± S.E.M of grouped mice (n=6).

# indicates significant difference ($p < 0.05$) compared to the vehicle (not sleep deprived) group.

* indicates significant difference ($p < 0.05$) compared to the vehicle + SD group. (One-way ANOVA followed by Student-Newman-Keuls post-hoc test).

VEH: Vehicle AXT: Astaxanthin, MNC: Minocycline SD: Sleep deprivation.

Effect of Minocycline on liver superoxide dismutase activity in mice subjected to sleep deprivation

As shown in Figure 7, sleep deprivation decreased the activity of superoxide dismutase in mice unlike in the control group. However, administration of minocycline (25 and 50 mg/kg) significantly ($p < 0.05$) increased liver superoxide dismutase activity produced by sleep deprivation.

Effect of Minocycline on liver malondialdehyde levels in mice subjected to sleep deprivation

The effect of minocycline on liver malondialdehyde levels in mice subjected to 72 hours REM sleep deprivation is shown in Figure 8. The result revealed that liver malondialdehyde was significantly elevated in sleep deprived mice, whereas Minocycline (25 and 50 mg/kg) significantly ($p < 0.05$) reduced the concentration of liver malondialdehyde in sleep deprived mice.

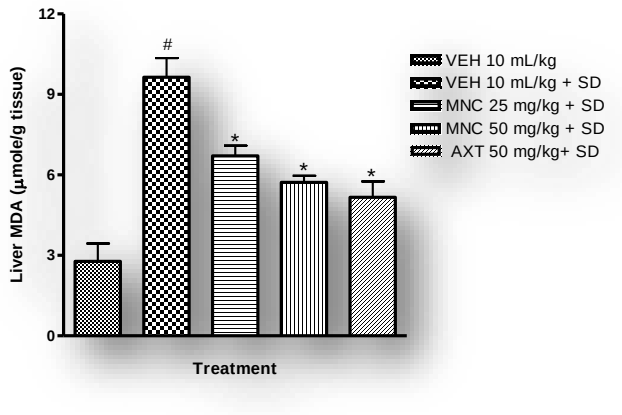

Figure 8: Effect of minocycline on liver malondialdehyde levels in mice subjected to sleep deprivation

Each result is expressed as mean ± S.E.M of grouped mice (n=6).

# indicates significant difference ($p < 0.05$) compared to the vehicle (not sleep deprived) group.

* indicates significant difference ($p < 0.05$) compared to the vehicle + SD group. (One-way ANOVA followed by Student-Newman-Keuls post-hoc test).

VEH: Vehicle AXT: Astaxanthin, MNC: Minocycline SD: Sleep deprivation.

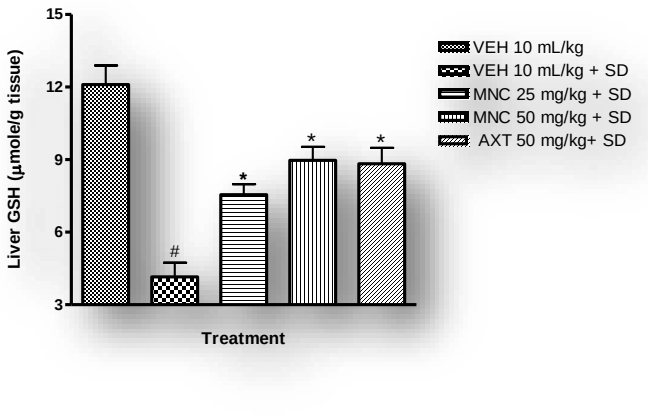

Figure 9: Effect of minocycline on liver glutathione levels in mice subjected to sleep deprivation

Each result is expressed as mean ± S.E.M of grouped mice (n=6).

# indicates significant difference ($p < 0.05$) compared to the vehicle (not sleep deprived) group.

* indicates significant difference ($p < 0.05$) compared to the vehicle + SD group. (One-way ANOVA followed by Student-Newman-Keuls post-hoc test).

VEH: Vehicle, AXT: Astaxanthin, MNC: Minocycline SD: Sleep deprivation.

Effect of Minocycline on liver glutathione levels in mice subjected to sleep deprivation

As indicated in Figure 9, sleep deprivation significantly ($p < 0.05$) decreased liver glutathione level in mice unlike the high levels seen in the non-sleep-deprived group. However, administration of minocycline significantly ($p < 0.05$) attenuated the decrease in liver glutathione produced by sleep deprivation.

Effect of Minocycline on relative organ weights of mice subjected to sleep deprivation

The effect of minocycline on organ weights in sleep deprived mice is shown in Table 1. Sleep deprivation significantly ($p < 0.05$) increased the weight of the adrenal gland and the liver in mice. However, administration of minocycline (25 and 50 mg/kg) significantly ($p < 0.05$) reversed the increases in the weight of the adrenal gland and the liver in mice caused by sleep deprivation.

Table 1: Effect of minocycline on relative organ weights of mice subjected to sleep deprivation.

Treatment	Relative organ weight (g per 100 g body weight)	
	Adrenal gland (mg)	Liver (X 10^3 mg)
VEH 10 mL/kg	3.53±0.62	0.89±0.02
VEH 10 mL/kg + SD	7.23±0.57#	1.12±0.04#
MNC 25 mg/kg + SD	5.37±0.32*	0.99±0.02*
MNC 50 mg/kg + SD	4.66±0.28*	0.91±0.04*
AXT 50 mg/kg+ SD	3.67±0.53*	0.96±0.04*

Each result is expressed as mean ± S.E.M of grouped mice (n=6).

# indicates significant difference ($p < 0.05$) compared to the vehicle (not sleep deprived) group.

* indicates significant difference ($p < 0.05$) compared to the vehicle + SD group. (One-way ANOVA followed by Student-Newman-Keuls post-hoc test).

VEH: Vehicle, AXT: Astaxanthin, MNC: Minocycline, SD: Sleep deprivation.

Effect of Minocycline on Alanine aminotransferase in mice subjected to sleep deprivation

As shown in Figure 10, sleep deprivation significantly ($p < 0.05$) increased alanine aminotransferase in mice unlike what is observed in the control group. However, administration of minocycline significantly ($p < 0.05$) attenuated the increases in alanine aminotransferase caused by sleep deprivation.

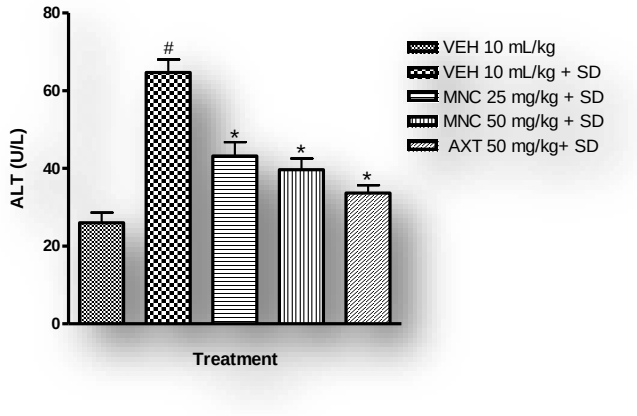

Figure 10: Effect of minocycline on alanine aminotransferase in mice subjected to sleep deprivation.

Each result is expressed as mean ± S.E.M of grouped mice (n=6).

# indicates significant difference ($p < 0.05$) compared to the vehicle (not sleep deprived) group.

* indicates significant difference ($p < 0.05$) compared to the vehicle + SD group. (One-way ANOVA followed by Student-Newman-Keuls post-hoc test).

VEH: Vehicle, AXT: Astaxanthin, MNC: Minocycline, SD: Sleep deprivation.

Effect of Minocycline on Aspartate aminotransferase in mice subjected to sleep deprivation

As shown in Figure 11, sleep deprivation significantly ($p < 0.05$) increased aspartate aminotransferase in mice. However, administration of minocycline (25 and 50 mg/kg) significantly ($p < 0.05$) attenuated the increases in aspartate aminotransferase caused by sleep deprivation.

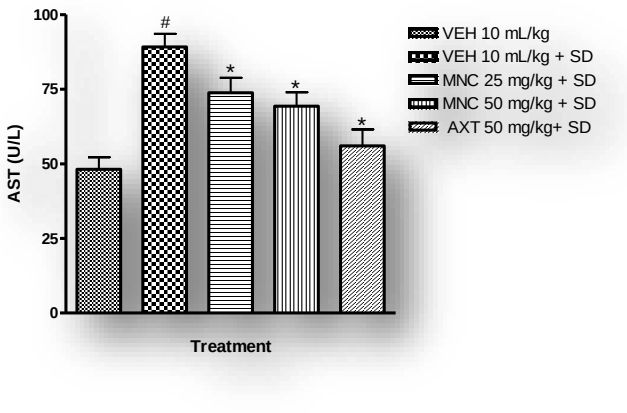

Figure 11: Effect of minocycline on aspartate aminotransferase in mice subjected to sleep deprivation.

Each result is expressed as mean ± S.E.M of grouped mice (n=6).

# indicates significant difference ($p < 0.05$) compared to the vehicle (not sleep deprived) group.

* indicates significant difference ($p < 0.05$) compared to the vehicle + SD group. (One-way ANOVA followed by Student-Newman-Keuls post-hoc test).

VEH: Vehicle, AXT: Astaxanthin, MNC: Minocycline, SD: Sleep deprivation.

Effect of Minocycline on alkaline phosphatase in mice subjected to sleep deprivation

The effect of minocycline on alkaline phosphatase in sleep deprived mice is shown in Figure 12. Sleep deprivation significantly ($p < 0.05$) increased alkaline phosphatase in mice. However, administration of minocycline (25 and 50 mg/kg) significantly ($p < 0.05$) attenuated the increases in alkaline phosphatase caused by sleep deprivation.

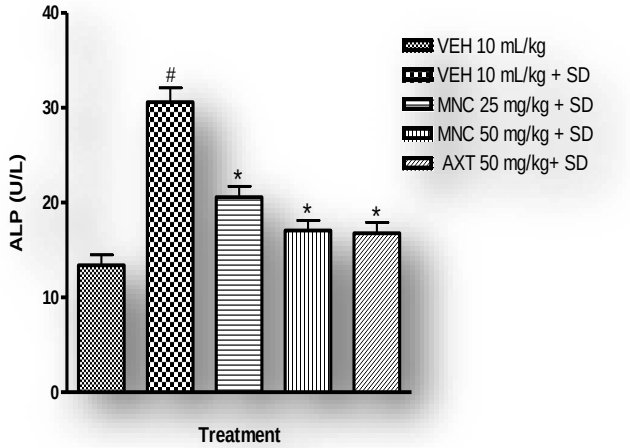

Figure 12: Effect of minocycline on alkaline phosphatase in mice subjected to sleep deprivation.

Each result is expressed as mean ± S.E.M of grouped mice (n=6).

# indicates significant difference ($p < 0.05$) compared to the vehicle (not sleep deprived) group.

* indicates significant difference ($p < 0.05$) compared to the vehicle + SD group. (One-way ANOVA followed by Student-Newman-Keuls post-hoc test).

VEH: Vehicle, AXT: Astaxanthin, MNC: Minocycline, SD: Sleep deprivation.

Effect of Minocycline on total bilirubin in mice subjected to sleep deprivation.

As shown in Figure 13, sleep deprivation significantly ($p < 0.05$) increased total bilirubin in mice. However, administration of minocycline significantly ($p < 0.05$) attenuated the increases in total bilirubin caused by sleep deprivation.

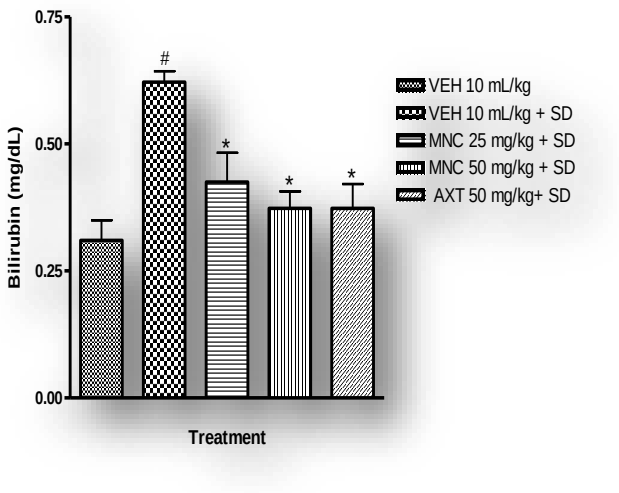

Figure 13: Effect of Minocycline on total bilirubin in mice subjected to sleep deprivation.

Each result is expressed as mean ± S.E.M of grouped mice (n=6).

# indicates significant difference ($p < 0.05$) compared to the vehicle (not sleep deprived) group.

* indicates significant difference ($p < 0.05$) compared to the vehicle + SD group. (One-way ANOVA followed by Student-Newman-Keuls post-hoc test).

VEH: Vehicle, AXT: Astaxanthin, MNC: Minocycline, SD: Sleep deprivation

DISCUSSION

This study investigated the effects of minocycline on blood glucose levels, serum lipid profile, liver enzymes and hepatic oxidative stress parameters in mice subjected to 72 hours rapid eye movement (REM) sleep deprivation.

Some studies have reported an association between REM sleep deprivation and lipid levels that is manifested as an increase in triglycerides (TG) levels or low high-density lipoproteins (HDL), suggesting that sleep deprivation may be a risk factor for cardiovascular diseases.[17] The present study is in line with existing literature as the results showed that the sleep deprived mice presented with a significant decrease in HDL and increased TG levels while the minocycline-treated groups (25 and 50 mg/kg) caused a significant reversal of these effects. Therefore, people who experience sleep deprivation may have fewer HDL – known as the "good" cholesterol – compared to those who have sufficient sleep.

Another significant relationship found in this study was between blood glucose levels and sleep deprivation. The results from this study showed that sleep deprived mice had higher blood glucose levels (i.e. hyperglycaemia) than the control group. Existing literature supports that sleep deprivation is associated with increase in blood sugar levels and these associations may be driven by obesity-related insulin resistance and pro-inflammatory milieu associated with poor sleep.[18] Furthermore, sleep deprivation is associated with the development of glucose intolerance and increased hepatic glucose production, suggestive of hepatic insulin resistance.[19] Also in this study, the organ weights were found to be significantly increased in the sleep deprivation group compared with the control group. Such organ weight elevation in the sleep deprived mice may be due to significant increase in plasma glucose concentration.[20] Therefore, sleep deprivation which increases the risk of hyperglycemia, hyperlipidemia and oxidative stress may play a crucial role in the pathogenesis of diabetes.[21] However, this increase in blood sugar level and organ weights was attenuated upon the administration of minocycline in a dose-dependent pattern.

Sleep deprivation can impair liver function and alter the pro-oxidant-antioxidant balance which results in increased free radical generation. An increase in free radicals causes overproduction of malondialdehyde (MDA)

and acutely increases nitric oxide (NO) systemically which may result in acute hepatic dysfunction and hepatocyte death which may lead to hepatic fibrosis or hepatic steatosis.[22] In the current study, liver NO and MDA levels were elevated in the sleep-deprived mice. The association between sleep deprivation and oxidative stress was significant in this study with results showing a decrease in antioxidant levels (measured by GSH, CAT SOD) and increase in pro-oxidant levels (measured by MDA and NO). A study by Rahman and colleagues showed that antioxidant levels decreased in peripheral tissues of animals that were deprived of total sleep for 5 and 10 days and it was restored to normal levels only after rebound recovery sleep.[23] There are also reports of decreased SOD activity in rats which were subjected to total sleep loss (3-14 days) in comparison to control groups.[24] Another study suggests that REM sleep deprivation of rats can increase reactive oxygen species (ROS) production in their hepatocytes which ultimately can induce apoptotic cell death[25] and a report by Wen, showed a sharp increase in lipid peroxidation in the hepatocytes of sleep deprived rats.[26]

Sleep occurs in all species and its loss which is also referred to as sleep deprivation leads to a drastic deterioration in various body functions. Sleep is believed to be characterized with antioxidant properties as well.[27] It is believed that oxidative stress plays important role in the development of vascular complications as well as pathogenesis of several diseases following exposure to stressful conditions and sleep disorders are closely associated with serious complications, such as hypertension and type 2 diabetes mellitus.[20] Diabetes is a prime risk factor for developing cardiovascular diseases and also leads to depletion of cellular antioxidant defence system with an increase in free radical production.[28] Several clinical and experimental studies have demonstrated this relationship.[25-29] In resonance with the present study, Viana et al.[29] demonstrated that minocycline administration for 5 days significantly lowered blood glucose level, and a longer treatment (up to 30 days) brought glucose levels to normal in diabetic rats. [29]

The liver is one of the major organs vulnerable to physiological stress.[30] Sleep deprivation activates many stress related pathways including the hypothalamic–pituitary–adrenal axis (HPA) which affects metabolism,[31] controls reactions to stress and regulates many body processes. Stress causes the adrenal gland to release adrenaline. This hormone prompts the pituitary gland to produce adrenocorticotropic hormone (ACTH) in response to biological stress. ACTH stimulates the adrenal gland to release cortisol, and other stress hormones. When the stress persists and becomes uncontrollable, there is a breakdown in adaptive mechanisms resulting in damage to various organs of the body.[32] In the current study, exposure of mice to sleep deprivation resulted in increase in liver enzyme activity, measured by alkaline phosphatase (ALP), aspartate aminotransferase (AST) and alanine aminotransferase (ALT) levels, when compared to the control group which suggests hepatic injury. This result is supported by existing data which showed that the severity of sleep deprivation was positively correlated with the prevalence rate of elevated liver enzymes.[33]

The results obtained in this study have validated our initial suggestions that minocycline significantly attenuates blood parameters; blood glucose and lipid profile as well as liver oxidative stress. Therefore, minocycline possesses the ability to mitigate oxidative stress-induced organ damage caused by stressors such as REM sleep deprivation.

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