

## ORIGINAL ARTICLE

# Minocycline Attenuated Depressive-Like Phenotype in Sleep-Deprived Mice via its Antioxidant Effect

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## ABSTRACT

**Introduction:** Central nervous system (CNS) neuropathologies are the most common effects of insufficient sleep/prolonged wakefulness. This puts people who undergo sleep deprivation in the course of their daily lives at risk of certain affective disorders such as depression. Minocycline is an antibiotic that can penetrate the CNS, which puts it at the forefront of numerous neuropathologies. This research was therefore intended to investigate into the possible anti-depressant effect of minocycline in mice exposed to chronic sleep deprivation. **Methods:** Thirty (30) mice were grouped into five (n=6) and treated with either distilled water, minocycline or astaxanthin. Four out of the five groups were exposed to the sleep deprivation set-up. After a 7-day treatment, all groups were subjected to behavioural tests. Subsequently, biochemical assays and histology of selected brain regions were carried out. Experimental data were analysed using one-way analysis of variance and another post-hoc test. **Results:** Results obtained suggest that minocycline attenuated the alterations in mood behaviour caused by sleep deprivation with a concurrent reversal of sleep-deprivation induced oxidative stress in mice. **Conclusion:** In conclusion, minocycline could be considered a reliable intervention in further studies on depression in sleep deprivation models.

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## INTRODUCTION

Sleep deprivation is the situation of not having enough sleep. Optimally, a human adult is expected to have a minimum 9 hours of nocturnal sleep. This value varies based on age and active status of the individual (1). In any case, the consequences of sleep deprivation on homeostatic functions include disruptions in internal circadian processes, mental impairments, hormonal imbalances and other medical problems (2,3). As such, sleep deprivation is considered a form of stress (4).

There is viable evidence that disturbed sleep patterns are associated with psychiatric disorders and is traditionally considered to be a symptom of depression (5). However, more recent research suggests that the relationship between sleep disruptions, in addition to being a symptom, may also be a risk factor to the development of mood disorders (6,7). Apart from its role in mood disorders, sleep deprivation is also considered in literature as a state that leads to the exacerbation of free

radical production which could incur oxidative stress (8,9). In line with the rising need to combat the adverse effects of stress on the body, especially the central nervous system, various substances with established brain-barrier crossing ability have been evaluated.

Minocycline is a known broad-spectrum antibiotic with benefits in inflammatory conditions and oxidative stress (10). It is lipid-soluble thereby enabling efficient transfer across the blood-brain barrier (BBB) (11,12). Evidence shows that minocycline demonstrated positive efficacy in cases of schizophrenia and psychotic depression (12,13). From the foregoing, this research aims to elucidate the potential antidepressant effect of minocycline on mice exposed to stress by sleep deprivation.

## MATERIALS AND METHODS

### Animals

Male Albino Swiss mice (22.0±2.0g body wt) used in the study were procured from the Faculty of Basic Medical Sciences animal house, Delta State University, Abraka and were housed in plastic cages at room temperature with equal hours of exposure to light and darkness. They were provided with adequate rodent pellet and water at liberty and all procedures were in accordance

with University ethics (approval number: REC/FBMS/DELSU/21/106) and NIH revised guidelines.

### **Treatment plan**

Mice received oral (i.e., p.o) doses of minocycline (Accord-UK Ltd), astaxanthin (Algatech, USA) or distilled water, using an oral gavage. Both drugs were initially dissolved in water to obtain the volumes used. Consequently, the mice were randomly distributed into five (5) treatment groups (n = 6): groups A and B received distilled water (10 mL/kg); groups C–D received minocycline (25 and 50 mg/kg, respectively) and group E received astaxanthin (50mg/kg). The animals were treated for 7-day duration and administrations were performed once each day. Mice in groups B-E were engaged in the 72-hour sleep deprivation protocol beginning from day 4 of treatment.

### **Experimental protocol**

Following the sleep deprivation protocol modified by Kumar and Kalonia (14) with minute modifications, mice were sleep deprived by placing them individually on the stands of a grid suspended on top water inside a plastic cage. Apart from depriving the mice from REM sleep, this method also subjects them to a significant level of stress (15). Twenty-four (24) hours after the treatment duration of 7 days, animals were then assessed once for any behavioural changes using the forced swimming, tail suspension, and social preference apparatus. Biochemical investigations and histological assay were subsequently carried out on the brain sections of mice.

### **Behavioural test**

#### ***Tail suspension test (TST)***

This test was carried out according to the procedure described by Can et al. (16) wherein a mouse is termed to be motionless or immobile when it hangs passively from a retort stand to which it was attached by its tail. The immobility duration (in seconds) was recorded during the last 4 min of a 6 min total observation period.

#### ***Social interaction test (SIT)***

According to the modified by Adebessin et al. (17), mice were individually kept in the three-way social interaction chamber with a mouse in one of its chambers and the other empty. Test mice was observed for interactions with the empty chamber and/or the occupied chamber. Mice were allowed to explore the setup for 6 min.

#### ***Forced swimming test (FST)***

In this test, mice were forced to swim individually following the method modified by Umukoro et al. (18) for 6 min. Total duration of immobility (in seconds) was recorded during the last 4 min of a 6 min observation period. A mouse was adjudged to be motionless when it only made slight movements good enough to keep its head above the water.

### **Biochemical investigation**

Soon after the behavioural tests, mice in the respective groups were euthanized and the whole brains were removed. Thereafter, each mouse brain was then weighed, homogenized with 10% w/v phosphate buffer (0.1M, pH 7.4) and centrifuged at 10,000 rpm for 15 min at 4°C. Specific aliquots of the resultant supernatants were used in the biochemical assay for the determination of glutathione concentration (19), malondialdehyde level (19), superoxide dismutase activity (19), catalase activity (20), and nitric oxide content (20) of each mouse.

### **Histology and estimation of neuronal density**

After the behavioural tests, paraffin wax-embedded tissue blocks of mice brains were prepared. Following identification of the cornu ammonis 1 (CA1) and prefrontal cortex (PFC), 5-6  $\mu$ m transverse sections were obtained using a microtome (Leica, Germany), fixed on glass slides and viewed with a light microscope at X400 magnification. Afterwards, These slides were then stained using Haematoxylin and Eosin staining pattern. Photomicrographs were subsequently obtained using a digital camera. Neuronal density or neuronal count was also extrapolated from the photomicrographs as a ratio of viable neuronal cell counts to the square area of the circular view in a section (21).

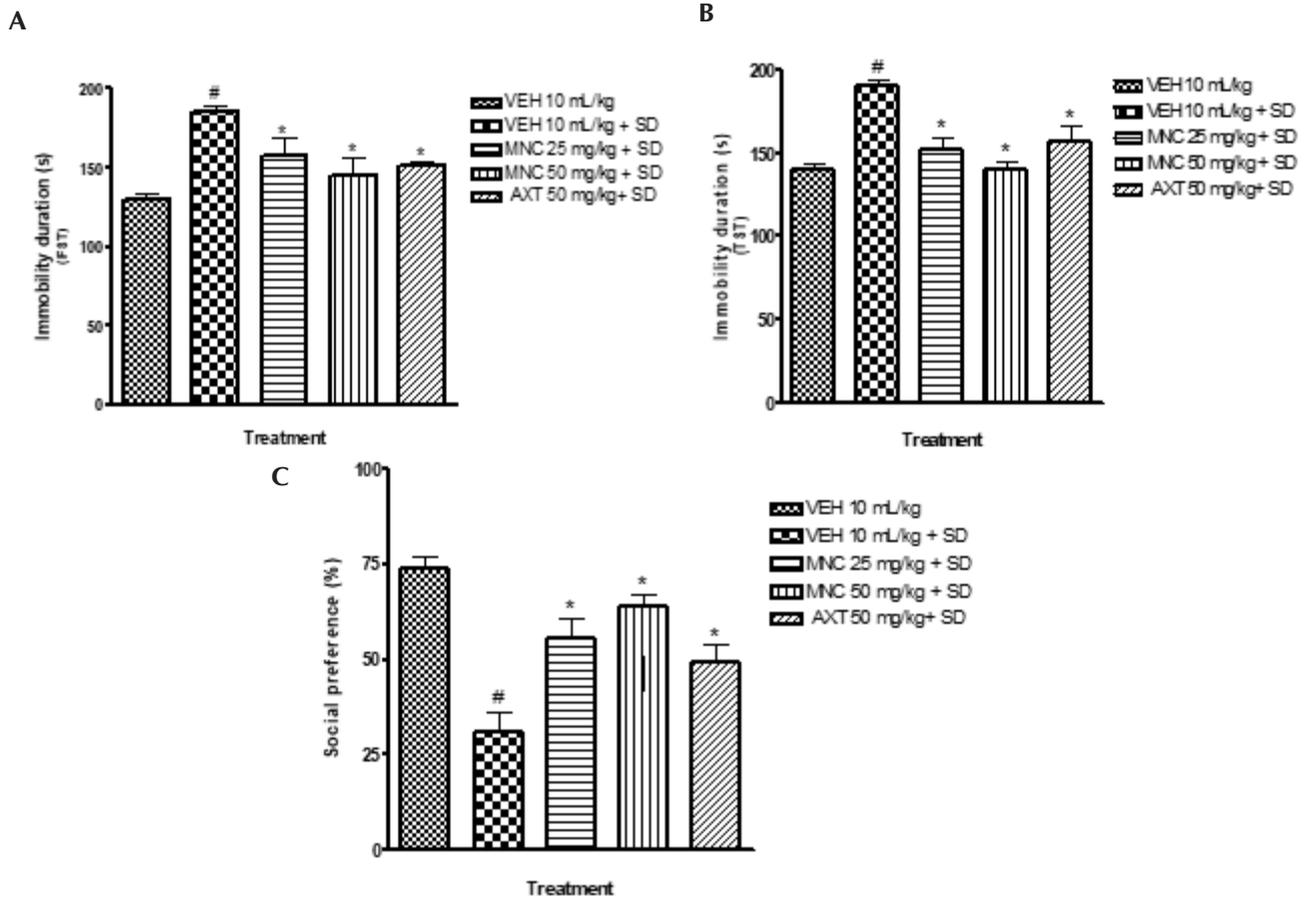
### **Statistical analysis**

All data are obtainable as Mean  $\pm$  S.E.M. The results were subsequently analysed by one-way analysis of variance and Student's Newman-Keuls post-hoc test was done in order to assess the source of significance using Graph Pad InStat® Biostatistics software. The level of significance for all tests was then set at  $p < 0.05$ .

## **RESULTS**

### **Effect of minocycline on depressive-like symptoms in sleep-deprived mice utilising behavioural paradigms**

From the FST, mice deprived of sleep showed a significant ( $p < 0.05$ ) increase in the duration of immobility when compared to control mice. However, oral administration of minocycline (25 and 50 mg/kg) significantly ( $p < 0.05$ ) mitigated this increase in immobility induced by sleep deprivation (Fig. 1A). From the TST, mice subjected to sleep deprivation showed a significant ( $p < 0.05$ ) increase in the duration of immobility when compared to control mice. However, administration of minocycline (25 and 50 mg/kg, p.o.) significantly ( $p < 0.05$ ) attenuated the increase in immobility induced by sleep deprivation (Fig. 1B). Also as observed in the SIT, sleep deprivation caused a significant ( $p < 0.05$ ) decrease in the preference of mice for the social chamber which is believed to be a sign of social withdrawal when compared to the control group. However, mice that were pre-treated with minocycline exhibited a significantly ( $p < 0.05$ ) higher preference for the social chamber in a pattern that insinuates dose-dependence (Fig. 1C).



**Figure 1: Effect of minocycline on depressive-like symptoms in sleep-deprived mice utilising behavioural paradigms.** A: FST, B: TST, C: Social preference. \* denotes significant difference ( $p < 0.05$ ) compared to vehicle + SD group. # denotes significant ( $p < 0.05$ ) difference compared to vehicle group (not sleep deprived). VEH: Vehicle, AXT: Astaxanthin, SD: Sleep deprivation, MNC: minocycline.

### Effect of minocycline on brain oxidants activity in sleep-deprived mice

The result shows that brain antioxidant levels (catalase, superoxide dismutase and glutathione) was significantly ( $p < 0.05$ ) decreased in sleep deprived mice when compared to non sleep-deprived group, whereas minocycline in both doses significantly ( $p < 0.05$ ) increased their levels in mice as effectively as astaxanthin. Otherwise, brain pro-oxidant levels (malondialdehyde and nitric oxide) were significantly ( $p < 0.05$ ) elevated in sleep deprived mice when compared to non sleep-

deprived group, whereas minocycline in both doses significantly ( $p < 0.05$ ) decreased the levels of brain pro-oxidant in mice as effectively as astaxanthin (Table I).

### Effect of minocycline on prefrontal cortex (PFC) neurons in sleep-deprived mice

Fig. 2 and 3 show the effect of minocycline on viable PFC neurons and neuronal density, respectively, in mice subjected to 72 hours sleep deprivation. The results revealed that sleep deprivation significantly ( $p < 0.05$ ) initiated degeneration of PFC neurons and consequently

**Table I: Antioxidant and prooxidant levels in sleep-deprived mice**

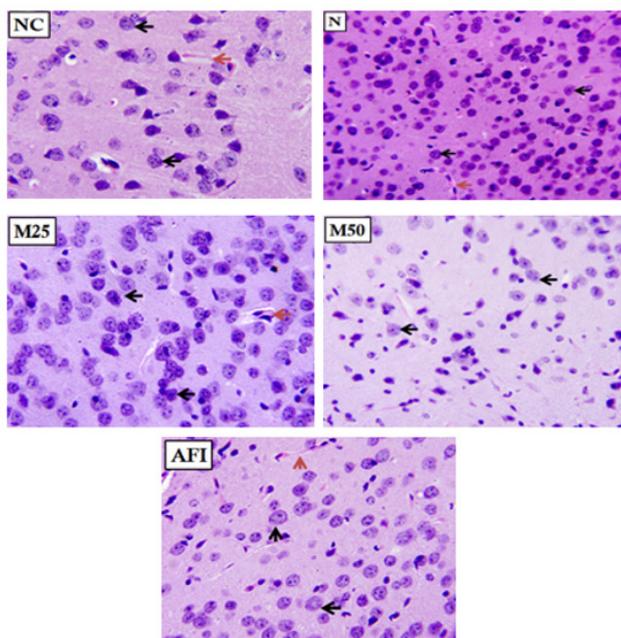
Treatment	GSH (mmol/g tissue)	SOD (Unit/mg protein)	CAT (Unit/mg protein)	MDA (mmol/g tissue)	Nitrites (mmol/mg tissue)
VEH 10 mL/kg	16.09±1.44	16.91±0.63	33.47±1.94	2.58±0.20	47.37±4.56
VEH 10 mL/kg + SD	6.22±0.51#	8.82±0.60*	15.87±1.95#	7.64±0.45#	76.59±6.37#
MNC 25 mg/kg + SD	9.45±0.59*	13.40±0.71*	22.25±1.77*	5.25±0.76*	56.95±4.80*
MNC 50 mg/kg + SD	11.67±0.43*	15.75±0.55*	25.68±1.63*	4.39±0.65*	52.91±3.98*
AXT 50 mg/kg+ SD	13.05±0.87*	15.61±1.07*	27.10±1.88*	5.01±0.48*	42.34±5.14*

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

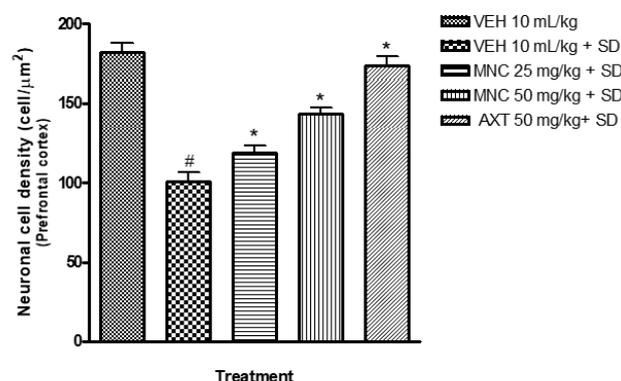
\* denotes significant difference ( $p < 0.05$ ) compared to vehicle + SD group.

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

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



**Figure 2: Photomicrograph of the prefrontal cortex of mice subjected to sleep deprivation.** Slide NC= VEH 10 mL/kg, Slide N= VEH 10 mL/kg + SD, Slide M25= MNC 25 mg/kg + SD, Slide M50= MNC 50 mg/kg + SD, Slide AFI= AXT 50 mg/kg + SD. VEH: Vehicle, AXT: Astaxanthin, SD: Sleep deprivation, MNC: Minocycline. Black arrow indicates: Regular neuronal cells. Red arrow indicates: Neuronal cells undergoing necrosis

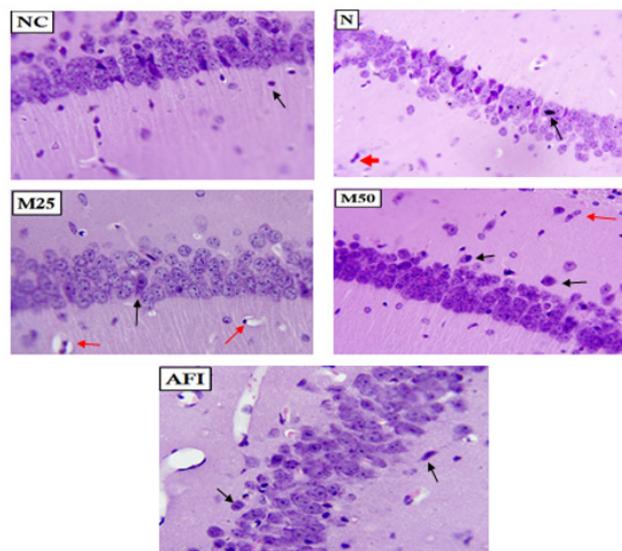


**Figure 3: Effect of minocycline on neuronal density of prefrontal cortex neurons in mice subjected to sleep deprivation.** Counts are based on values obtained using pre-calibrated Image J software. Dataset is expressed as mean ± S.E.M for grouped mice (n=3). \* indicates significant (p < 0.05) difference compared to the vehicle + SD group. # indicates significant (p < 0.05) difference compared to the vehicle group (not sleep deprived). VEH: Vehicle, AXT: Astaxanthin, SD: Sleep deprivation, MNC: minocycline.

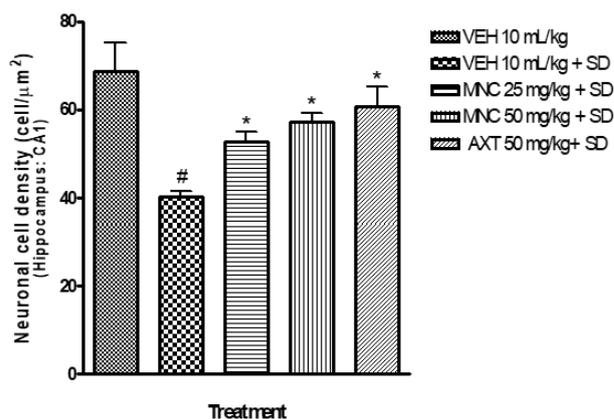
reduced the number of viable neurons in comparison to the non sleep-deprived group. These effects were significantly reversed by minocycline (25 and 50 mg/kg).

**Effect of minocycline on CA1 neurons in sleep-deprived mice**

Fig. 4 and 5 show the effect of minocycline on viable CA1 neurons and neuronal density, respectively, in mice subjected to 72 hours sleep deprivation. The



**Figure 4: Photomicrograph of the hippocampal CA1 of mice subjected to sleep deprivation.** Slide NC= VEH 10 mL/kg, Slide N= VEH 10 mL/kg + SD, Slide M25= MNC 25 mg/kg + SD, Slide M50= MNC 50 mg/kg + SD, Slide AFI= AXT 50 mg/kg + SD. VEH: Vehicle, AXT: Astaxanthin, SD: Sleep deprivation, MNC: Minocycline. Black arrow indicates: Regular neuronal cells. Red arrow indicates: Neuronal cells undergoing necrosis



**Figure 5: Effect of minocycline on neuronal density of hippocampal CA1 neurons in mice subjected to sleep deprivation.** Counts are based on values obtained using pre-calibrated Image J software. Dataset is expressed as mean ± S.E.M for grouped mice (n=3). \* indicates significant (p < 0.05) difference compared to the vehicle + SD group. # indicates significant (p < 0.05) difference compared to the vehicle group (not sleep deprived). VEH: Vehicle, AXT: Astaxanthin, SD: Sleep deprivation, MNC: minocycline.

results revealed that sleep deprivation significantly (p<0.05) initiated the degeneration of CA1 neurons and consequently reduced the number of viable neurons in comparison to the non sleep-deprived group. These effects were significantly reversed by minocycline (25 and 50 mg/kg).

**DISCUSSION**

This study was designed to evaluate the probable benefit of minocycline on depression-like behaviours and oxidative stress induced by chronic sleep deprivation in

mice. The results from the current study depicted that sleep deprivation induced oxidative stress by enhancing production and activity of reactive oxygen species, a mechanism that has been linked to a depression-like phenotype in earlier studies (21, 22).

Depression-like phenotype was assessed in this study using behavioural tests such as forced swimming, tail suspension and social interaction test. In these tests, sleep deprived mice exhibited depressive-like symptoms indicated by increased duration of immobility in tail suspension and forced swimming tests, and increased need to be alone in the social interaction test when compared to the normal control group which received astaxanthin, a known adaptogen. This is in resonance with previous studies that have ascribed such mood-lowering responses in experimental tests to the outset of mood disorders such as depression (23, 24).

Also, certain pro-oxidants (malondialdehyde, nitric oxide) and antioxidants (glutathione, catalase, and superoxide dismutase) were measured in sleep-deprived mice. The results revealed that sleep deprivation caused a significant increase in pro-oxidant levels with a concomitant decrease in antioxidant level/activity. This imbalance in oxidant levels has been recognized as one of the mechanisms for the production of oxidative radicals (25). Since free radicals accumulate more during prolonged durations of wakefulness (26), excessive sleeplessness (or sleep deprivation) has therefore been associated with increased oxidative stress (27,28).

On the other hand, mice that were pre-treated with minocycline prior to exposure to sleep deprivation exhibited opposite effects to the sleep-deprivation only group. Minocycline attenuated depression-like behaviour in the behavioural tests as well as attenuated the increase in oxidative free radicals caused by sleep deprivation as seen in previous studies (29). Furthermore, histological analysis of the PFC and CA1 also support the potential antidepressant role of minocycline in brains of sleep-deprived mice, since both brain regions have been previously implicated in the outset of mood disorders such as depression (20,30). The effects observed in this research concur with previous studies that have posited that minocycline may improve mood and alleviate depression through its anti-inflammatory function (31) or inhibition of the nitric oxide pathway (32).

## CONCLUSION

In conclusion, the results of this study provide evidence that just like astaxanthin, minocycline possesses antidepressant and free-radical scavenging activity in mice exposed to chronic sleep deprivation. However, further studies that focus on long-term use of minocycline on a wider experimental scale is required to expatiate on the benefits of minocycline in depression.

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