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Research Article

Minocycline abrogates lung oxidative damage and haematological perturbations in mice exposed to hypoxia

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Abstract

Objectives: Hypoxia results from the unavailability of sufficient oxygen to tissues. Since previous studies have shown that hypoxia results in inflammation, this research has found it necessary to investigate the impact of chronic hypoxia on the lungs and blood of mice and also the potential benefit of minocycline which is a well-known antibiotic.

Design: Thirty mice were split into 5 groups of 6 animals each. A physical model of hypoxia was used. Here, mice were locked in a 250 mL airtight container for 20 mins each day for seven consecutive days.

Interventions: One hour before exposure to hypoxia, mice in all groups except the vehicle group, were treated with varying doses of minocycline (10, 20, 40 mg/kg, p.o.).

Main Outcome Measures: The mice in the positive control group expressed a sign of lung inflammation with an increase in myeloperoxidase activity, as well as lung oxidative stress with an increase of prooxidants over antioxidants. While in the blood, white blood cells were significantly increased whereas red blood cell count depreciated as blood volume did too.

Results: The various effects of hypoxia on the blood and lungs of the mice were significantly prevented and attenuated to an acceptable degree by minocycline

Conclusion: In conclusion, minocycline expresses an acceptable benefit in the respiratory and circulatory systems of mice in hypoxic conditions.

Keywords: Hypoxia, Lungs, Blood, Haematology, Oxidative stress, Minocycline

INTRODUCTION

Hypoxia is a condition that results when the cellular supply of oxygen does not meet the demand. Most commonly, previous research have focused on investigating this condition and its effect on the brain since the brain is considered the organ with the greatest need for oxygen.¹ Other studies had also investigated the potential benefit of minocycline on such hypoxic conditions in the brain (such as hypoxia-ischemia) due to the fact that the drug is highly lipophilic and crosses the blood-brain barrier.² In those studies, the benefit of minocycline was linked to its intrinsic anti-inflammatory activity.

Minocycline is a tetracycline antibiotic with highly profitable pharmacokinetic properties (good tolerance, safety, broad spectrum of action, and high efficacy). Apart from its known antibiotic activity, various studies have elucidated a potential role of minocycline in oxidative stress attenuation which has been linked to its anti-inflammatory ability.¹⁻⁴

From the foregoing, the main objectives of the current study were to elucidate the potential benefit of minocycline on lung tissue damage and blood dyscrasia induced by chronic hypoxia in mice.

METHODS

Procurement of Animals

Thirty (30) healthy albino mice (male; weight=24.0±2.0 g) which were used in this research were procured from the animal house of the Basic Medical Sciences faculty of the institution. The NIH protocols for animal experimentation (under the *Guide for Care and Use of Laboratory Animals*; publication 85-23) were followed with minute modifications in housing and feeding the mice.

Drug Preparation and Treatment groups

Minocycline (100 mg) was dissolved in an appropriate volume of distilled water to obtain the stock solution. Further serial dilutions were carried out to acquire the three dose levels used in this study (10, 20, 40 mg/kg). Therefore, the mice were allotted into five (5) treatment groups of six animals each (n = 6) based on the treatment they received:

Group 1 (vehicle group) received 10 mL/kg distilled water only;
Group 2 (positive control) received 10 mL/kg distilled water;
Group 3 received 10 mg/kg minocycline;
Group 4 received 20 mg/kg minocycline;
Group 5 received 40 mg/kg minocycline.

The animals were treated for 7 consecutive days and all administrations were oral.

Experimental protocol

Hypoxia-induced stress was initiated in mice using a physical model. One hour after mice were orally treated with either distilled water or minocycline, mice in groups 2–5 only were subjected to the hypoxic stress design by locking them individually in an airtight cylindrical container of ~250 mL capacity for 20 min daily for the total duration of treatment i.e., seven (7) days.⁵ On day 8, haematological assays and biochemical assays of the lungs were carried out. Histology of the lungs was also done.

Biochemical assay of lungs

Following euthanization, each mouse lung was harvested, weighed and homogenized with phosphate buffer and cold centrifuged for 15 min. Aliquots of the resulting supernatant were then used for each of the biochemical assays.

Reduced glutathione (GSH) concentration and glutathione peroxidase (GPx) activity

GSH concentration and GPx activity were determined using an established method.⁶

Malondialdehyde (MDA) content

MDA content was estimated following previous protocol.⁷

Superoxide dismutase (SOD) activity

SOD activity was determined by a modified method.⁸

Catalase (CAT) activity

CAT activity was assayed based on the method described.⁹

Nitrite concentration

Lung nitrite concentration (μM) was estimated using Greiss reagent.¹⁰

Myeloperoxidase (MPO) activity

MPO activity in the lungs was determined according to the method previously described.^{11,12}

Lung tissue histology

Lung tissue obtained for histological studies from the mice in each group was prepared and subjected to H&E staining following the method of Akinluyi et al.¹³ with slight modifications. Micrographs were then obtained digitally.

Haematological assays

Subsequent to euthanasia, blood was obtained from mice via cardiac puncture into EDTA tubes. A full blood analysis was carried out according to previously described methods.¹⁴

Statistical analysis

All data are presented as means $\pm$ SEM. Statistical comparisons were made using one way-ANOVA followed by a Bonferroni's post-test using the Graph Pad Biostatistics software. The level of significance for all tests was set at $p < 0.05$.

RESULTS

Oxidative stress profile of the lungs in hypoxia-induced stress

Figures 1 and 2 reveal that hypoxia-induced stress resulted in a significant hike in prooxidant levels in the lungs. The former represented MDA content and the latter depicted nitrites concentration. In both figures also, minocycline-treated groups showed signs of attenuation of this effect.

On the other hand, the antioxidant molecules of the mice exposed to hypoxia alone were significantly diminished with reduced activity when compared to the vehicle group. Figures 3-6 showed reduced SOD activity, reduced CAT activity, reduced GSH concentration, and reduced GPx activity, respectively. However, minocycline administration significantly attenuated these effects.

Myeloperoxidase (MPO) activity in lung of mice exposed to hypoxia-induced stress

Mice in the positive control group showed a significant increase in MPO activity during the biochemical assays (Figure 7). However, minocycline groups responded oppositely by presenting with significant reductions in MPO activity.

Histology of the lungs of mice exposed to hypoxia-induced stress

Lungs of mice in the positive control group (depicted as slide G) showed the presence of fewer pneumocytes and the shrinking of capillaries more than the mice of the vehicle group (depicted as slide N). However, the tables turned when the mice which received any of the doses of minocycline showed an improved architecture of the alveoli (slides A-C; see Figure 8).

White blood cell count in mice exposed to hypoxic stress

Figure 9 revealed the significant increase in number of white blood cells of mice in the positive control when compared to the vehicle group. Minocycline treatment caused a significant suppression in this increase.

Red blood cell count in mice exposed to hypoxic stress

Figure 10 revealed the significant depletion in white blood cell count of mice in the positive control when compared to the vehicle group. Minocycline treatment caused a significant attenuation in this decrease.

Haemoglobin content in mice exposed to hypoxic stress

Haemoglobin content of mice exposed to hypoxia-induced stress was significantly reduced when compared to mice in the vehicle group. However, minocycline administration caused a significant increase in haemoglobin content in a manner resembling dose dependence (Figure 11).

Blood volume markers of mice exposed to hypoxic stress

From Table 1, it is easy to deduce that hypoxic stress caused a significant reduction in blood volume markers – PCV, MCV, and MCHC – when compared to the vehicle only group. However, minocycline caused a significant boost in blood volume towards normal.

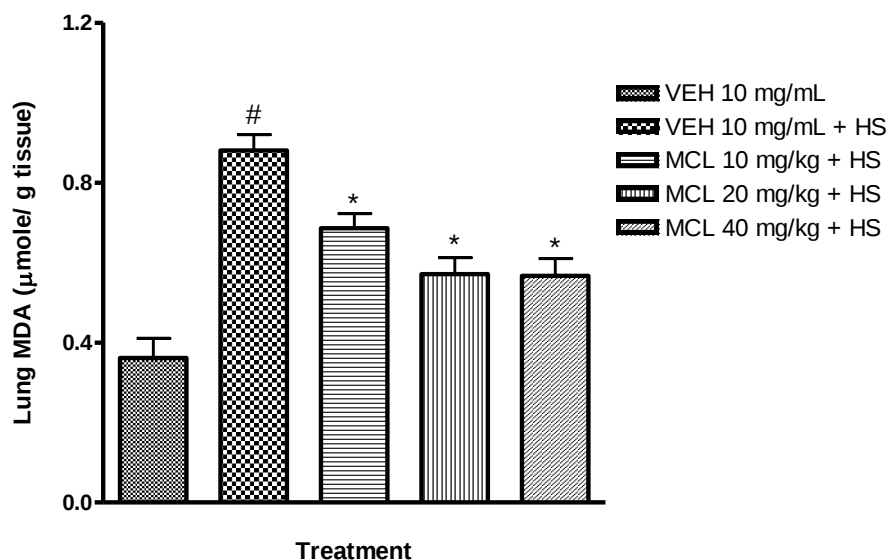
Table 1: Effect of minocycline on blood volume markers of mice exposed to hypoxic stress

Treatment	PCV (%)	MCV (fl)	MCHC (pg/dL)
VEH 10 mL/kg	54.10±3.12	67.33±5.16	40.72±2.57
VEH 10 mL/kg + HS	26.33±2.20 [#]	29.80±3.77 [#]	15.20±2.13 [#]
MCL 10 mg/kg + HS	34.50±3.35	43.20±3.81	19.83±1.62
MCL 20 mg/kg + HS	40.97±2.87 [*]	52.63±3.15 [*]	24.67±1.41 [*]
MCL 40 mg/kg + HS	45.50±2.64 [*]	55.47±4.39 [*]	27.50±1.80 [*]

Each result is presented as mean ± S.E.M of grouped mice (n=3).

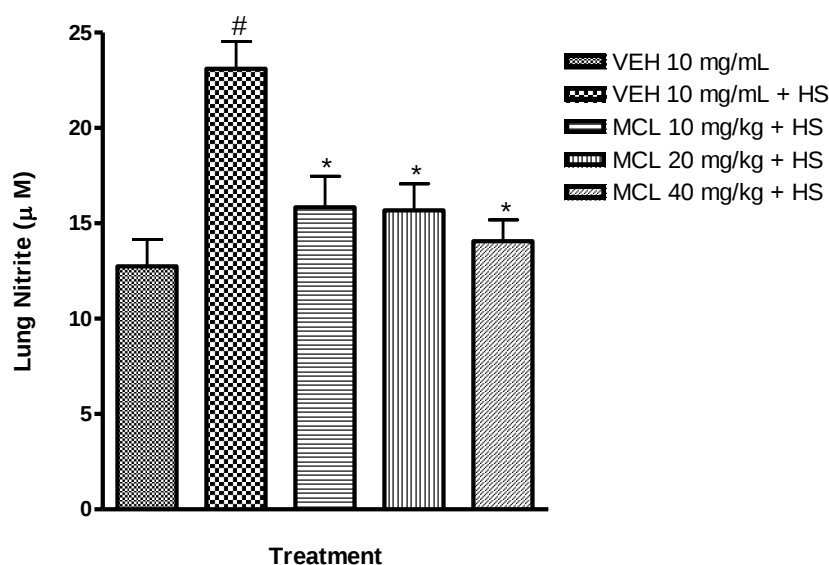
[#] indicates significant difference (p<0.05) compared to the vehicle group.

^{*} indicates significant difference (p<0.05) compared to the vehicle + HS group. **VEH** – Vehicle, **MCL** – Minocycline, **HS** – Hypoxic Stress

**Figure 1: Effect of minocycline on lung malondialdehyde level in mice exposed to hypoxic stress**

[#] indicates significant difference (p<0.05) compared to the vehicle group.

^{*} indicates significant difference (p<0.05) compared to the vehicle + HS group. **VEH** – Vehicle, **MCL** – Minocycline, **HS** – Hypoxic Stress

**Figure 2: Effect of minocycline on lung nitrite level in mice exposed to hypoxic stress**

[#] indicates significant difference (p<0.05) compared to the vehicle group.

^{*} indicates significant difference (p<0.05) compared to the vehicle + HS group. **VEH** – Vehicle, **MCL** – Minocycline, **HS** – Hypoxic Stress

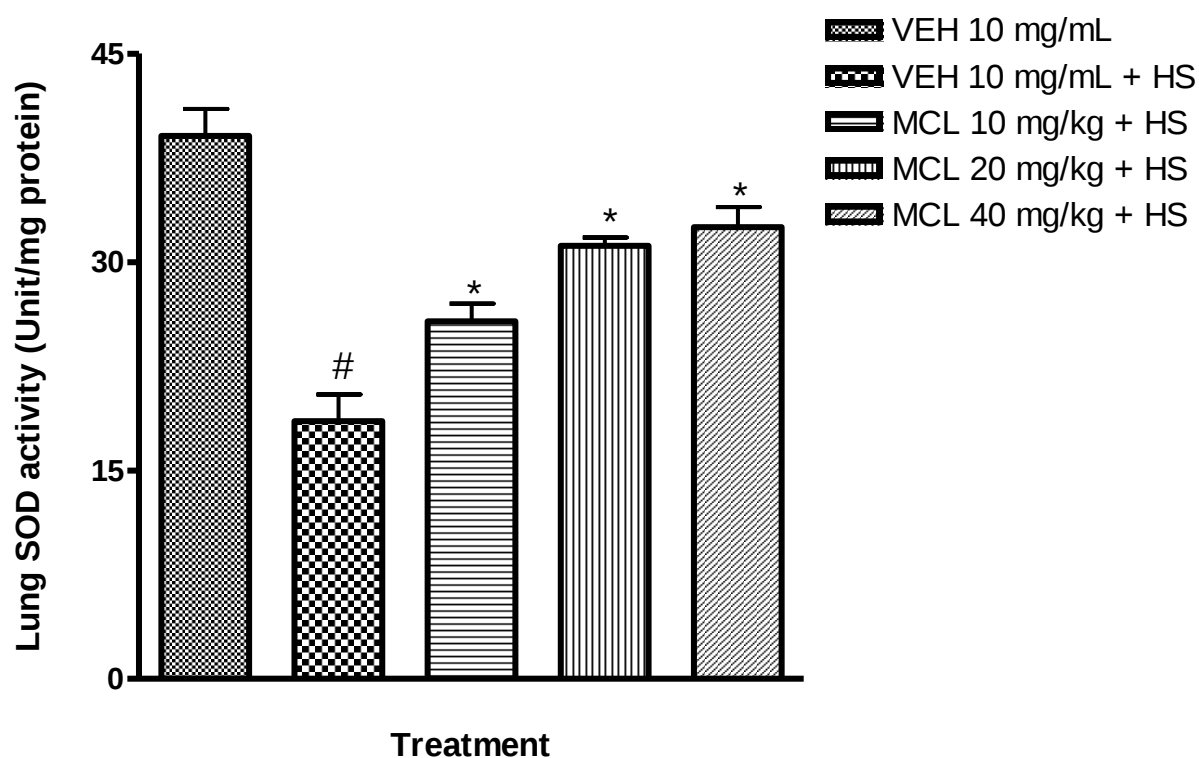


Figure 3: Effect of minocycline on lung superoxide activity in mice exposed to hypoxic stress

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

* indicates significant difference ($p < 0.05$) compared to the vehicle + HS group. VEH – Vehicle, MCL – Minocycline, HS – Hypoxic Stress

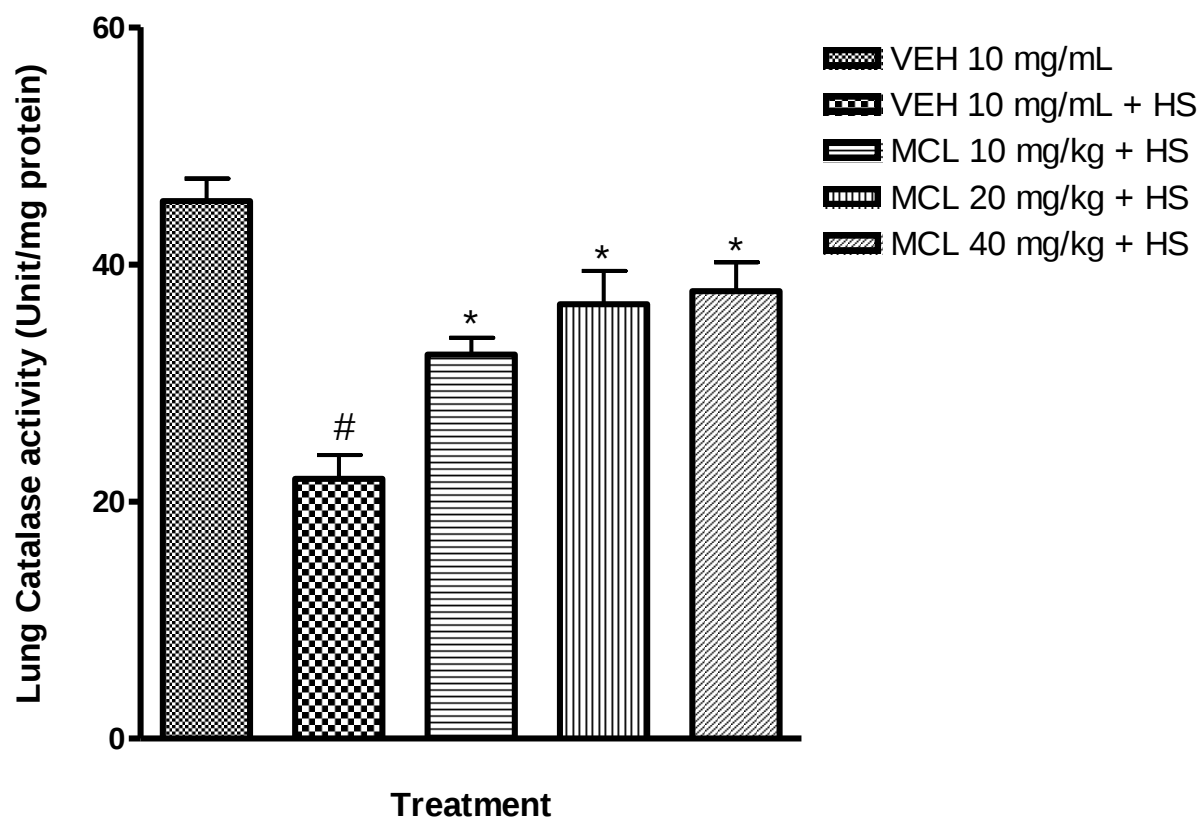


Figure 4: Effect of minocycline on lung catalase activity in mice exposed to hypoxic stress

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

* indicates significant difference ($p < 0.05$) compared to the vehicle + HS group. VEH – Vehicle, MCL – Minocycline, HS – Hypoxic Stress

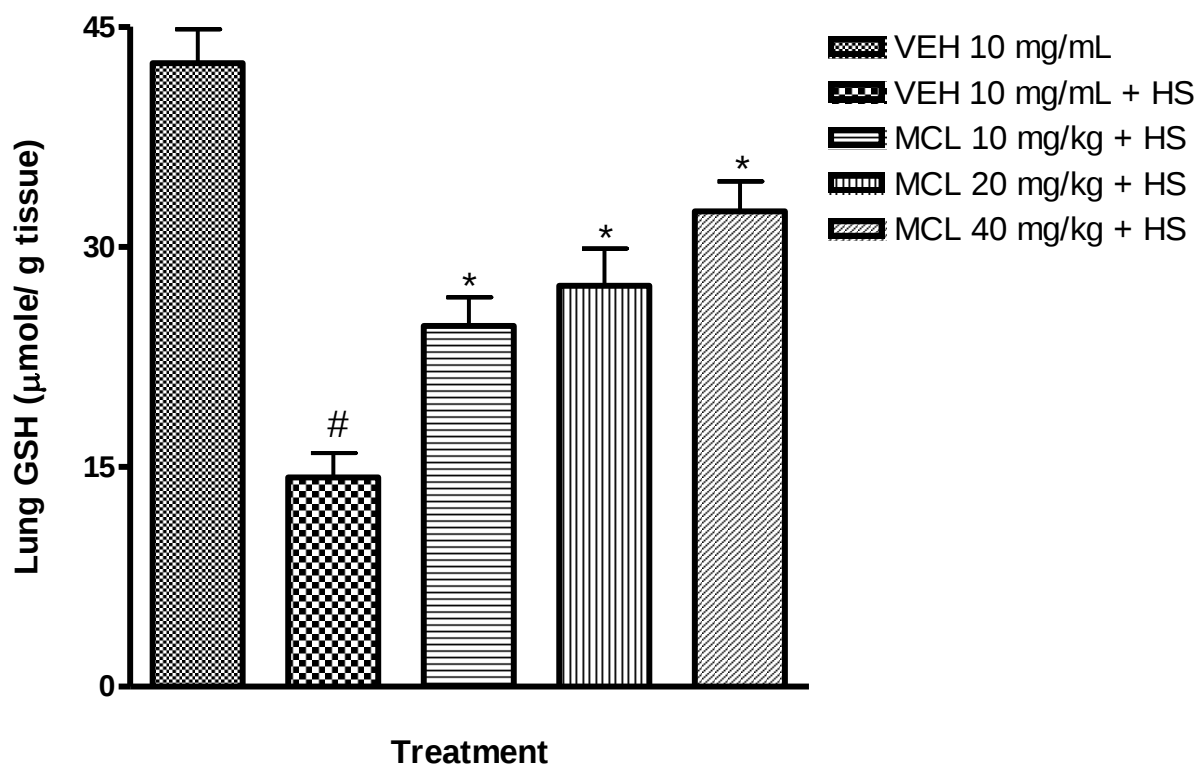


Figure 5: Effect of minocycline on lung glutathione level in mice exposed to hypoxic stress

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

* indicates significant difference ($p < 0.05$) compared to the vehicle + HS group. **VEH** – Vehicle, **MCL** – Minocycline, **HS** – Hypoxic Stress

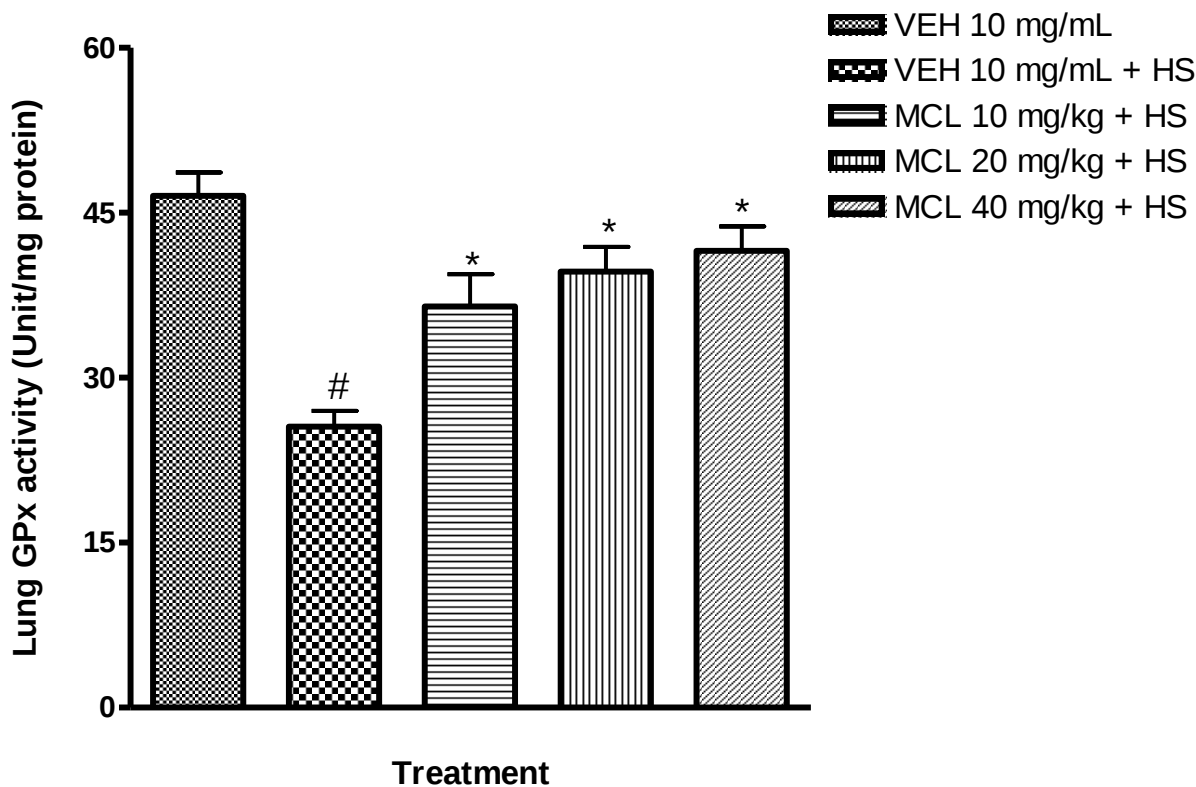


Figure 6: Effect of minocycline on lung glutathione peroxidase activity in mice exposed to hypoxic stress

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

* indicates significant difference ($p < 0.05$) compared to the vehicle + HS group. **VEH** – Vehicle, **MCL** – Minocycline, **HS** – Hypoxic Stress

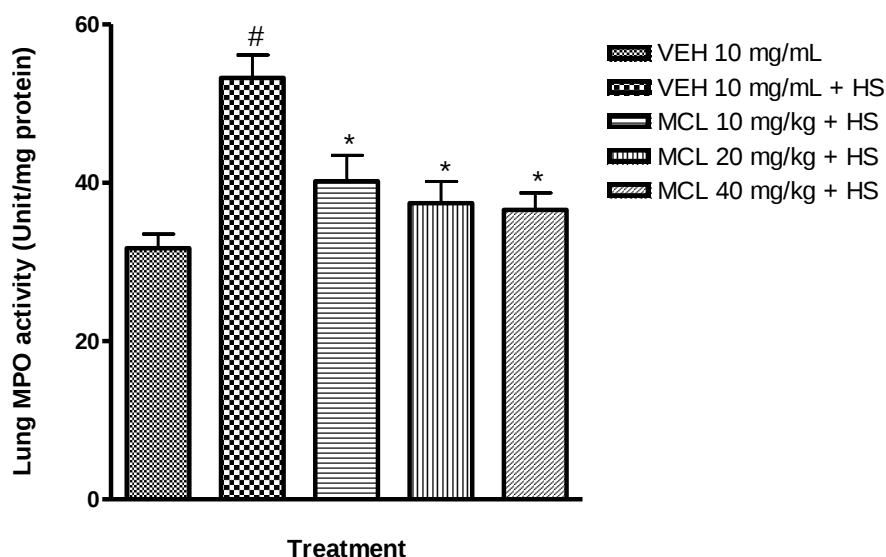


Figure 7: Effect of minocycline on lung myeloperoxidase activity in mice exposed to hypoxic stress

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

* indicates significant difference ($p < 0.05$) compared to the vehicle + HS group. **VEH** – Vehicle, **MCL** – Minocycline, **HS** – Hypoxic Stress

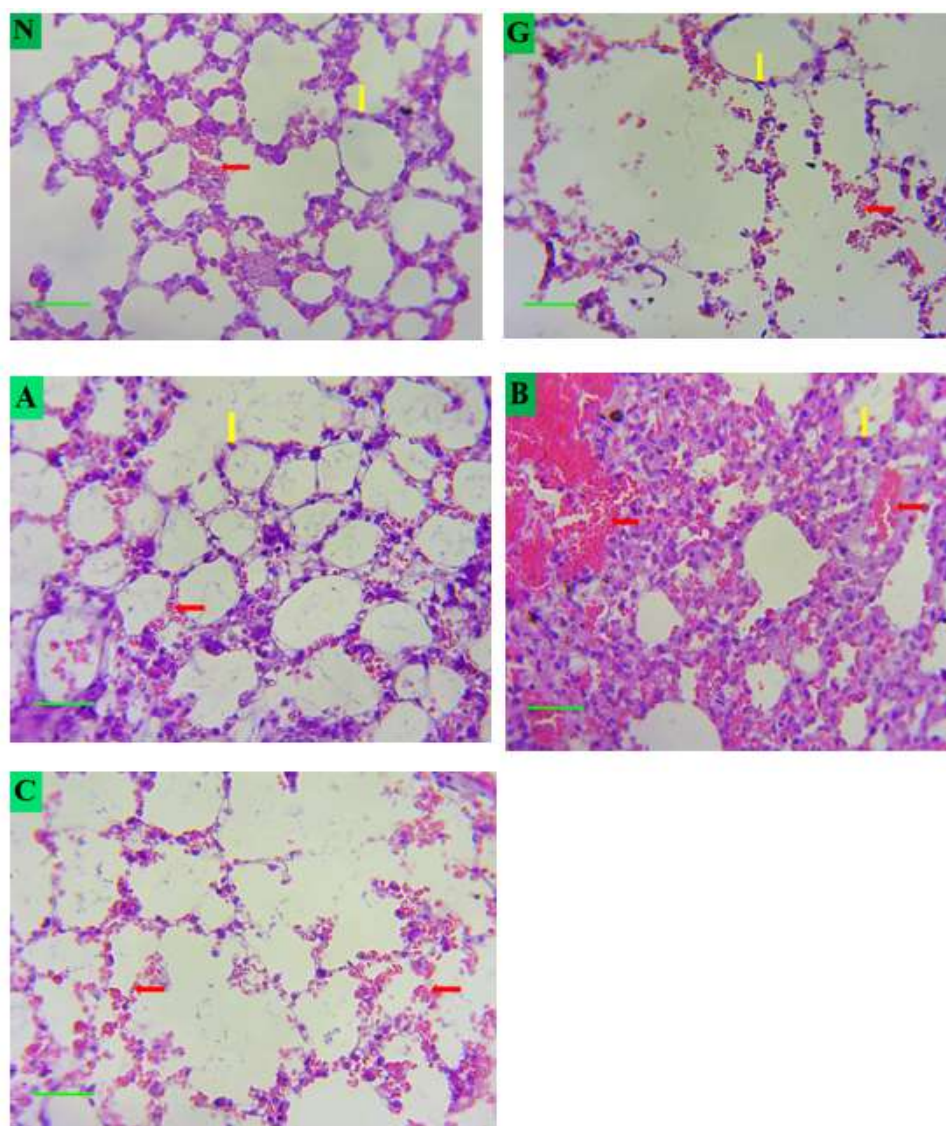


Figure 8: Histology of the lung tissue of mice exposed to hypoxic stress

Key:

Slide **N** – VEH 10 mL/kg

Slide **G** – VEH 10 mL/kg+HS

Slide **A** – MCL 10 mg/kg+HS

Slide **B** – MCL 20 mg/kg+HS

Slide **C** – MCL 40 mg/kg+HS

Red arrow: Capillaries.

Yellow arrow: Pneumocytes

VEH – Vehicle, **MCL** – Minocycline, **HS** – Hypoxic Stress

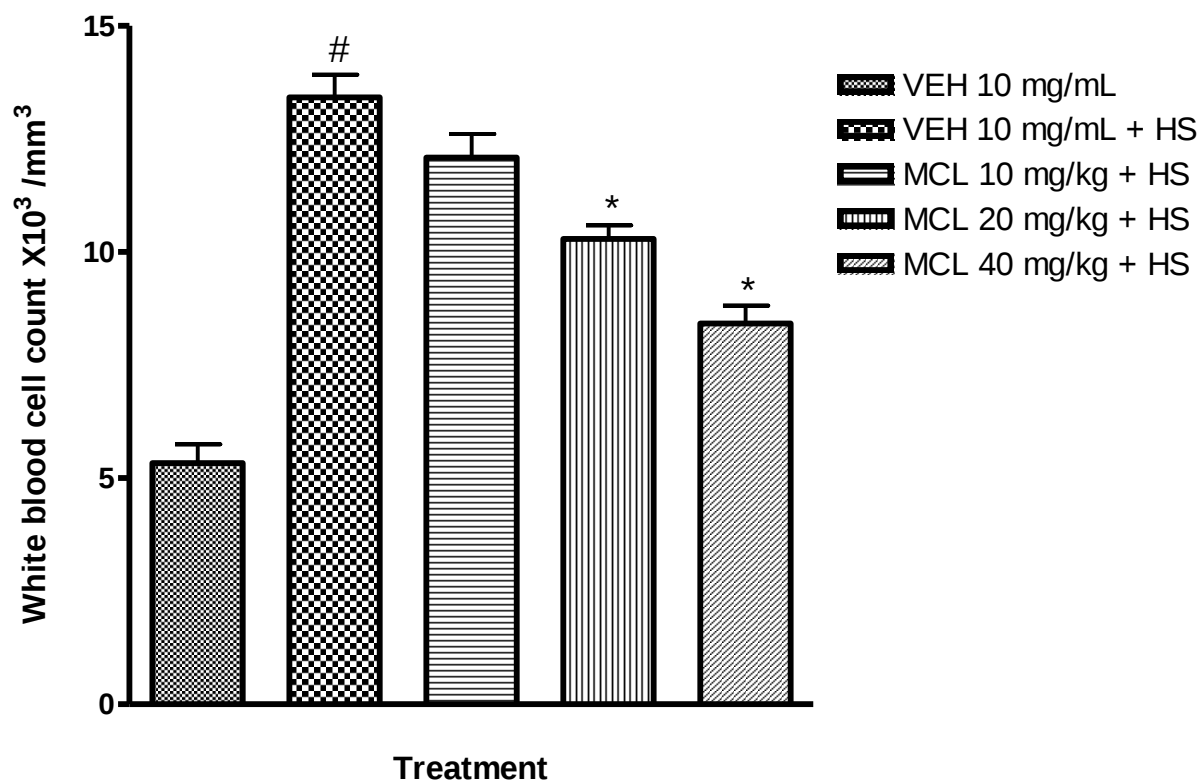


Figure 9: Effect of minocycline on white blood cell count in mice exposed to hypoxic stress

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

* indicates significant difference ($p < 0.05$) compared to the vehicle + HS group. VEH – Vehicle, MCL – Minocycline, HS – Hypoxic Stress

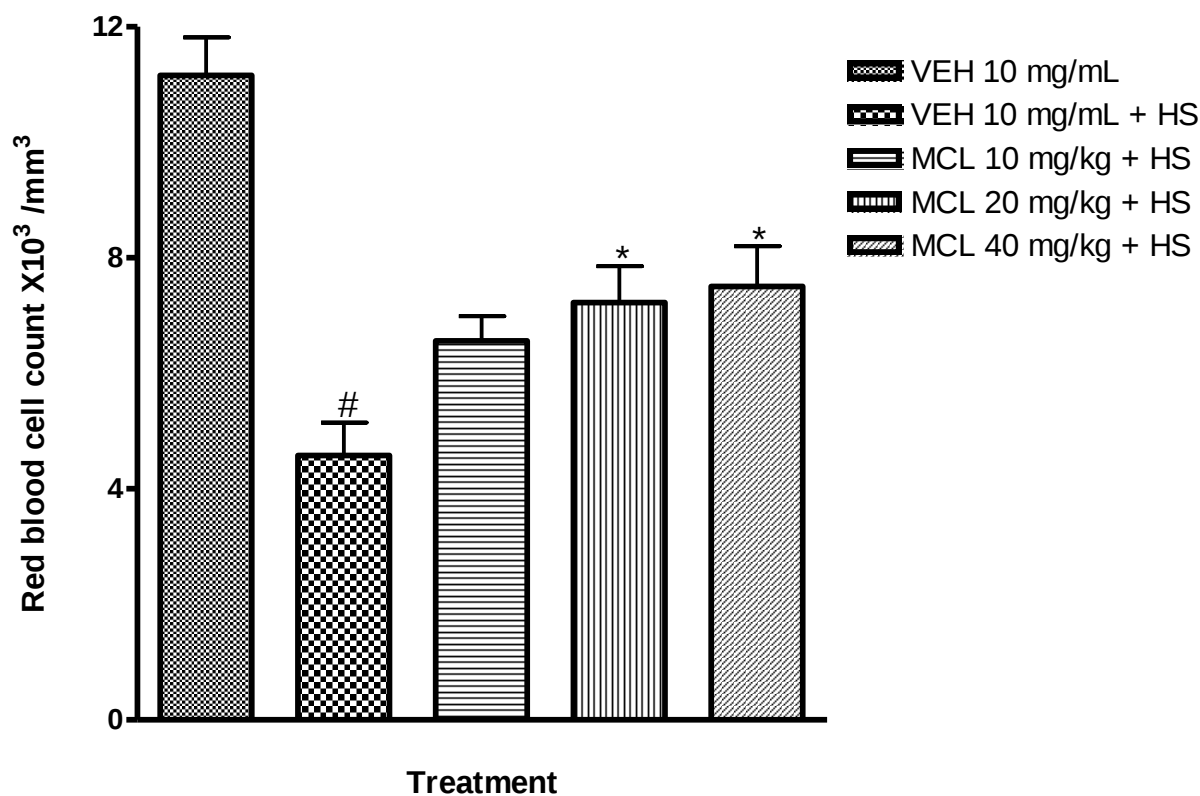


Figure 10: Effect of minocycline on red blood cell count in mice exposed to hypoxic stress

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

* indicates significant difference ($p < 0.05$) compared to the vehicle + HS group. VEH – Vehicle, MCL – Minocycline, HS – Hypoxic Stress

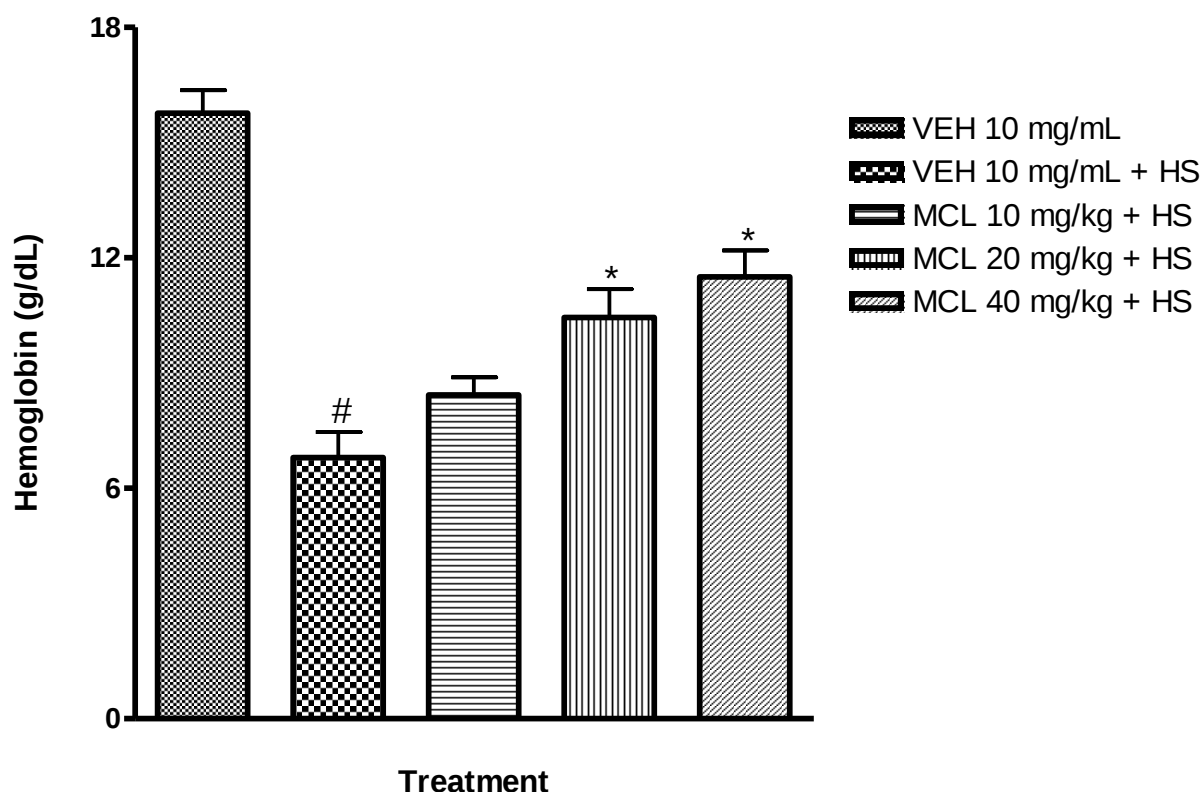


Figure 11: Effect of minocycline on haemoglobin content in mice exposed to hypoxic stress

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

* indicates significant difference ($p < 0.05$) compared to the vehicle + HS group. **VEH** – Vehicle, **MCL** – Minocycline, **HS** – Hypoxic Stress

DISCUSSION

In this study, we have demonstrated that minocycline modulates blood components, pulmonary oxidative stress and lung tissue malformations through a pathway linked to anti-inflammation and antioxidant activity.

Hypoxic condition in the current study triggered a significant rise in myeloperoxidase (MPO) activity and white blood cell count as well as a significant reduction in red blood cell count, haemoglobin content and packed cell volume (PCV). This is in line with previous studies explained below.

Blood comprises of red blood cells, white blood cells and platelets. The red blood cells are responsible for the oxygenation of blood due to their haemoglobin-pairing ability, while the white blood cells are one of the innate immune response mechanisms to any insult or inflammation.¹⁵ Both cell types require the presence of oxygen in order to function within acceptable standards. However, in the absence of sufficient oxygen, the production of red blood cells (i.e., erythropoiesis) slows down. This in turn stimulates an intrinsic inflammatory response in the body which can be signified by a rise in white blood cell count.¹⁶ Also, the haemoglobin content would reduce due to insufficient red blood cells to bind to and the volume of oxygenated blood (as measured by PCV) will subsequently reduce.

In the lungs, the current study revealed that hypoxia initiated oxidative stress by causing a significant reduction in antioxidant activity which enhanced the level of prooxidants in the lungs. Also, the cellular structures of the alveoli were disrupted by hypoxia. This agrees with previous studies which showed that chronic hypoxia diminishes the activity of antioxidants through the activation of microglia in the

respiratory centres of the brainstem.¹⁷ We can therefore agree that the oxidative stress pattern observed in hypoxic conditions has roots in inflammation.

Finally, mice that were pre-treated with minocycline before exposure to hypoxia expressed suppressed negative effects of hypoxia in the lungs and blood alike. Minocycline is believed to counter these negative effects through its anti-inflammatory/antioxidant rather than its antibiotic ability. In the blood, minocycline attenuated hypoxic effects by causing a rapid drop in white blood cell count and enhanced red blood cell count in line with established studies.^{18,19} Also in the lungs, the activity of the inflammatory biomarker, MPO, was significantly reduced in minocycline –treated groups when compared to the hypoxia only group. This agrees with existing studies which had shown that hypoxia can trigger astrocyte and microglia activation which are precursors to inflammation and that minocycline can inhibit this activation thereby suppressing inflammation.^{7,17}

CONCLUSION

Minocycline has once again proven to be a potent anti-inflammatory agent in chronic hypoxia in lungs and blood. However, a greater extent of research is needed to ascertain the dose and safety profile of minocycline in such conditions.

Conflict of interest: None declared

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